ETHNOBOTANICAL SURVEY AND *IN VITRO* EVALUATION OF ANTIMICROBIAL ACTIVITY OF PLANTS TRADITIONALLY USED AS HERBS AND SPICES FROM KABBE CONSTITUENCIES IN ZAMBEZI REGION, NAMIBIA

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ABSTRACT

The aims of this study were to conduct an ethnobotanical survey on indigenous knowledge of plants traditionally used as herbs and spices in Kabbe constituencies of the Zambezi region, evaluate their antimicrobial activity, determine their synergistic effects, and further elucidate their potential mechanism of action. Using semi-structured interviews and closed-end questionnaires, ethnobotanical data were collected from local informants in fourteen villages of Kabbe constituencies between December 2018 and April 2019. Plant samples were extracted with 1:1 ratio of dichloromethane:methanol (DCM:MeOH) and double distilled water before evaluated against laboratory strains of *Candida albicans*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella sonnei*, *Pseudomonas aeruginosa* and *Proteus vulgaris* using disc diffusion assay, minimum inhibitory concentration (MIC), time-kill synergistic study, permeability of cell membrane, and measurement of release of 260 nm absorbing materials and proteins. Twenty-three plant species belonging to 16 plant families were documented. *Cleome gynandra* (83.8) and *Hibiscus mechowii* (77.9) were widely used species. DCM:MeOH extracts of *C. gynandra* had the lowest MIC value of 6.25 mg/mL against both *B. cereus*, *E. faecalis*, *E. coli*, *C. perfringens*, *S. typhimurium*, *P. aeruginosa*, *P. vulgaris*, *C. albicans* and *S. cerevisiae*. Meanwhile, the water extracts of *Eucalyptus* sp. showed the lowest MIC value of 6.25 mg/mL against both *E. coli*, *S. typhimurium* and *P. vulgaris*. Twenty-three synergistic effects were observed with *S. cerevisiae*, *S. typhimurium*, *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, and *C. perfringens*. The 2 × MIC of *Nymphaea lotus* exhibited the greatest electric conductivity at 4 and 6 hours, leaked DNA materials and proteins at 4, 8, 12 and 24 hours against *C. albicans*. Our results contributed data to the gap in the knowledge and availability of scientific information regarding plants traditionally used as herbs and spices in Kabbe constituencies of the Zambezi region. Toxicity studies are needed to support the safe usage of these plants as food additives and natural preservatives for food safety applications.

LIST OF PUBLICATION/ CONFERENCES PROCEEDINGS

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LIST OF ABBREVIATIONS AND/OR ACRONYMS

ANOVA	Analysis of variance
BC	Before Christ
BSA	Bovine Serum Albumin
CAE/g	Catechin hydrate equivalents per gram
CFU/ml	Colony-Forming Units per millilitre
dd-H_2O	Double Distilled Water
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
FC	Number of informants who mentioned the uses of a plant
FI	Frequency index
FIC index	Fractional Inhibitory Concentration index
FL	Fidelity level
GAE/g	Gallic acid equivalent per gram
H_0	Null hypothesis
H_1	Alternative hypothesis
HIV/AIDS	Human immunodeficiency virus infection and acquired immune deficiency syndrome
IBM	International Business Machines

IC_{50}	50 % Growth Reduction
ICF	Informant consensus factor
MET	Ministry of Environment and Tourism
MIC	Minimum Inhibitory Concentration
N	Total number of informants
NBRI	Namibian Botanical Research Institute
NCRST	National Commission on Research, Science and Technology
Np	Number of informants that claim a use of a plant species
Nt	Number of the species used
Nur	Number of use citations in each category
OD_{260nm}	Optical Density of 260 nm
PBS	Phosphate-buffered saline
PCA	Plate Count Agar
R^2 value	R-squared value
RFC	Relative frequency of citation index
sp.	Single species within the genus
spp.	Several species within the genus
SPSS	Statistical Package for the Social Sciences tool
TB	Tuberculosis

TEK	Traditional Ethnobotanical Knowledge
UNAM	University of Namibia
UV	Use value

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DEDICATION

I dedicate this study to my late best friend, brother, business partner and mentor, Tangyh James Salomon. This project reminds me of our childhood memories from years ago. How much you looked up to me as your bigger brother. Through the words of God, we hold out hope that we will meet again. May the Lord and Saviour continue to grant you, Tangyh, an eternal rest and peace! I LOVE YOU ALWAYS BROTHER!

EIMBILO 653 MEHANGANO.

DECLARATIONS

I, Abner Tomas, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

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Name of student	**Signature**	**Date**

1. CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Herbs and spices have a long history of traditional usage for both culinary and medicinal purposes (Embuscado, 2018). They constitute the edible parts of plants that are added to foods or beverages for their natural flavourings, aroma, visual appearance, preservative, and medicinal purposes (Asowata-Ayodele, et al., 2016). Several plants parts have a long record of accomplishment of safe usage as herbs or spices (Dini, 2018). The commonly used plant parts are leaves, flowers, stems, seeds, fruits, roots, barks and vegetable substances (Embuscado, 2015). Specifically, *Moringa* (*Moringa* spp.) leaves, cloves (*Syzygium aromaticum*), cinnamon (*Cinnamomum* spp.), star anise (*Illicium verum*), red pepper (*Capsicum annuum*), ginger (*Zingiber officinale*), and basil (*Ocimum basilicum*) are some of the widely used herbs and/or spices. Historically, herbs and spices were valued as a symbol of health, wealth, power, influence, control and good food (Peter, 2001). They were traded between nations similarly to the same way gold, silver and oil are in recent years (Uhl, 2010). Hence, herbs and spices were regarded to be the early drivers of globalisation in terms of trade and state formation (Sánchez & Kelley, 2019). In agreement, literature shows that most herbs and spices were introduced to various parts of the world by means of trade, war, slavery, colonialism, occupation and immigration (Nunn & Qian, 2010; Pinchin, 2014).

A study by Uhl (2010) has indicated that herbs and spices are found in different forms depending on the specific uses, application, and shelf life. The most common forms in which herbs or spices are found are fresh, dried, whole, ground, crushed, pureed, pastes, extracts, or infusions (NIIR Board of Consultants & Engineers, 2006).

Moreover, herbs and spices are of particular interest due to their ability to flavour food and provide aroma, texture, as well as colour (Frawley, 2019). Also, they have possible ameliorative or preventive agents for chronic health disorders (Srinivasan, 2016). In traditional medicine, herbs and spices are known to possess modulatory effects against atherosclerosis, cancer, diabetic, obesity, inflammation, arthritis, immune deficiency, free radicals, microbes, ageing and mental health (Hussain, et al., 2015). These modulatory effects have become an important research area in twenty-first century (Amrita, et al., 2009). Scientific studies have suggested that herbs and spices are rich in antioxidants, and subsequently they are potent inhibitors of tissue damage and inflammation caused by high levels of blood sugar as well as circulating lipids (Anbazhagi, et al., 2009; Ene-Obong, et al., 2016). Therefore, they have very low calorie content and other potential bioactive compounds in diet (Witkowska, et al., 2013). Additionally, the demand for high quality, preservative-free, less processed foods and beverages with extended shelf life have increased recently (Ahmed, et al., 2013). In the light of the above context, interest in using natural antimicrobial compounds, including extracts of herbs and spices, is increasing to replace synthetic chemical compounds used in food preservation (Hintz, et al., 2015).

The Zambezi region has more than one third of its population regarded as poor and living in rural areas (Colpaert, et al., 2013; NPC, 2016). These people exhibit more traditional values as well as cultural, as part of their everyday life (Chinsembu, et al., 2014). They largely depend on wild and cultivated plant species for their survival (NSA, 2013). This is because they are easily accessible, affordable, have low or not known side effects (Maroyi, 2013). Sometimes, they are the only source of herbs and spices that are available (Sachdeva, et al., 2018). Besides, medicinal plants used to treat livestock diseases and manage human immunodeficiency virus infection and

acquired immune deficiency syndrome (HIV/AIDS) opportunistic infections in Zambezi region have been extensively documented (Chinsembu, et al., 2014; Chinsembu & Hedimbi, 2010). In spite of the previous work reported on Zambezi region indigenous plants, there are no documented records of plants traditionally used as culinary herbs and spices to the best of our knowledge. Therefore, this study aimed to survey and document the indigenous knowledge of plants traditionally used as culinary herbs and spices in Kabbe constituencies of Zambezi region. In addition, the present study aimed to evaluate *in vitro* antimicrobial activity, determine the synergistic effects of herb and spice extracts against selected foodborne pathogens, and elucidate their potential mechanism of action.

1.2 Statement of the Problem

Despite a good number of reports on herbs and spices, their uses, forms, preparation as well as their applications in foods or beverages worldwide (Embuscado, 2015; Witkowska, et al., 2013); little attention has been given to the inventory of traditionally used herbs and spices and their contributions toward food safety in Kabbe constituencies of Zambezi region. To the best of our knowledge, there had been no empirical studies that have evaluated the *in vitro* antimicrobial activity and potential mechanism of action of plants from the Kabbe constituencies traditionally used as herbs and/or spices against foodborne pathogens.

1.3 Objectives of the Study

The specific objectives of the study were:

a) To conduct an ethnobotanical survey of indigenous knowledge of plants traditionally used as herbs and/or spices in Kabbe North and Kabbe South constituencies of Zambezi region, Namibia.

b) To screen and evaluate *in vitro* antimicrobial activity of plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region against foodborne pathogens.

c) To determine whether any synergistic effects exist for various combinations of plant extracts traditionally used as herbs and/or spices against selected foodborne pathogens.

d) To elucidate the potential mechanism of action of plant extracts against selected foodborne pathogens using the permeability of cell membrane assays and the release of 260 nm absorbing materials and proteins.

1.4 Hypotheses of the Study

This study investigated the following hypotheses:

a) H_0: There is no indigenous knowledge on plants traditionally used as herbs and\or spices in Kabbe North and Kabbe South constituencies of Zambezi region.

H_1: There is indigenous knowledge on plants traditionally used as herbs and/or spices in Kabbe North and Kabbe South constituencies of Zambezi region.

b) H_0: Plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have no antimicrobial activity against foodborne pathogens.

H_1: Plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have antimicrobial activity against selected foodborne pathogens.

c) H_0: Various combinations of plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have no synergistic effects against selected foodborne pathogens.

H_1: Various combinations of plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have synergistic effects against selected foodborne pathogens.

d) H_0: Plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have no mechanism of action against foodborne pathogens.

H_1: Plant extracts traditionally used as herbs and/or spices from Kabbe constituencies in Zambezi region have mechanism of action against foodborne pathogens.

1.5 Significance of the Study

The study contributed to the documentation on plants traditionally used as herbs and spices in Kabbe constituencies of Zambezi region. Deciphering putative scientific evidence of these plants could lead to the documentation and understanding of their potential *in vitro* antimicrobial activity against selected foodborne pathogens, which can serve as natural preservatives to control foodborne pathogens. These in turn can be used as substrates in the formation of new antimicrobial agents, functional foods or nutraceuticals. Moreover, this study recommended small to large-scale cultivation, marketing, and usage of these plants that can improve the primary health, socio-economic and employment creation in Zambezi region because of their availability, low cost and simplicity.

2. CHAPTER TWO: LITERATURE REVIEW

2.1 Herbs and Spices

2.1.1 The Use of Herbs and Spices in Ancient Civilization

For thousands years, different plant parts have been used as herbs and/or spices by most if not all cultures around the globe (Embuscado, 2015). Recent literature has reported that various wild and cultivated plants have a long history of uses in traditional medicine and food industry (Sánchez & Kelley, 2019). For instance, herbs and spices were mentioned used as part of everyday lives during the Biblical times. The earliest mention of herbs and spices in the Bible occurs in Genesis 2:12 in reference to the bdellium, a fragrant gum resin from *C. mukul*, a shrub-like tree found growing in arid regions of India, Bangladesh, and Pakistan (Frawley, 2019). In ancient civilization, herbs and spices like anise (*P. anisum*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*), myrrh (*C. myrrha*) and hyssop (*Hyssopus officinalis*) were used as medicine, cosmetic ointments, aromatic oils, perfumes, fumigation, religious rituals, embalming, and in cooking (Raghavan, 2006). In addition, they were recorded as enormous trade values (Vasanthi & Parameswari, 2010). For example, records dating from the 25th century BC have indicated how cinnamon (*Cinnamomum* spp.), rosemary (*Salvia rosmarinus*), sage (*S. officinalis*), sesame (*Sesamum indicum*), tamarind (*Tamarindus indica*), and turmeric (*Curcuma longa*) were imported to Egypt from China and Southeast Asia (Haw, 2017; Oguntade, et al., 2013).

Furthermore, herbs and spices have play significant roles in maintaining human health, improving the quality of human life, and served us with valuable components of seasoning, beverages, cosmetics, dyes, and medicines (Fritts, et al., 2018). Herbs are primarily used treat and manage illnesses as well as disorders, although they possess

secondary functions. Herbs are also used to flavour and garnish food by providing aroma (Spencer, 2017). Furthermore, they are used as aromatic oils (Frawley, 2019). Garlic (*A. sativum*) and hyssop (*H. officinalis*) are good examples of herbs that used for their aroma and aromatic oils, respectively (Parthasarathy, et al., 2008). According to a study by Hussain et al. (2015), herbs are broadly categorized into three categories based on their use and toxicity: "food herbs", "medicinal herbs", and "poisonous herbs". Based on this study, "food herbs" have very low toxicity and are usually applied to food without causing adverse response when consumed. In much similar way, the "medicinal herbs", are used as drug for specific medical conditions, usually for a limited period of time as prescribed by a medical practitioner. On the other hand, the "poisonous herbs" have strong potential for either acute or chronic toxicity; however, they need to be utilized strictly under the supervision of trained clinicians (Rajkovic, 2014).

Spices are primarily used to flavour food by providing aroma, texture, and colour (Srinivasan, 2016). They provide savoury, spicy, sweet, pungent, bitter, or sour notes to foods and beverages (Shan, et al., 2007). Spices also provide secondary effects, such as preservative, nutritional, cosmetic, and health functions (Uhl, 2010). Additionally, spices were used in religious functions and ceremonial events in many cultures, since most believed that they had magical properties (Sánchez & Kelley, 2019). A study by Torku (2017) have illustrated that thorny perennial desert trees and bushes like bdellium (*Commiphora mukul*), myrrh (*C. myrrha*), and frankincense (*Boswellia* spp.) were used in embalming, as perfumes, medicine, anointing oils as well as burned for incense offerings in fumigation, to please the ancient gods, and to banish evil spirits, insects, pests and serpents. Moreover, spices like cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), marjoram (*Origanum majorana*), cassia (*C. cassia*), and

cinnamon (*Cinnamomum* spp.) were commonly used in Egypt and India by the upper classes to ward off the foul odours of the crowded lower classes, and appease the gods of death by embalming the bodies of important personages against decay (Peter, 2001; Rosengarten, 1969).

2.1.2 Forms of Herbs and Spices

Recent studies have indicated that herbs and spices are found in different forms. They can either be in a fresh, dried, whole, ground, crushed, pureed, pastes, extracts, or infusions form (Raghavan, 2006). Of course, each of these forms has its advantages and disadvantages, depending on the specific application, processing parameters, and shelf life (Kong, et al., 2007). The form of fresh herbs and spices provide appealing fresh taste which overall come from their flavor, aroma, and texture in comparison with the dried form (Dini, 2018; NIIR Board of Consultants & Engineers, 2006). According to Uhl (2010), these could be due to volatile components in fresh plants parts. These volatile components in fresh herbs and spices have high vapor pressure at a given temperature compared with a nonvolatile compound (Wyllie & Ryabchikov, 2000).

In comparison to fresh forms, the dried forms of herbs and spices are easier to process, have longer shelf life, and lower cost (Sharif, et al., 2018). Moreover, some dried herbs and spices have flavor intensified compared to when they are fresh (Raghavan, 2006). This could be attributed to less moisture and aroma because of lost volatile components during the drying process (Fritts, et al., 2018). This has made dried herbs and spices to have advantages of being able to withstand higher temperatures and processing conditions better than fresh herbs and spices (Guldiken, et al., 2018). However, they create undesirable appearance in finished product such as discoloring (Shan, et al., 2007).

2.1.3 Preparation and Processing of Herbs and Spices

Herbs and spices have unique chemical compounds that create sensual qualities such as aroma, color, flavor, and sometimes texture to food (Parthasarathy, et al., 2008). Fresh herbs and spices require to undergo preliminary preparations before their application uses due to volatile components which can be lost during harvesting, storing, processing, or handling (Shan, et al., 2007). Therefore, in order for fresh herbs and spices to give these optimal flavor sensations, appropriate preparation techniques are required. According to Uhl (2010), herbs and spices can be prepared by either grounding, slicing, roasting, toasting, frying, or boiling in order for them to release their characteristic flavors. A study by Hui and Sherkat (2005) has shown that these preparatory methods release common sensory characteristics such as bitter, salty, sour, sweet, cooling, earthy, floral, fruity, herbaceous, hot, nutty, piney, pungent, spicy, sulfury, and woody to food and beverage substances. For instance, volatile oils are prepared from spices through grinding, cutting, and heating. These oils have sensory impression on food, which is primarily determined by the chemical senses of taste and smell (Bajpai, et al., 2009). The volatile oils are often prepared from herbs and spices for uses in aromatherapy (El-Massry, et al., 2008). Therefore, herbs and spices are prepared to suit specific applications by creating very different flavour profiles (Raghavan, 2006). For example, a full and broad-spectrum flavor of herbs and spices is achieved by dry roasted, fried in oil, deep fried, simmered, pickled, braised, barbecued, or boiled in water (Dini, 2018). During these preparatory methods, the flavor is enhanced, intensified, or changed due high temperature applied (Fritts, et al., 2018).

2.1.4 Common Bioactive Ingredients in Herbs and Spices

Herbs and spices have natural bioactive phytochemicals, which plays a role in disease preventing, foods and beverages preservation, as well as in fragrances (Tassou, et al., 2012). The common characterised active phytochemicals from herbs and spices include the alkaloids, anthraquinones, bitters, essential oils, flavonoids, terpenoids, lignans, sulfides, polyphenols, carotenoids, coumarins, saponins, plant sterols, tannins, uramins, and phthalates (Embuscado, 2018; Srinivasan, 2016). The associated benefits of these bioactive compounds are summarized in **Table 1** below. Although most of these bioactive compounds are associated with potential benefits, fresh herbs and spices possess challenges in deducing their true health benefits (Braca, et al., 2018). Some of these phytochemicals are volatile and can be lost during harvesting, storing, processing, or handling (Shan, et al., 2007). Thus, preliminary preparations must be taken to ensure these herbs and spices give their true health benefits and optimal flavour sensations.

Table 1: The classification of bioactive compounds, their associated benefits and examples of herb and/or spice sources.

Classification of Bioactive Compounds	Associated Benefits	Examples of herbs and/or spices containing bioactive compounds	References
Alkaloids	• antimalarial agents • anticancer agents • blood promoting circulation agents	Peppers (*Capsicum* spp.) Saffron (*Colchicum autumnale*)	(Aniszewski, 2007; Guldiken, et al., 2018; Nyambe, 2018)
Anthraquinones	• anticancer • anti-inflammatory • diuretic • antiarthritic • antifungal • antibacterial • antimalarial	Aloe (*Aloe* spp.) Cascara (*Rhamnus purshiana*) Rhubarb (*Rheum rhabarbarum*) Senna (*Senna* spp.)	(Bolen, 2019; Deitersen, et al., 2019; Diaz-Muñoz, et al., 2018; Simpson & Amos, 2017)

Bitters	• antioxidant • antimicrobial • antineoplastic (antitumor) • anti-inflammatory • antidiabetic properties • relaxing effect on the nervous system • enhance appetite • improve digestion and nutrients absorption	Angelica (*Angelica* spp.) Chamomile (*Matricaria chamomilla*) Dandelion (*Taraxacum* spp.) Goldenseal (*Hydrastis canadensis*) Horehound (*Marrubium vulgare*) Peppermint (*Mentha × piperita*)	(Guldiken, et al., 2018; Hussain et al., 2015; Yashin, et al., 2017)
Flavonoids	• anti-allergic • antiviral • anticancer • antioxidant	Parsley (*Petroselinum crispum*) Rosemary (*Salvia rosmarinus*) Thyme (*Thymus* spp.) Onions (*Allium cepa*)	(Embuscado, 2018; Nishiumi, et al., 2011; Yashin, et al., 2017)
Saponins	• hypoglycemic activity • lowering of serum cholesterol levels	Aubergine (*Solanum melongena*) Peppers (*Capsicum* spp.)	(Hussain, et al., 2015; Savage, 2003; Yücekutlu & Bildacı, 2008)

	• lowering of low-density lipoprotein-cholesterol levels • stimulation of the cell-mediated immune system • inhibition of the growth of cancer cells • antifungal activity • antioxidant effects • virucidal activity • neurotrophic and neuroprotective effects	Fenugreek (*Trigonella foenum-graecum*) Ginseng (*Panax* spp.)	
Tannins	• anti-inflammatory • analgesic • antilymphocytic • antimicrobial • antileishmanial	Hibiscus (*Hibiscus* spp.) Chamomile (*Matricaria chamomilla*) Cinnamon (*Cinnamomum* spp.) Cloves (*Syzygium aromaticum*)	(Hussain, et al., 2015; Pietta, et al., 2003; Shirmohammadli, et al., 2018)

	• antioxidants • immunomodulatory • neuroprotective • antihypertensive • antidiarrheal activities • treat ulcerative colitis	Guarana (*Paullinia cupana*)	
Essential oils	• antiseptic • antimicrobial • anti-inflammatory • antispasmodic • anticancer • enhancing the appetite, digestion and absorption of food, • stimulate the heart and circulatory system	Thyme (*Thyme* spp.) Oregano (*Origanum vulgare*) Rosemary (*Salvia Rosmarinus*) Cumin (*Cuminum cyminum*) Pepper (*Capsicum* spp.) Sage (*Salvia officinalis*)	(El-Massry, et al., 2008; Hussain, et al., 2015; Plant, et al., 2019; Srinivasan, 2016; Tajkarimi, et al., 2010; Uhl, 2010)

2.1.5 Antimicrobial Activity of Herb and Spice Extracts

In a number of studies, herbs and spices have been reported as the main sources of antimicrobial activities for many decades (Kong, et al., 2007). These antimicrobial activities play major roles in food systems by controlling natural spoilage processes (food preservation) and microbial growth (food safety) (Tajkarimi, et al., 2010). Several studies have analysed the antimicrobial activity of individual and combined extracts of different herbs and spices against pathogenic and food spoilage microorganisms (Fukai, et al., 2005; Mostafa, et al., 2018). In particular, a study by Baljeet et al. (2015) evaluated the antibacterial and antifungal activity of individual as well as in a combination of cumin (*C. cyminum*), ginger (*Zingiber officinale*) and garlic (*A. sativum*) against bacterial strains of *Bacillus subtilus*, *Pseudomonas fluroscens*, *Salmonella* typhi and fungal strains of *Candida albicans* and *Rhizopus azygosporus*. These herbs and spices had MIC of 12.5 mg/mL against all the tested microorganisms as well as strong microbial growth inhibition by the combinations of cumin and garlic with MIC fluctuated from 3.8 to 6.7 mg/mL. Based on these observations by Baljeet et al. (2015), it was suggested that there were herbs and spices with effective antimicrobial activity.

Moreover, literature has reported that antimicrobial activities in herbs and spices are due to the presences of phytochemicals, which naturally are used to provide defence mechanism in plant species (Ananias, 2015; Embuscado, 2015; Guldiken, et al., 2018). For instance, herbs like aloe (*Aloe* spp.), hibiscus (*Hibiscus* spp.), thyme (*Thyme* spp.), peppers (*Capsicum* spp.), and angelica (*Angelica* spp.) contain anthraquinones, flavonoids, essential oils, saponins, and tannins which are responsible for biological activities such as antiarthritic, antifungal, antibacterial, and antimalarial (Embuscado, 2018; Diaz-Muñoz, et al., 2018; Hussain et al., 2015; Srinivasan, 2016). Several

studies have studied the relationship between antimicrobial activity and phytochemical compounds in these herbs and spices (Eymar, et al., 2016; Guldiken, et al., 2018). These phytochemicals found in most herbs and spices possess broad antimicrobial properties against food spoilage bacteria and fungi (Naz & Bano, 2013). Similarly, herb and spice extracts with phenolic and polyphenols possess antimicrobial properties against *Actinobacillus* spp., *Actinomyces* spp., *Aspergillus* spp., *Bacillus* spp., *Candida* spp., *Enterococcus* spp., *Escherichia* spp., *Lactobacillus* spp., *Listeria* spp., *Micrococcus* spp., *Porphyromonas* spp., *Prevotella* spp., *Pseudomonas* spp., *Salmonella* spp., *Serratia* spp., *Staphylococcus* spp., *Streptococcus* spp., and *Xylella* spp. (Bouarab-Chibane, et al., 2019; Maddox, et al., 2010). The broad antimicrobial properties of phenolic and polyphenols were reported caused by various antimicrobial mechanisms of action such as microbial membranes disruption and enzymes inactivation (Kong, et al., 2007). Because of these, herbs and spices have crucial roles as natural food preservatives in food safety (Ahmed, et al., 2013).

2.1.6 Possible Mechanism of Action of Herbs and Spices

Natural plant compounds possess antimicrobial actions and have been used as bactericide and fungicide (Yamada, et al., 1979). Studies have reported the relationship between these bioactive ingredients and antimicrobial action (Huang, et al., 2019; Miksusanti, et al., 2008). According to literature, plant phytochemical compounds have specific biochemical interactions that disrupt phospholipid bilayer, and cause leakage of cytoplasmic material (Shukla, et al., 2016; Tagousop, et al., 2018). This leakage is considered an indicative of gross and irreversible damage to the cytoplasmic membrane (Tagousop, et al., 2018). Therefore, it cause cell lysis. This mode of action was reported similar to that of broad-spectrum antibiotics, membrane-active disinfectants and preservatives (Wal, et al., 1995).

Moreover, literature have reported various methods that are used to determine mechanism of action (Fetz, et al., 2016; Schenone, et al., 2013; Yamada, et al., 1979; Zhang, et al., 2017). These methods include microscopy-based methods, direct biochemical methods, computation inference methods, omics based methods, assay of potassium and phosphate ions efflux, measurement of extracellular adenosine triphosphate (ATP) concentration, and membrane potential. For instance, a study by Zhang, et al. (2017) has examined changes in *E. coli* microstructure using scanning and transmission electron microscopes, permeability of cell membrane, release of cell constituents, and the membrane potential assays after it was exposed to black pepper essential oil (*Piper nigrum* L.). Thus, the choice of mechanism of action method depend on the resources available.

2.2 Spice and Herbs Possibilities of Fighting Foodborne Diseases

Foodborne diseases have always been a major concern in both developing and developed countries around the globe (Weerakkody, et al., 2010). As a matter of fact, Kalyoussef and Feja (2014) study describes foodborne diseases as diseases that arise from consuming contaminated food or beverage by microbial pathogens, naturally produced toxins, or other chemicals. Three main types of food contaminations, which contribute to foodborne diseases, are physical, chemical, and biological food safety hazards (Gutierrez, 2013). These food safety hazards contaminate food or beverage and cause diseases or injury (Kalyoussef & Feja, 2014). This can range from diarrhoea to more serious symptoms, such as cancer depending on which hazard has contaminated the food. Moreover, two most common forms of foodborne diseases are acute gastroenteritis and toxin-mediated food poisoning (Seventer & Hamer, 2017). They can range in severity from mild to serious, even resulting in death.

2.3 Foodborne Pathogens

According to Martinović et al. (2016), foodborne pathogens are bacteria, fungi, viruses, prions or protozoa that cause foodborne illnesses by contaminating food during production, processing, storage, transportation, or just before consuming. These pathogens have raised food safety concerns on both consumers and food producers in recent years (Alshannaq & Yu, 2017). This is because most of these foodborne pathogens secrete different components, including toxins, which are thermostable even during typical food preparation methods (Rajkovic, 2014). Above all, the most common foodborne pathogens are *Bacillus cereus*, *Candida albicans*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, Norovirus, *Salmonella typhimurium*, *Shigella sonnei*, and *Staphylococcus aureus* (Weerakkody, et al., 2010). These pathogens are the biggest cause of foodborne illness; due to severity of the sickness or the number of cases of illness, they cause (Albrecht & Sumner, 1992).

2.3.1 Foodborne Fungi

Yeasts and moulds are common type of foodborne fungi, which cause very large economic losses, especially on the stored food and beverage (Miescher, et al., 2011). Even so, these fungi themselves might not directly contaminate food or beverage (Johannessen & Torp, 2005). However, the toxic secondary metabolites, called mycotoxins, produced by certain foodborne fungi contaminate food and cause acute to chronic toxicity in human and animals when consumed (Alshannaq & Yu, 2017). For example, the fungal genera of *Fusarium*, *Alternaria*, *Penicillium*, and *Aspergillus* are the major mycotoxin producers and are responsible for causing unpredictable and ongoing food safety problems worldwide (Ismaiel & Papenbrock, 2015).

2.3.2 Foodborne Bacteria

Generally, bacteria are a common cause of foodborne illnesses and food poisoning. According to Eymar et al. (2016) bacterial-related foodborne illnesses can be classified as either intoxications or infections. In brief, foodborne intoxications occurred by ingesting food containing odourless and tasteless toxins or poisons from bacteria, as a result of bacterial growth in the food item. The most important pathogens that cause bacterial intoxications are *C. botulinum*, *S. aureus*, and *B. cereus*. They are capable of causing disease even if they have been eliminated in food items (Alshannaq & Yu, 2017). In comparison, foodborne infections are caused by ingesting food containing live foodborne bacteria in a large amount, which are capable of producing or discharging a toxin once they are ingested (Albrecht & Sumner, 1992). The *Salmonella* spp., *C. perfringens*, and *Campylobacter* spp. are the most common cause of foodborne infections (Eymar, et al., 2016).

3. CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Study Areas

The Zambezi region is one of the 14 regions of Namibia located in the extreme north east of the country. It is well known for its diversity in plant species, traditions and cultures (Chinsembu & Hedimbi, 2010). The study was conducted in Kabbe constituency (**Figure 1**). The Kabbe constituency had a population of 14 979 people in 2010 (NSA, 2013).

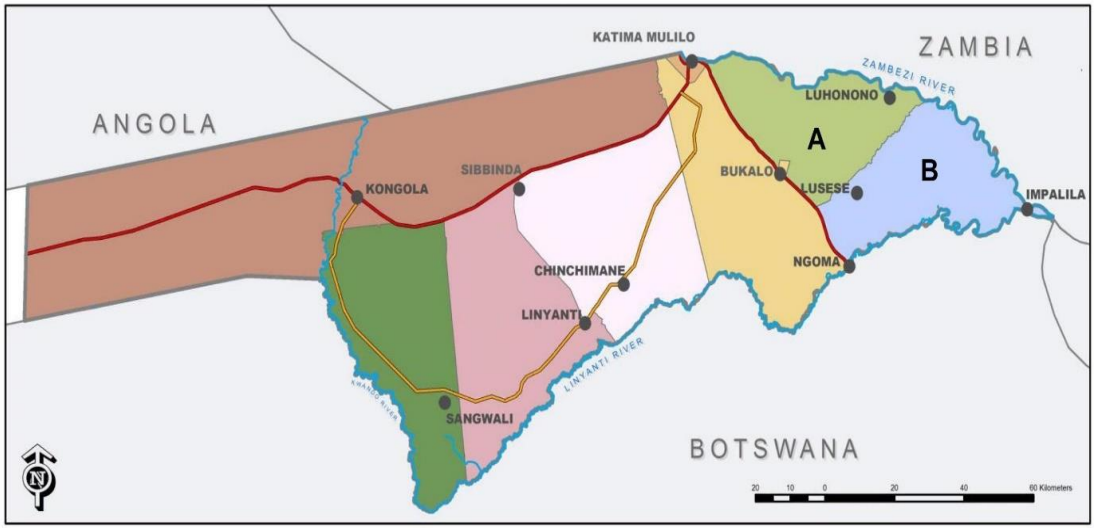


Figure 1: The map of Zambezi region showing the location of Kabbe North (A) and Kabbe South (B) constituencies (EIS, 2015).

3.2 Ethnobotanical Data Collection

The ethnobotanical data and plant collection were collected in six villages (Bukalo, Mukungu, Saili, Kasheshe, Sikuzwe, and Mudaniko) of northern Kabbe constituency and eight villages (Kandiyana, Kabula, Sinchembe, Kachepati, Muchenje, Libuyu, Lizauli, and Lifelo) of the southern Kabbe constituency. Most of these villages are situated in the flood area of the Zambezi River and are rich in animals and plants diversity. The people in these villages have strong dependency on traditional spices

and medicinal plants (Chinsembu, et al., 2014). The ethnobotanical data and plant collection were done between December 2018 and April 2019 with a total of 68 informants. The ethnobotanical data collection method was done according to Chinsembu et al. (2014), with few modifications. Simple random sampling was applied to select informants in Kabbe constituencies. The objectives of the research were explained, and their consent obtained before informants signed the prior informed consent agreement form and ethical confidentiality agreement (**Appendix 1 & 2**). Only then, they were individually engaged in semi-structured interviews supplemented with open-ended questionnaire (**Appendix 3**). The semi-structured face-face interviews intended to address details on the traditional uses, preparation methods and mode of administration of herbs and spices remedies found and used in the Kabbe constituency (**Figure 2**). Additionally, vernacular names of the plant species, parts, and dosage use were also recorded. Furthermore, all interviews were conducted in local languages, with the help of Silozi-English translators.

The ethical clearance certificate and research permit were obtained from the National Commission on Research, Science and Technology (NCRST), Kabbe constituency office, and University of Namibia Research Ethics Committee, respectively (**Appendix 4 & 5**).

Figure 2: The research team conducting ethnobotanical surveys in villages of Kabbe constituencies.

3.3 Plant Collection, Handling and Identification

Plant parts traditionally used as herbs and/or spices mentioned by at least three independent participants were collected and photographs of putative herbs and spices were taken in the field (**Figure 3**). Upon collection, the plant specimens were given voucher numbers and transported in plant presses to UNAM and later they were taken to the Namibian Botanical Research Institute (NBRI) for taxonomic identification (**Appendix 6**).

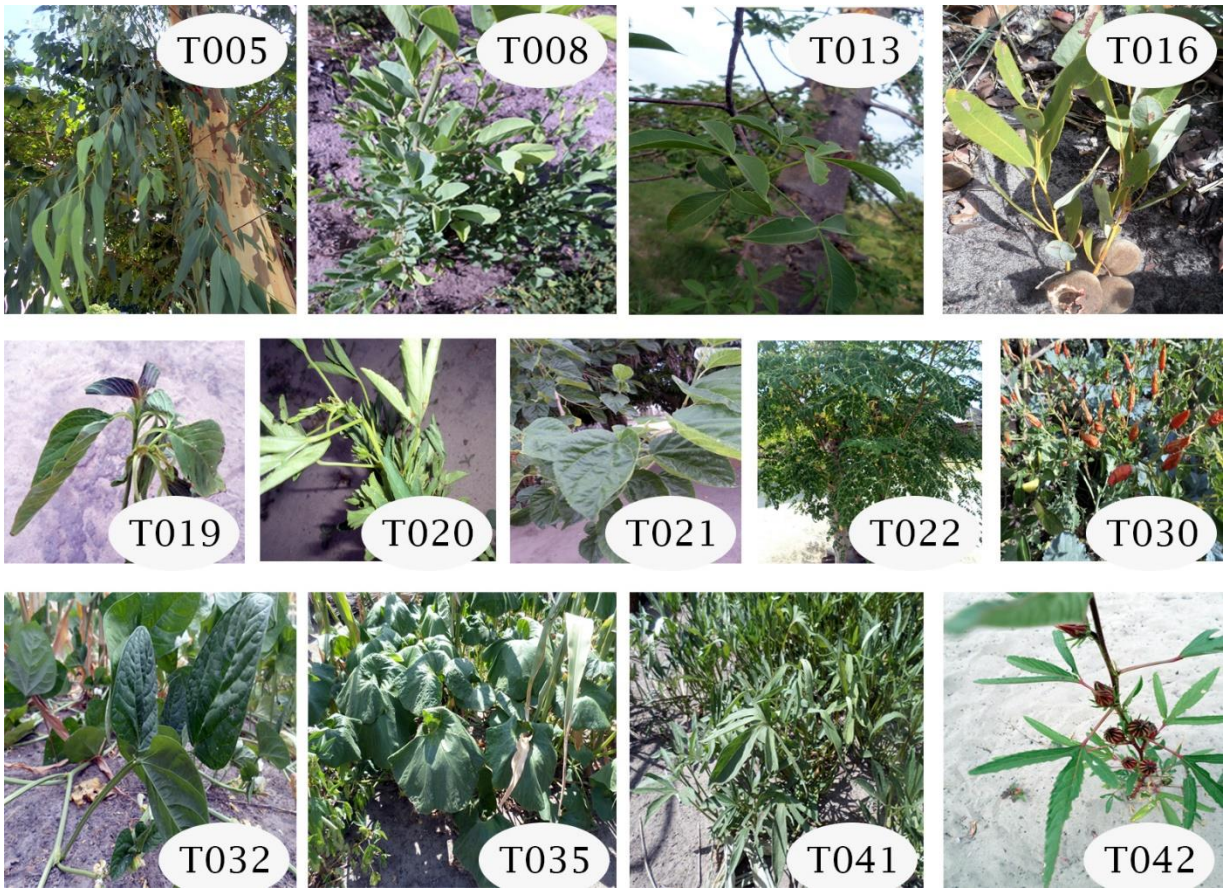


Figure 3: Photographs with voucher numbers of some of the collected plant species traditionally used as herbs and spices in Kabbe constituencies, Zambezi Region.

3.4 Plant Materials and Crude Extracts Preparation

The collected plant parts were rinsed in running tap water to remove unwanted particles, shade-dried at room temperature, milled to fine powder in a clean grinder, wrapped in aluminium foil and stored in closed containers until extraction (Weerakkody, et al., 2010).

Dichloromethane and methanol (DCM:MeOH) extracts were prepared according to the method described by Mostafa et al. (2018) with modifications. About 10 g fine powder of each plant part collected was soaked in a 250 mL of 1:1 ratio of DCM:MeOH followed by continuous shaking with an orbital shaker (Digital Flask laboratory orbital shaker, MRC, UK) at a speed of 100 rpm in the dark for 36 hours at

room temperature. This step was followed by filtration using filter papers (Whatman Grade 4 filter paper, Whatman, England), into separate flasks. The marc (residue) of each plant was re-soaked in fresh DCM:MeOH, as mentioned before, for about 36 hours. The filtrates of all portions were pooled for the respective plants and concentrated with a Hei-VAP Core Rotary evaporator (Heidolph Instruments, German), under reduced pressure at 40 °C. The resulting concentrated extracts of each plant material were transferred to 1.5 mL Eppendorf tubes and refrigerated at 4 °C until testing for antimicrobial activity. Preparation of water extracts was done using the similar procedure except that, double distilled water (dd-H_2O) was used. In addition, the filtrates were concentrated with a Rotary evaporator under reduced pressure at 90 °C instead of 40 °C.

3.5 Microbial Strains and Cultivation

Laboratory strains of fungi *Candida albicans* and *Saccharomyces cerevisiae*; Gram-positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Enterococcus faecalis*; Gram-negative bacteria *Escherichia coli*, *Salmonella typhimurium*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* were obtained from the Department of Biological Sciences, UNAM.

Microbial stock cultures were revived in Tryptic Soy Broth (TSB) medium and maintained following standard microbiological techniques described by Kong et al. (2007) with modifications. Enumeration of indicator strains using plate count method (Viable count) was performed from subcultures grown overnight on Plate Count Agar (PCA) and Tryptone Soya Agar (TSA) at 37 °C. The working cultures were prepared from 18 to 24 hours subcultures adjusted to 0.5 McFarland standard equivalents of 10^7 CFU/mL in TSB medium.

3.6 Antimicrobial Assay

3.6.1 Disc Diffusion Assay

Prior to antimicrobial screening, McFarland standard corresponding to 0.5 was prepared as discussed by Donay et al. (2007). The disc diffusion method used to test the antimicrobial activities of the herb and spice extracts was conducted according to a method described by Tadeg et al. (2005) with some modifications. For susceptibility testing, 200 mg/mL of each crude extract concentration was prepared in dimethyl sulfoxide (DMSO). Sterile discs (Whatman, 6 mm) were impregnated with 100 µL of the reconstituted extract, and dried at 37 °C overnight in an incubator. Each test microorganism (*C. albicans*, *S. cerevisiae*, *S. aureus*, *B. cereus*, *L. monocytogenes*, *C. perfringens*, *E. faecalis*, *E. coli*, *S. typhimurium*, *S. sonnei*, *P. aeruginosa*, and *P. vulgaris*) was adjusted to 0.5 McFarland standard equivalents of 107 CFU/mL before inoculated onto Muller-Hinton agar. Only then, discs impregnated with the reconstituted extract were gently pressed onto the inoculated Muller-Hinton agar to ensure complete contact with the medium. The 20 µg standard antibiotic disc of ampicillin (Oxoid, England) was used as positive control and DMSO as a negative control. The plates were then incubated at 37 °C for 18-24 hours. Inhibition zones were measured after incubation, using a ruler to the nearest millimetre and recorded as the diameter of growth-free zones. The strength of inhibition was classified as follows: for diameter ≥ 25 mm (very strong), diameters ranging from 24 – 15 mm (strong), diameters ranging from 14 – 11 (moderate) and diameters ranging from 10 – 8 mm (weak), while diameter ≤ 8 mm (negative) (Nematollahi, et al., 2011). The agar disc diffusion test was done in triplicates and repeated twice.

3.6.2 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) were determined for all plant extracts, using a modified macro dilution broth method by Weerakkody et al. (2010). Briefly, serial dilutions ranging from 100 to 1.25 mg/mL of plant extracts were prepared in solvents. Distilled water was used as a solvent for water extracts, whereas 70 % methanol was used for DCM:MeOH extracts. About 200 mg of concentrated plant extracts was weighed in test tubes and 2 mL of the solvents was added to make the concentration of 100 mg/mL. Extracts with the concentration of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 5 mg/mL, 2.5 mg/mL and 1.25 mg/mL extracts were then prepared using two-fold serial dilutions.

About 1 mL of each overnight test microorganisms (adjusted to 0.5 McFarland standard equivalents of 10^7 CFU/mL in TSB medium) was added to test tubes containing prepared plant extracts concentrations and incubated for overnight at 37 °C. After 18-24 hours of incubation, 100 µL of culture/extract was spread on TSA plates and incubated for another 24 hours at 37 °C. The ampicillin was used as positive control and 70 % methanol as a negative control. After incubation period, the MIC value for each plant extract was observed by examining the presence of the test microbial colonies at each concentration. The lowest concentration of the plant extract that completely inhibited the growth of a particular test microorganism was recorded as MIC. The experiments were replicated three times on different occasions with duplicate samples analysed per replicate.

3.6.3 50 % Growth Reduction (IC_{50})

Likewise, the 50 % Growth Reduction (IC_{50}) values were determined for all plant extracts using a modified macro-dilution broth method by Weerakkody et al. (2010). Similar procedures for MIC preparation (Section 3.6.2) were followed. However, after

incubation period, the IC_{50} value for each plant extract was observed by examining the presence of the test microbial colonies at each concentration. The concentration that inhibited 50 % of test microbial growth (in comparison with the negative control) was recorded as IC_{50}. The experiments were replicated three times on different occasions with duplicate samples analysed per replicate.

3.6.4 Time-Kill Assay

The time-kill assay was carried out on the plant extracts exhibiting the strongest antimicrobial activity (6.25 mg/mL or below), using a modification of the viable cells count method of Joray et al (2011). It was carried out to assess the *in vitro* reduction of a microbial population of *C. albicans, B. cereus, E. faecalis, E. coli, S. typhimurium, S. sonnei* and *P. vulgaris* after exposure to test plant extracts. The TSB broth (5 mL) was inoculated with overnight suspension of microbial culture to approximately 10^6 CFU/mL. Equal volumes of the extracts (5 mL), with concentrations adjusted to obtain final concentrations of 6.25 mg/mL, were added to the TSB tubes and mixed using a vortex for 30 seconds. All tubes were incubated at 37 °C. At different time intervals (0, 2, 4, 18 and 24 hours) of exposure, 0.1 mL of 10-fold dilutions suspension was spread in duplicate on separate TSA plates (Oxoid, Basingstoke, UK) which were incubated for 24 hours. The numbers of colonies were counted and compared with that of the control where extracts were replaced with sterile distilled water with addition of 5 % DMSO. The experiments were replicated three times on different occasions and the results were expressed as means of triplicate analyses in log CFU/mL. Antimicrobials were considered microbicidal when a ≥3 log10 decrease in CFU/mL was reached compared with the initial inocula (Joray, et al., 2011).

3.6.5 Time-Kill Synergy Study

The time-kill synergy study was performed on seven DCM:MeOH crude extracts exhibiting the strongest antimicrobial activity (6.25 mg/mL) and twenty-one combinations of these plant extracts. The single plant study was done using similar above mentioned time-kill study by Joray et al (2011) with modifications. By contrast, the combination study was performed according to a previously reported method by Bremmer et al. (2016) with modifications. In short, the TSB broth (5 mL) was inoculated with overnight suspension of microbial culture (*B. cereus*, *S. cerevisiae*, *C. albicans*, *S. typhimurium*, *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, and *C. perfringens*) adjusted to 0.5 McFarland standard equivalents of 10^7 CFU/mL. 5 mL combinations of plant extracts (1:1 ratio with concentrations adjusted to 6.25 mg/mL each) was then added to the inoculated TSB broth tubes and mixed using a vortex for 30 seconds. All tubes were incubated at 37 °C. Test microbial growth was quantified after 0, 4, 18 and 24 hours incubation at 37 °C by plating 0.1 mL of 10-fold dilutions onto the TSA plates (Oxoid, Basingstoke, UK) which were incubated for 24 hours. The numbers of colonies were counted and compared with that of the control where extracts were replaced with sterile distilled water with addition of 5 % DMSO.

The time-kill synergy studies were replicated three times on different occasions with duplicate samples analysed per replicate in both single plant and combination studies. The results were expressed as means of triplicate analyses in $\log_{10}$ CFU/mL after 24 hours of incubation at 37 °C. The synergistic, indifferent, and antagonistic activities were defined according to Bremmer et al. (2016). The individual plant extracts were considered microbicidal when a ≥ 3 $\log_{10}$ decrease in CFU/mL was reached compared with the initial inocula. The synergy of the plant extracts combination was defined as a ≥ 2 $\log_{10}$ decrease in colony count at 4, 18 or 24 hours with the antimicrobial

combination compared to the most active single plant extract. The indifference was defined as a <2 $\log_{10}$ increase or decrease in colony count at 4, 18 or 24 hours with the combination compared with the most active plant extract alone. The antagonism was defined as a ≥2 $\log_{10}$ increase in colony count at 4, 18 or 24 hours with the combination compared with that by the most active plant extract alone.

3.6.6 Determination of Permeability of Cell Membrane

In order to understand the antimicrobial activity and mechanism of action of selected herbs and spices, the permeability of cell membrane was determined when different concentrations of DCM:MeOH plant extracts with the lowest MICs and microbicidal effects were introduced to selected test microorganisms. It was expressed in the relative electric conductivity according to the method described by Zhang et al. (2017) with some modifications. The *B. cereus*, *C. albicans*, *E. coli*, *E. faecalis* and *S. typhimurium* were each separated by centrifuging at 3 000 x g for 10 minutes and washed with 5 % glucose until the electric conductivity was closer to that of 5 % glucose. The electric conductivity of 5 % glucose indicated the case of isotonic microbes, and was measured by an electrical conductivity meter (CON 11, Eutech Instruments, Singapore). The extracts were each prepared at three different concentrations: control (no plant extract), 1 x MIC (6.25 mg/mL), and 2 x MIC (12.5 mg/mL); before added into 5 % glucose. The electric conductivity of the mixtures was measured and marked as L_1. In addition, different concentrations of plant extracts (control, 1 x MIC, and 2 x MIC) were added into the isotonic microbes, respectively. They were mixed, and the samples were incubated at 37 °C for 24 hours with the conductivity measured at 2, 4, 6, and 24 hours and marked as L_2. The electric conductivity of microbes in 5 % glucose treated in boiling water for 5 minutes was used as the control, and was marked as L_0. The following formula was used to calculate

the permeability of cell membrane: Relative electric conductivity (%) = 100 x (L_2 - L_1)/L_0 (Zhang, et al., 2017).

3.6.7 Measurement of Release of 260 nm Absorbing Materials and Proteins

The measurement of the release of 260 nm absorbing materials from selected test microorganisms (*B. cereus*, *C. albicans*, *E. coli* and *S. typhimurium*) were performed using a method described by Du et al. (2012) with some modifications. In summary, the microbial cells in the 100 mL suspension were collected by centrifugation at 3 000 x g for 20 minutes, washed three times with 0.1 M Phosphate-buffered saline (PBS) (pH 7.4), and re-suspended in 0.1 M PBS (pH 7.4). The 100 mL of cell suspensions were each incubated at 37 °C for 4 hours in the presence of DCM:MeOH plant extracts with the lowest MICs and microbicidal effects (1:1 ratio) at three different concentrations (control, 1 x MIC, and 2 x MIC). After 4 hours. The suspensions were centrifuged at 3 000 x g for 10 minutes. The supernatants were diluted with 0.1 M PBS. The SpectraMax M2 Microplate Readers (Molecular Devices, USA) was used to measure the absorption at 260 nm of supernatants every after 4 hours for 16 hours. Results were expressed in terms of optical density of 260 nm (OD_{260nm}) absorbing materials.

Moreover, the concentration of proteins in supernatant was determined according to the method described by Zhang et al. (2017). The above steps were repeated two times and the absorbance was measured at 280 nm. The amount of released protein was calculated by a standard curve ($y = 0.0272x + 0.1689$) using Quick Start Bovine Serum Albumin Standard #5000206 (BSA) (Bio-Rad Laboratories, United States of America) (**Appendix 7A**). This standard curve was prepared as a control for the Bradford protein assay and the absorbance was measured at 595 nm using the Microplate Readers. The

results were expressed in terms of protein concentration (μg/mL) at the absorbance of 595 nm.

3.7 Determination of Total Phenol and Total Flavonoid Contents

Total phenolic content in plant extracts was estimated using the Folin-Ciocalteu colorimetric oxidation/reduction method described by Skerget et al. (2005), with some modifications. In brief, aliquots (0.5 mL) of appropriate dilutions of extracts were oxidized for 4 minutes with 2.5 mL of Folin-Ciocalteu's reagent (Sigma-Aldrich, Germany) diluted 10 times with water, and the reaction was neutralized with 2 mL of sodium carbonate (75 g/L). The samples were incubated for 20 minutes at 25 °C, and the absorbance of the resulting blue colour was measured at 760 nm with a UV-1600 PC Spectrophotometer (VWR International, Radnor, China). For a control sample, 0.5 mL of distilled water was used. A standard curve was generated using various concentrations of Gallic acid (3.125, 6.25, 12.5, 25, 50, 100, 200 μg/mL) (**Appendix 7B**). The equation of standard curve was $y = 0.0027x + 1.2159$ and the R-squared (R^2) value was 0.9012. Results were expressed as mg of Gallic acid equivalent per gram (mg GAE/g) of plant extract. All values were presented as means of triplicate analyses with duplicate samples analysed per replicate.

Total flavonoid content was measured with the aluminium chloride colorimetric assay based on the method by Kamtekar et al. (2014), with few modification. The catechin hydrate solution (1000 μg/mL) stock solution was prepared by dissolving 100 mg of Catechin hydrate in 100 mL of absolute methanol. 1 mL of aliquots and 1 mL standard Catechin hydrate solution (100, 200, 400, 600, 800, 1000 μg/mL) was positioned into test tubes and 4 mL of distilled water and 0.3 mL of 5 % sodium nitrite solution was added into each. After 5 minutes, 0.3 mL of 10 % aluminium chloride was added. At 6^{th} minute, 2 mL of 1 M sodium hydroxide was added. Finally, volume was made up

to 10 mL with distilled water and mixed well. The orange yellowish colour was developed. The absorbance was measured at 510 nm with the UV-1600 PC Spectrophotometer (VWR International, Radnor, China). The blank was performed using distilled water. The samples were performed in triplicates. The calibration curve was plotted using standard Catechin hydrate, which resulted in an R^2 value of 0.9836 (**Appendix 7C**). The equation of standard curve was $y = 0.0003x + 0.0403$. The data of total flavonoids of plant extracts were expressed as mg Catechin hydrate equivalents per gram (mg CAE/g) of dried weight.

3.8 Data Analysis

3.8.1 Ethnobotanical and Demographic Data Analysis

The indigenous knowledge of plants traditionally used as herbs and/or spices in Kabbe North and Kabbe South constituencies of Zambezi region, Namibia was analyzed using the following formulas:

3.8.1.1 Use value (UV)

The use value (UV) demonstrates the relative importance of plants known locally. It was calculated using the formula (Tardío & Pardo-De-Santayana, 2008):

$$UV = \Sigma Ui / N$$

Where Ui is the number of uses mentioned by each informant for a given species, and N is the total number of informants.

3.8.1.2 Frequency index (FI)

The frequency index (FI) was calculated for each plant species used as herb and/or spice collected to compare the relative importance of each plant species. The FI is defined as a numerical expression of the percentage frequency of citation for a single

plant species by informants. The following formula was used to calculate FI (Mahwasane, et al., 2013):

$$FI = FC/N \times 100$$

Where FC is the number of informants who mentioned the use of the plant species, and N is the total number of informants (68 informants) in Kabbe North and Kabbe South constituencies.

The FI was high when a particular plant mentioned by many informants and low when there was few reports.

3.8.1.3 Informant consensus factor (ICF)

The Informant consensus factor (ICF) was calculated according to the following formula (Sargın, et al., 2013):

$$ICF = Nur\text{-}Nt / Nur\text{-}1.$$

Where Nur refers to the number of use citations in each category, and Nt to the number of the species used.

The ICF values of the culinary/therapeutic uses for plant species documented in this survey were conferred. The plant species that are presumed to be effective against different culinary/therapeutic use groups have higher ICF values. This method is to check homogeneity of the information (Sargin, 2015).

3.8.1.4 Fidelity level (FL)

The fidelity level (FL), which is defined as the percentage of informants claiming the use of a certain plant for the same major purpose, was calculated for the most

frequently reported culinary/therapeutic uses as following (Cheikhyoussef, et al., 2011):

$$FL\ (\%) = Np/N \times 100$$

Where Np is the number of informants that claim a use of a plant species as a culinary herb or spice, and N is the number of informants that use the plants as a culinary/therapeutic uses.

Prior to the calculation of FL, reported ailments were grouped into major culinary/therapeutic uses categories, which are leafy vegetable, seasoning, preservation, and traditional medicines. Generally; plants which the proportion of informants agreed on its use against a given culinary/therapeutic uses categories are more likely to be biologically active.

3.8.2 Statistical Analysis

The disc diffusion, MIC, IC_{50}, total phenol and total flavonoid contents data were stored in Microsoft Excel 2013 (Microsoft Corporation, Redmond, USA) before analysed for normality using the Shapiro-Wilk test ($p < 0.05$) in the International Business Machines Statistical Package for the Social Sciences tool (IBM SPSS) version 25.0 (IBM, New York, USA). The normally distributed data were analysed using One way Analysis of variance (ANOVA) test, whereas those that were not normally distributed were analysed using nonparametric statistics (either Mann-Whitney U or Kruskall-Wallis tests) ($p < 0.05$).

4. CHAPTER FOUR: RESULTS

4.1 Socio-Demographic Information

Sixty-eight informants in Kabbe North and Kabbe South constituencies between the ages of 23-89 participated in the present study. Most of the informants (42.6 %) were recorded in the 23-39 years age group followed by 60-69 years age group (22.1 %) (**Table 2**). In addition, the highest number of plants mentioned during the ethnobotanical data collection were recorded in the 23-39 years age group with 44.0 %. Male informants participated in the ethnobotanical survey were fewer in comparison to females (35.3 %).

Table 2: Overall number of informants by age group participated in the ethnobotanical survey and proportion percentage (%) of plant species mentioned.

	Age Group (Years)						**Total (%)**
	23-39	**40-49**	**50-59**	**60-69**	**70-79**	**80-89**	
Female	15	7	3	10	4	5	64.7
Male	9	5	2	5	2	1	35.3
Proportion %	44	15	4	24	4	9	100

Two dominant ethnic groups were reported during the ethnobotanical survey living in Kabbe constituencies. The Masubia was the largest ethnic group with 74.4 % of the total informants participated in this study followed by the Mafwe ethnic group with 17.9 % (**Figure 4**). Other ethnic groups including the Mayeyi, Matotela and Mashi made up 7.7 % of the total informants participated in the present study.

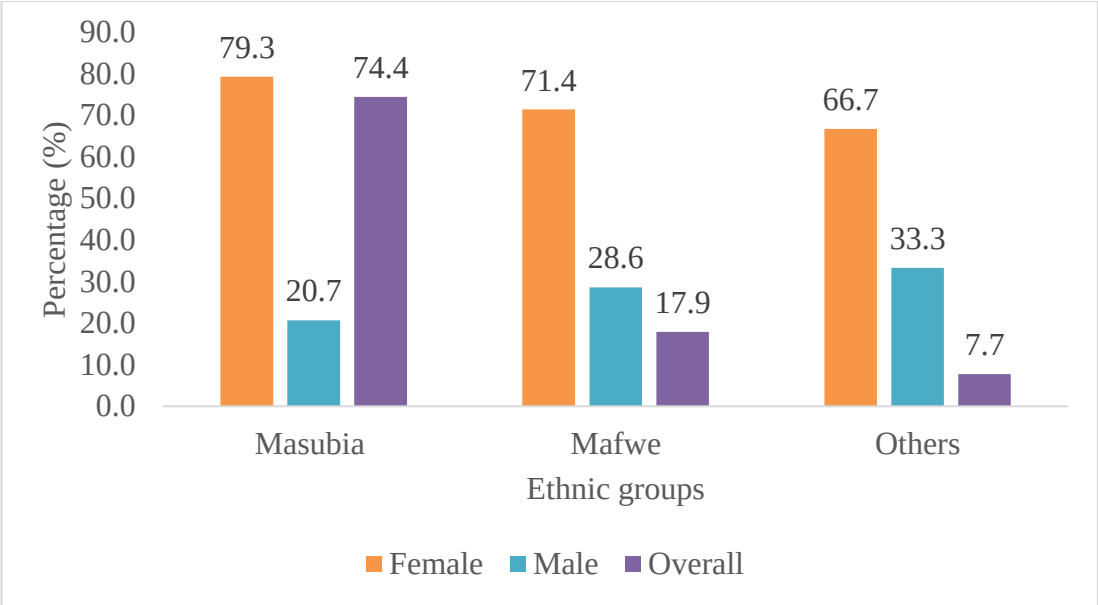


Figure 4: Percentage of informants by ethnic groups that participated in the ethnobotanical survey in Kabbe constituencies, Zambezi Region.

4.2 Ethnobotanical Uses of Collected Plants

The 23 plant species were collected and taxonomically identified (**Table 3**) as follow: we had hypothesized that there is indigenous knowledge on plants traditionally used as herbs and/or spices in Kabbe North and Kabbe South constituencies of Zambezi region. This has been supported by our results. The *Capsicum* sp., *Cucurbita* sp., *Cucurbita* sp., *Eucalyptus* sp., *Hibiscus* sp., and *Morus* sp. were only identified until genus only.

4.2.1 Botanical Description of Collected Herbs and Spices

The collected plant species traditionally used as herbs and spices in Kabbe constituencies belong to 16 plant families (**Figure 5**). The largest proportion of plants documented belong to the family *Malvaceae* which includes plant species like *Adansonia digitata*, *Corchorus tridens*, *Hibiscus fuscus*, *H. mechowii* and *Hibiscus* sp.. The *Amaranthaceae*, *Cucurbitaceae*, *Fabaceae* and *Moraceae* family were each represented by two plant species. The rest of plant families had one species each.

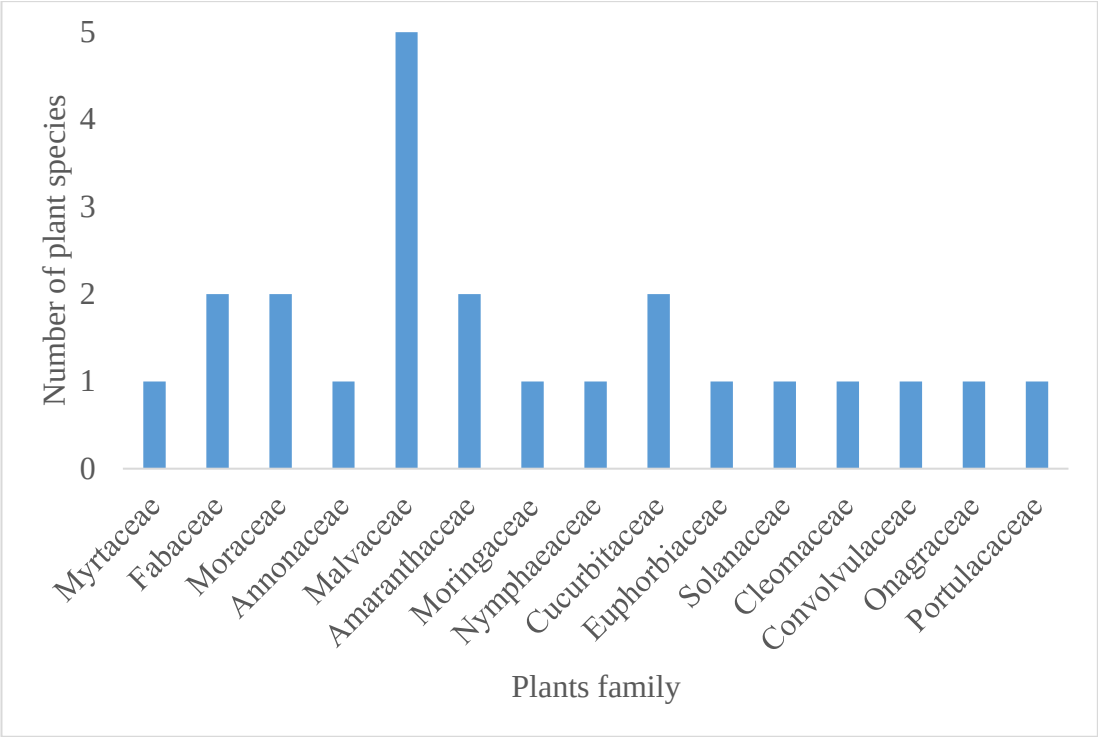


Figure 5: Overall distribution of collected plant species across different plant families.

Different plant parts were used as herbs and/or spices. Leaves were the most commonly used part (54 %) followed by roots (22 %) and fruits (11 %) **(Figure 6)**.

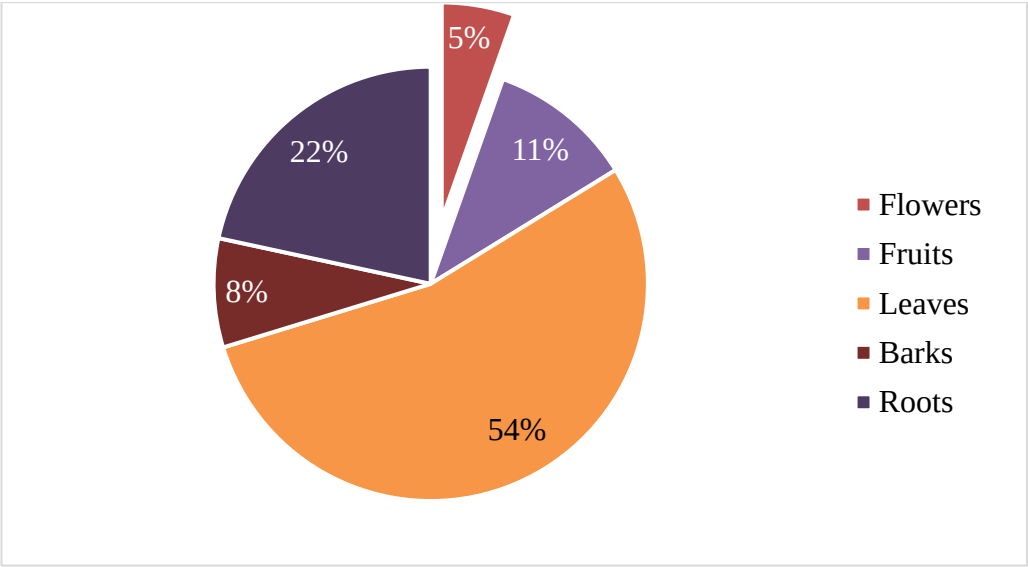


Figure 6: Percentage of plant parts traditionally used as herbs and spices in Kabbe constituencies, Zambezi Region.

4.2.2 Culinary/Therapeutic Plant Uses and Methods of Preparation

In general, plants are culinary/therapeutic used for food and medicine. Most plants documented in this study were culinary and/or therapeutic used as food preservative (30 %) or seasoning (30 %) (**Figure 7**). Medical uses were recorded with 24 % of total plants collected in this study followed by leafy vegetable with 16 %.

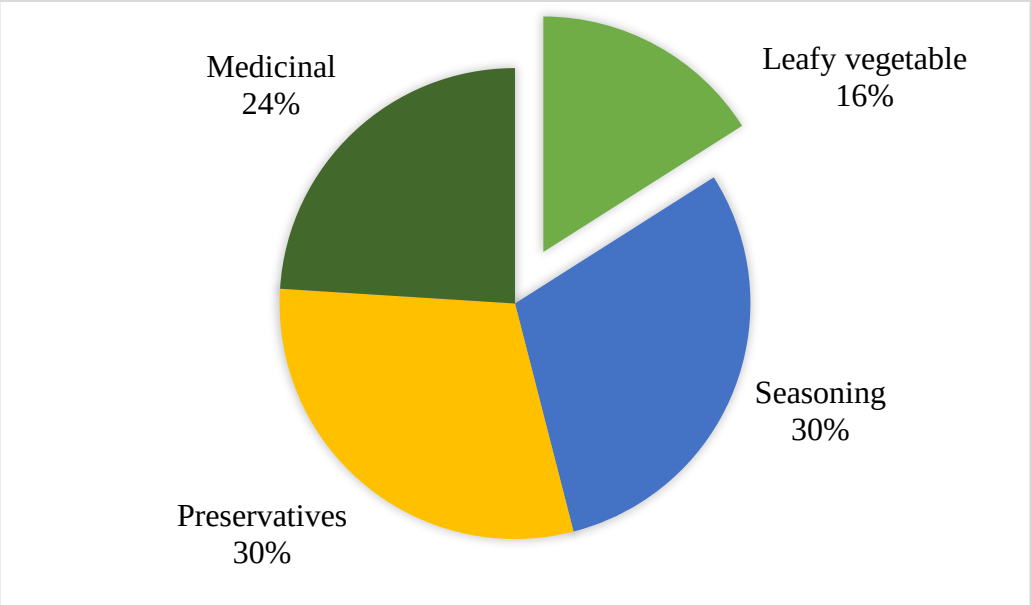


Figure 7: Percentage of frequency plant uses in Kabbe constituencies of Zambezi region.

Traditional systems of medicines, herbal remedies are prepared using various methods depending on the plant utilized and condition being treated. In Kabbe constituencies of Zambezi region, the recorded methods utilized for the preparation of the collected plants therapeutically were infusion (52 %), decoction or infusion (39 %) and decoction (9 %) (**Figure 8**).

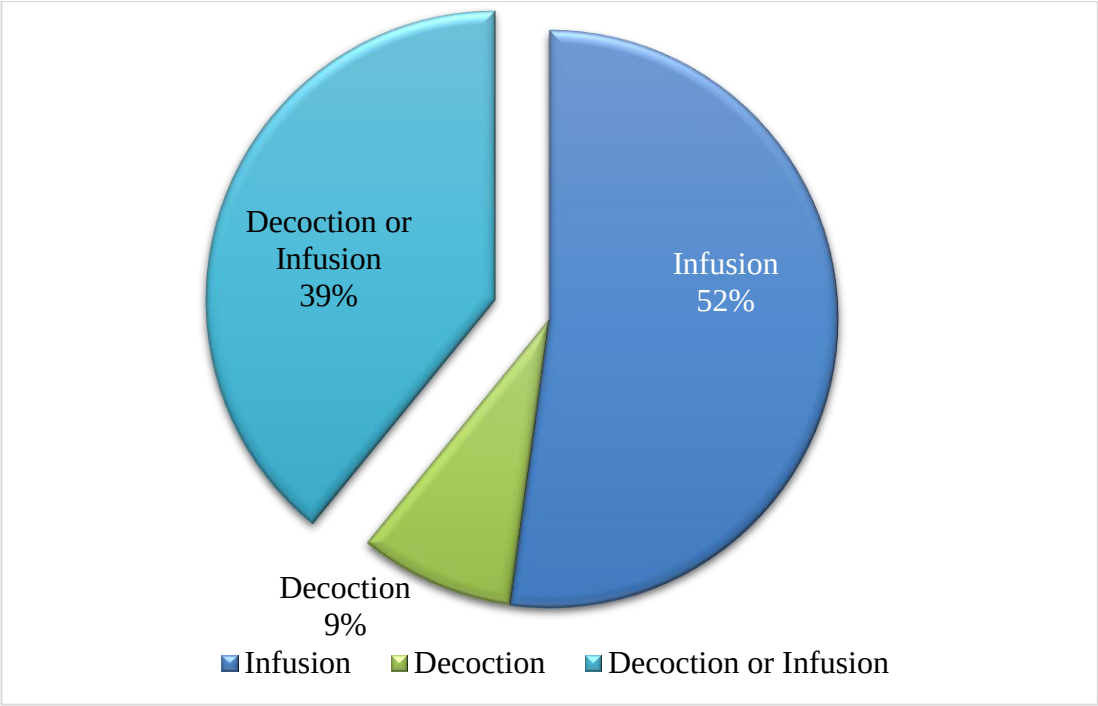


Figure 8: The percentage of traditional methods of preparation for plants used as herbs and/or spices.

Table 3: Plant species traditionally used as herbs and/or spices in Kabbe constituencies, Zambezi Region.

Family	Scientific name	Voucher number	Local name of plant	Plant parts used	UV	FI	Culinary/Therapeutic uses	Mode of preparation
Amaranthaceae	*Amaranthus thunbergii* Moq.	T020	Libowa/ Ihato	Leaves	0.79	79.41	Leafy vegetable. Medical uses. Help to expel parasitic worms and other internal parasites. Help increased production of urine. Used to treat kidney infections and Tuberculosis (TB) sores.	Infusion
Amaranthaceae	*Amaranthus hybridus* L. var. hybridus	T023	Lindowa	Leaves, Roots	0.19	19.12	Leafy vegetable. Medical uses. Used to make green salad. Expel parasitic worms and other internal parasites from the body, and treat diarrheal.	Infusion
Annonaceae	*Annona senegalensis* Pers.	T016	Malolo	Fruits, Roots	0.10	10.29	Seasoning uses. Preservative uses. Medical uses. Fresh fruits used to make juice and salad. Roots used as herbal tea. Treat stomach-ache and Malaria.	Decoction

Cleomaceae	*Cleome gynandra* L.	T040	Sishungwa	Leaves	0.84	83.82	Leafy vegetable. Medical uses. Used to treat diseases such as epilepsy, earache, headache, conjunctivitis, and worm infections.	Infusion
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	T041	Kalembula (Leaves) Ngulu (Tubes)	Leaves, Roots (Tubers)	0.40	39.71	Seasoning uses. Preservative uses. Leaves used as relish. Tubes grounded to make flour. Help to strengthen bones and treat.	Decoction or Infusion
Cucurbitaceae	*Cucurbita* sp.	T027	Mangambwa	Leaves	0.77	76.47	Seasoning uses. Preservative uses. Medical uses. Leaves used as relish. Used to treat joint pain, connective tissues and swelling.	Infusion
Cucurbitaceae	*Cucurbita* sp.	T035	Nalumanga	Leaves	0.27	26.47	Leafy vegetable.	Infusion
Euphorbiaceae	*Manihot esculenta* Crantz.	T028	Mwanja	Leaves, Roots	0.63	63.24	Seasoning uses. Preservative uses. Leaves used as relish. Roots grounded to make flour.	Infusion
Fabaceae	*Baphia massaiensis* Taub	T008	Muvunje	Roots	0.12	11.77	Seasoning uses. Preservative uses. Medical uses. Roots used	Decoction

							as herbal tea/coffee. Roots are used as toothbrush.	
Fabaceae	*Vigna unguiculata*	T032	Nyangu/ Manawa	Fruit (Seeds), Leaves	0.56	55.88	Seasoning uses. Preservative uses. Fruits and leaves used to make soup and relish.	Decoction or Infusion
Malvaceae	*Adansonia digitata* L.	T013	Mubuyu	Leaves, Bark, Roots	0.21	20.59	Seasoning uses. Preservative uses. Medical uses. Barks and roots are help to boost appetite and general immune system. Leaves and roots used to treat diarrhoea and Malaria.	Decoction or Infusion
Malvaceae	*Hibiscus fuscus* Garcke	T019	Sindambi	Leaves	0.32	32.35	Leafy vegetable. Medical uses. Treat diarrhoea, mouth and throat sores. Boost low blood pressure.	Infusion
Malvaceae	*Corchorus tridens* L.	T024	Delele	Leaves	0.63	63.24	Leafy vegetable. Help boost appetite.	Decoction or Infusion
Malvaceae	*Hibiscus* sp.	T034	Mundambi	Leaves	0.37	36.77	Leafy vegetable. Medical uses. Used to make jams, sauces, and tea.	Decoction or Infusion

Malvaceae	*Hibiscus mechowii* Garcke.	T042	Sindambi/ Mundambi	Leaves	0.78	77.94	Leafy vegetable. Medical uses. Treat diarrhoea, mouth and throat sores.	Infusion
Moraceae	*Ficus exasperate*	T014	Mukwiyu/ Muchaba	Leaves, Bark	0.35	35.29	Seasoning uses. Preservative uses. Medical uses. Leaves used as herbal tea, boost low blood pressure and treat TB. Barks improve appetite, treat diarrhea and Malaria.	Decoction or Infusion
Moraceae	*Morus* sp.	T021	Murobeni	Leaves	0.16	16.18	Leafy vegetable. Medical uses. Boiled as herbal tea. Can be used as relish.	Infusion
Moringaceae	*Moringa ovalifolia* Dinter & A.Berger	T022	Mulinga	Flowers, Fruit, Seeds, Leaves, Roots	0.25	25.00	Medical uses. Seasoning uses. Preservative uses. Used as relish, herbal tea, and treat various infections such as diarrhoea.	Decoction or Infusion
Myrtaceae	*Eucalyptus* sp.	T005	Kapulanga	Leaves, Bark	0.09	8.82	Seasoning uses. Preservative uses. Medical uses. Barks are added to food as food supplement. Barks and leaves	Decoction or Infusion

							used as toothbrush. Leaves are used to treat symptoms of coughs, colds, and congestion.	
Nymphaeaceae	*Nymphaea lotus* L.	T025	Lisoto	Flowers (Lilies), Roots (Rhizome, Tubers)	0.31	30.88	Seasoning uses. Preservative uses. Flowers used to provide aroma. Rhizomes and tubers grounded and mixed with maize flour.	Decoction or Infusion
Onagraceae	*Ludwigia leptocarpa* (Nutt.) Hara	T043	Lifulanvunvu	Leaves	0.43	42.65	Seasoning uses. Preservative uses. Leafy vegetable. Source of salty taste and as a dye.	Infusion
Portulacaceae	*Talinum arnotii* Hook.f.	T044	Nasilele	Leaves	0.29	29.41	Seasoning uses. Preservative uses. Leafy vegetable.	Infusion
Solanaceae	*Capsicum* sp.	T030	Mbili-mbili	Fruits, Leaves	0.38	38.24	Seasoning uses. Preservative uses. Medical uses. Used to add heat to food, and make soup. Help to expel parasitic worms, treat cough with mucus, and sore throat.	Decoction or Infusion

* UV= use value, FI= frequency index

4.2.3 The Use Value (UV) and Frequency Index (FI)

The highest calculated use value (UV) and frequency index (FI) in this study were recorded for *C. gynandra* with 0.84 and 83.82, followed by *H. mechowii* with 0.78 and 77.94, respectively (**Table 3**). The lowest calculated UV and FI were noted for *Eucalyptus* sp. with 0.09 and 8.82, respectively.

4.2.4 The Informant Consensus Factor (ICF)

A sum of 243 use reports have been recorded in this study. This was categorized in eleven different culinary and/or therapeutic use groups. The joint/connective tissues pain, kidney infections, and stomach-ache categories have the greatest ICF value of 1.00 each while the diarrheal group has the lowest ICF score of 0.62 (**Table 4**). The diarrheal category had the smallest ICF value of 0.62.

Table 4: Informant Consensus Factor (ICF) values of category of culinary/therapeutic uses of spices and herbs in Zambezi region.

Culinary/Therapeutic use category	Citation number (Nur)	Number of taxa (Nt)	ICF value (Nur-Nt / Nur-1)
Appetite	33	3	0.94
Blood pressure	14	2	0.92
Coughs/ colds/ congestion	6	2	0.80
Diarrheal	14	6	0.62
Internal parasites	26	3	0.92
Joint pain/ connective tissues	40	1	1.00
Kidney infections	46	1	1.00
Malaria	10	3	0.78

Mouth/ throat sores	30	3	0.93
Stomach-ache	6	1	1.00
TB sores	18	2	0.94

4.2.5 The Fidelity Level (FL)

Fidelity level (FL) was calculated for plants species, which have been cited by four, or more informants for being used against a given culinary and/or therapeutic use categories. The following species: *A. digitata*, *A. thunbergii*, *A. senegalensis*, *Capsicum* sp., *C. tridens*, *Cucurbita* sp., *Eucalyptus* sp., *F. exasperata*, and *M. ovalifolia* scored the highest values above 70.0 % (**Table 5**). The lowest FL value was recorded for *H. mechowii* with 56.6 %.

Table 5: Fidelity level (FL) values of plants used as herbs and spices cited by four or more informants for being used to treat given ailment.

Plant name	**Culinary/Therapeutic use category**	**Number of Informants**	**Fidelity level value (%)**
A. digitata	Malaria	10	71.4
A. thunbergii	Kidney infections	46	85.2
A. senegalensis	Stomach-ache	6	85.7
Capsicum sp.	Internal parasites	26	100.0
C. tridens	Appetite	33	76.7
Cucurbita sp.	Joint pain/ connective tissues	40	76.9
Eucalyptus sp.	Coughs/colds/ congestion	6	100.0
F. exasperate	TB symptoms	18	75.0
H. fuscus	Blood pressure	14	63.6
H. mechowii	Mouth & throat sores	30	56.6
M. ovalifolia	Diarrheal	14	82.4

4.3 Antimicrobial Assay

4.3.1 Disc Diffusion Assay

The disc diffusion method was performed on twenty-four plant parts. Overall, the test normality using the Shapiro-Wilk test revealed that the disc diffusion results of plant extracts against test microorganisms were not normally distributed ($p < 0.05$) (**Table 6**). The strength of inhibition of each plant extract was different from the other against tested microorganisms. The Independent-Sample Kruskal-Wallis test revealed that there was significant difference between the inhibition zones ($p < 0.05$) and plant extracts.

Based on the classification of the strength of inhibition discussed by Nematollahi et al. (2011), the DCM:MeOH barks and roots extracts of the *A. digitata* at 200 mg/ml showed strong inhibition strength against *C. albicans* with 24.0±3.559 and 23.0±6.976 mm, respectively (**Appendix 8.1**). The dd-H_2O roots extracts of *B. massaiensis* at 200 mg/mL showed strong inhibition strength of 18.7±0.471 mm and 15±4.546 against *B. cereus* and *L. monocytogenes,* respectively, as well as 19.7±2.494 mm, 18.3±0.471 mm, 17.3±0.943 mm, 18.3±0.943 mm, and 18.0±1.633 mm against *E. coli*, *S. typhimurium*, *S. sonnei*, *P. aeruginosa*, and *P. vulgaris*, respectively (**Appendix 8.2**). On average, the barks of *Eucalyptus* sp. had strong strength of inhibition (15.1±0.78 mm) against the tested microorganisms. However, the average mean showed that leaves of *A. digitata* had weak strength of inhibition (9.2±0.50 mm) against the tested microorganisms.

In addition, the Independent-Sample Kruskal-Wallis test revealed that there was significant difference across the inhibition zones and test microorganisms ($p < 0.05$) (**Table 7**).

The *C. albicans* was more susceptible to plant extracts than any other test microorganisms. For instance, the dd-H_2O extracts of *Morus* sp. and *Capsicum* sp. showed strong inhibition strength at 200 mg/ml against *C. albicans* with zones of inhibition diameter ranging from 16.3±3.091 mm and 17.7±0.471 mm, respectively. On the other hand, the *E. faecalis* was less susceptible to plant extracts compared with other test microorganisms (10.5±0.69 mm) (**Appendix 8.2**). The DCM:MeOH extracts of *H. fuscus*, *A. thunbergii* and *M. ovalifolia* both had negative inhibition strength against *E. faecalis*.

There was a significant difference between plant extracts extracted using different solvents. The Independent- Sample Mann-Whitney U test revealed that there was significant difference across the inhibition zones and solvents used ($p < 0.05$) (**Table 8**). The DCM:MeOH extracts had significantly more antimicrobial activity than dd-H_2O extracts.

Table 6: Antimicrobial activity of plant species traditionally used as herbs and/or spices using disc diffusion method.

Plant species (voucher numbers)	Mean zone of inhibition (mm ± SE)	Normality test (Wilk-Shapiro test)
Eucalyptus sp. (T005a)	14.6±0.67	
Eucalyptus sp. (T005b)	15.1±0.78	
B. massaiensis (T008)	13.1±0.75	
A. digitata (T013a)	9.2±0.50	*p= 0.000*
A. digitata (T013b)	11.6±1.03	Data are not normally distributed: $p < 0.05$
A. digitata (T013c)	10.6±0.85	
A. senegalensis (T016a)	11.3±0.54	
A. senegalensis (T016b)	11.7±0.73	
H. fuscus (T019)	10.5±0.64	
A. thunbergii (T020)	9.6±0.50	
Morus sp. (T021)	10.3±0.72	
M. ovalifolia (T022)	13.8±0.58	

C. tridens (T024)	13.3±0.85
N. lotus (T025)	13.2±0.71
Cucurbita sp. (T027)	12.6±0.88
Capsicum sp. (T030)	12.7±0.54
Cucurbita sp. (T035)	11.4±0.44
C. gynandra (T040)	13.1±1.01
I. batatas (T041)	13.1±0.68
L. leptocarpa (T043)	12.4±0.43
Ampicillin	23.4±0.94
DiMe:MOH	6.3±0.13
dd-H_2O	6.2±0.09

Independent- Sample Kruskal-Wallis test: $p = 0.000$, H_0 rejected; significant difference: $p < 0.05$

*SE= Standard Error

Table 7: The mean of the zone of inhibition (mm) of test microorganisms.

Test Microorganisms	Means zone of inhibition (mm ± SE)	Normality test (Wilk-Shapiro test)
S. aureus	11.8±0.63	
B. cereus	11.8±0.65	
L. monocytogenes	11.8±0.61	
C. perfringens	12.1±0.78	
E. faecalis	10.5±0.69	p= 0.000,
E. coli	12.4±0.81	Data are not normally distributed: $p < 0.05$
S. typhimurium	12.8±0.70	
S. sonnei	12.8±0.69	
P. aeruginosa	12.3±0.60	
P. vulgaris	13.5±0.75	
C. albicans	14.1±0.63	
S. cerevisiae	13.0±0.65	
Independent- Sample Kruskal-Wallis test: $p = 0.001$, H_0 rejected; significant difference: $p < 0.05$		

*SE= Standard Error

Table 8: Mean zone of inhibition (mm) for DCM:MeOH and dd-H_2O extraction methods.

Extraction methods	Means zone of inhibition (mm ± SE)	Normality test (Wilk-Shapiro test)
DCM:MeOH extraction	13.7±0.25	p= 0.003
dd-H_2O extraction	10.6±0.17	Data are not normally distributed: $p < 0.05$
Independent- Sample Mann-Whitney U test: $p = 0.000$, H_0 rejected; significant difference: $p < 0.05$		

*SE= Standard Error

4.3.2 Minimum Inhibitory Concentration (MIC)

The DCM:MeOH leaves extracts of *C. gynandra* (T040) had the lowest MIC values of 6.25 mg/mL against six test microorganisms (*B. cereus*, *E. faecalis*, *E. coli*, *S. typhimurium*, *P. vulgaris*, and *C. albicans*) (**Appendix 9.1**). The DCM:MeOH extracts of *C. gynandra*, *Eucalyptus* sp., *Morus* sp. and *N. lotus* had both the lowest MIC value at 6.25 mg/mL against *S. typhimurium* and *C. albicans*. In contrast, the DCM:MeOH extracts of *A. digitata*, *A. senegalensis*, *H. fuscus*, *A. thunbergii*, *M. ovalifolia*, *C. tridens*, *N. lotus*, *Cucurbita* sp. (T027), *Capsicum* sp. (T030), *Cucurbita* sp. (T035) and *I. batatas* had the MIC value at or above 50 mg/mL against *S. aureus*, *B. cereus*, *L. monocytogenes*, *C. perfringens*, *E. faecalis* and *E. coli*.

The dd-H_2O extracts of *Eucalyptus* sp., *B.* massaiensis and *A. digitata* showed the lowest MIC values of 6.25 mg/mL against *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *P. vulgaris* and *C. albicans* (**Appendix 9.2**). The dd-H_2O *H. fuscus*, *A. thunbergii*, *Morus* sp. and *M. ovalifolia* extracts had MIC values at or above 100 mg/mL against *S. sonnei*, *P. aeruginosa*, *P. vulgaris*, *C. albicans* and *S. cerevisiae*.

Based on the test normality using the Shapiro-Wilk test ($p < 0.05$), the MIC values of plant extracts against test microorganisms were not normally distributed: $p= 0.000$ (**Table 9**). The average MIC data did not come from a normal distribution; therefore, the strength of inhibition of each plant extract was different from the other against tested microorganisms. However, the Independent-Sample Kruskal-Wallis test revealed that there was significant difference between MIC values ($p < 0.05$).

Table 9: Mean of antimicrobial activity of plant species traditionally used as herbs and/or spices using MIC.

Plant species (voucher numbers)	**MIC (mg/mL ± Standard Error)**	**Normality test (Wilk-Shapiro test)**
Eucalyptus sp. (T005a)	94.3±16.7	
Eucalyptus sp. (T005b)	63.8±14.0	
B. massaiensis (T008)	79.2±14.5	
A. digitata (T013a)	136.5±15.4	*p= 0.000*
A. digitata (T013b)	156.8±14.5	Data are not normally distributed:
A. digitata (T013c)	151.3±13.9	$p < 0.05$
A. senegalensis (T016a)	133.6±14.7	
A. senegalensis (T016b)	144.5±14.5	
H. fuscus (T019)	176.0±11.4	
A. thunbergii (T020)	170.3±12.6	
Morus sp. (T021)	127.1±15.2	

M. ovalifolia (T022)	158.3±12.6
C. tridens (T024)	164.5±13.1
N. lotus (T025)	116.6±16.8
Cucurbita sp. (T027)	176.0±10.1
Capsicum sp. (T030)	136.1±14.5
Cucurbita sp. (T035)	181.2±8.9
C. gynandra (T040)	109.3±18.3
I. batatas (T041)	175.0±11.9
L. leptocarpa (T043)	128.9±16.9

Independent- Sample Kruskal-Wallis test: $p = 0.000$, H_0 rejected; significant difference: $p < 0.05$

4.3.3 50 % Growth Reduction (IC_{50})

The DCM:MeOH leave extracts of *C. gynandra* had the lowest IC_{50} values of 5 mg/mL against *B. cereus*, *E. faecalis*, *E. coli*, *S. typhimurium*, *P. vulgaris* and *C. albicans* (**Appendix 10.1**). The *H. fuscus*, *M. ovalifolia*, *C. tridens*, *Cucurbita* sp. (T027), *Cucurbita* sp. (T035) and *I. batatas* extracts showed the highest 50 % growth reduction concentrations above 50 mg/mL against all test microorganisms. Moreover, none of the plant extract managed to reduce the growth of *L. monocytogenes* by 50 % at the concentration below 50 mg/mL.

Similarly, the dd-H_2O extracts of the *Eucalyptus* sp., *B.* massaiensis and *A. digitata* had the lowest IC_{50} values between 5 and 6.25 mg/mL against *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *P. vulgaris* and *C. albicans* (**Appendix 10.2**). The *H. fuscus* extract was the only aqueous extracts that managed to reduce the concentration of *L. monocytogenes* by 50 %, even at the concentration of 12.5 mg/mL. The water extracts of *H. fuscus* and *A. thunbergii* had the highest IC_{50} values above 50 mg/mL against *E. coli*, *S. typhimurium*, *S. sonnei*, *P. aeruginosa*, *P. vulgaris*, *C. albicans* and *S. cerevisiae*.

Based on the test normality using the Shapiro-Wilk test ($p < 0.05$), the IC_{50} values of plant extracts against test microorganisms were not normally distributed: $p = 0.000$ (**Table 10**). The average IC_{50} data did not come from a normal distribution; therefore, the strength to reduce 50 % growth of each plant extract was different from the other against tested microorganisms. In addition, the Independent-Sample Kruskal-Wallis test revealed that there was significant difference between plant extracts ($p < 0.05$).

Table 10: Mean of antimicrobial activity of plant species traditionally used as herbs and/or spices using IC_{50}.

Plant species (voucher numbers)	**IC_{50} (mg/mL ± Standard Error)**	**Normality test (Wilk-Shapiro test)**
Eucalyptus sp. (T005a)	52.2±13.2	
Eucalyptus sp. (T005b)	35.4±13.0	
B. massaiensis (T008)	35.0±9.5	
A. digitata (T013a)	72.8±12.8	*p= 0.000*
A. digitata (T013b)	77.6±13.1	Data are not normally distributed:
A. digitata (T013c)	67.9±13.5	$p < 0.05$
A. senegalensis (T016a)	80.4±16.7	
A. senegalensis (T016b)	87.1±16.4	
H. fuscus (T019)	109.1±14.9	
A. thunbergii (T020)	103.3±14.6	

Morus sp. (T021)	62.2±11.3
M. ovalifolia (T022)	114.0±15.7
C. tridens (T024)	96.3±12.8
N. lotus (T025)	75.7±14.9
Cucurbita sp. (T027)	115.1±13.4
Capsicum sp. (T030)	80.2±14.1
Cucurbita sp. (T035)	135.9±14.3
C. gynandra (T040)	78.3±16.1
I. batatas (T041)	149.4±14.5
L. leptocarpa (T043)	74.2±13.6

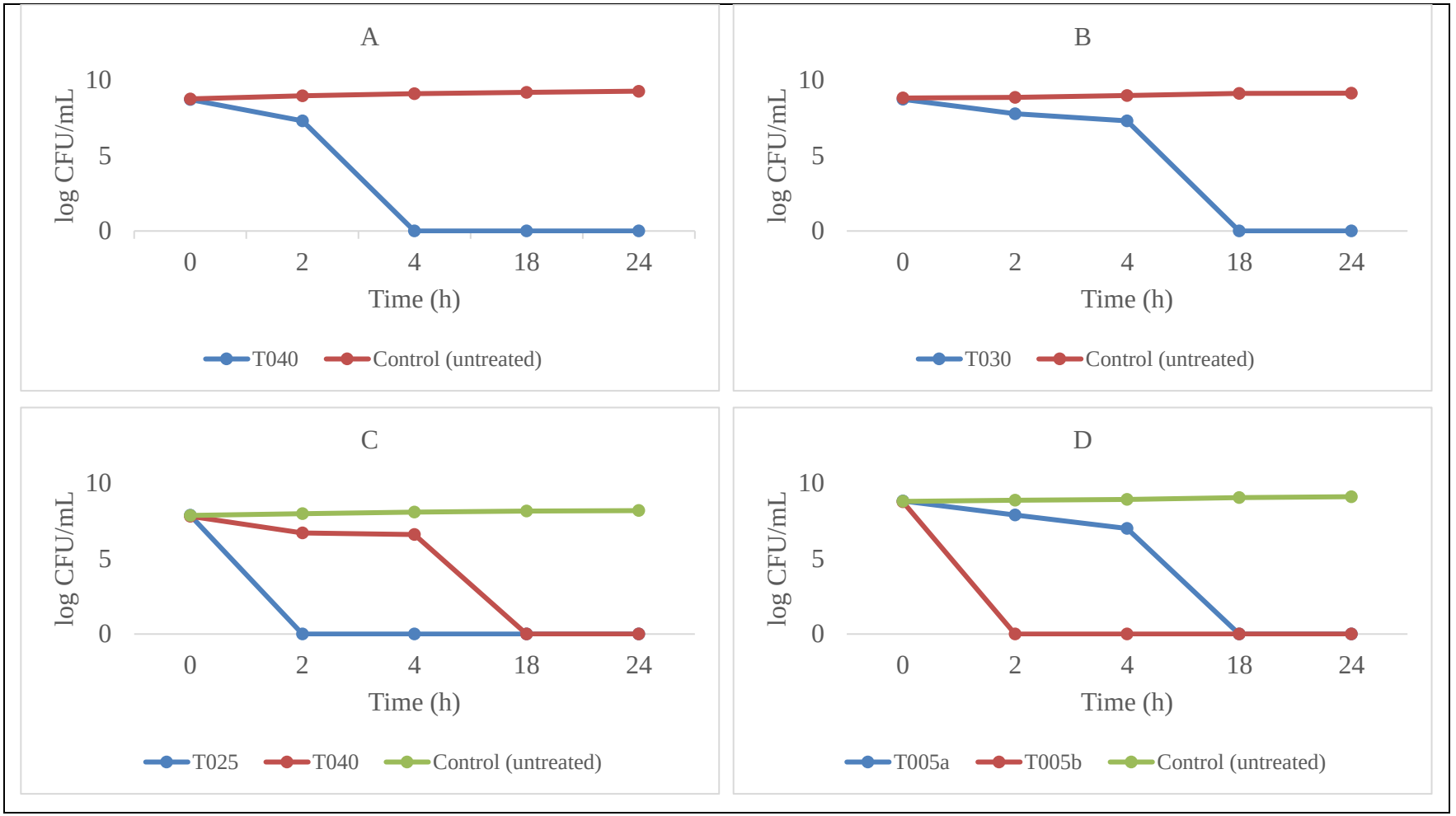
Independent- Sample Kruskal-Wallis test: $p = 0.000$, H_0 rejected; significant difference: $p < 0.05$

4.3.4 Kill-Time Study

The survivor curves for *C. albicans, B. cereus, E. faecalis, E. coli, S. typhimurium, S. sonnei* and *P. vulgaris* against the DCM:MeOH plant extracts were documented in **Figure 9**. The DCM:MeOH extracts of *Eucalyptus* sp., *Morus* sp. and *N. lotus* were particularly effective against *C. albicans, S. typhimurium, E. faecalis* and *S. sonnei*. Their microbicidal effects were observed after 2 hours of exposure and counts remained undetectable until 24 hours of exposure. The *Capsicum* sp., *C. gynandra,* and *Eucalyptus* sp. extracts also displayed bactericidal effects after 18 hours of contact against *E. faecalis, P. vulgaris, E. coli,* and *S. sonnei*.

Not only that, the survivor curves for *C. albicans, S. aureus, E. coli, S. typhimurium, P. aeruginosa* and *P. vulgaris* in the TSB broth at MIC 6.25 mg/mL against dd-H_2O extracts were also documented (**Figure 10**). The extracts of *Eucalyptus* sp. showed bactericidal effects after 24 hours of exposure against *E. coli*. Treatment of *S. aureus* with *A. digitata* or *A. senegalensis* extracts resulted in reduction of initial population levels to 7.7-8.6 log CFU/mL after 24 hours of exposure. On the other hand, when *C. albicans,* and *S. typhimurium* were exposed to *Eucalyptus* sp. or *B. massaiensis* extracts, the numbers of viable cells increased to 8.0-9.1 log CFU/mL after 24 hours of treatment, respectively.

Based on the test normality using the Shapiro-Wilk test ($p < 0.05$), the kill-time mean log CFU/mL of test microorganisms placed in different plant extracts were not normally distributed: $p= 0.000$. The survivor curves for *C. albicans, B. cereus, E. faecalis, E. coli, S. typhimurium, S. sonnei* and *P. vulgaris* against the DCM:MeOH plant extracts was different (Independent-Sample Kruskal-Wallis test: $p = 0.000$).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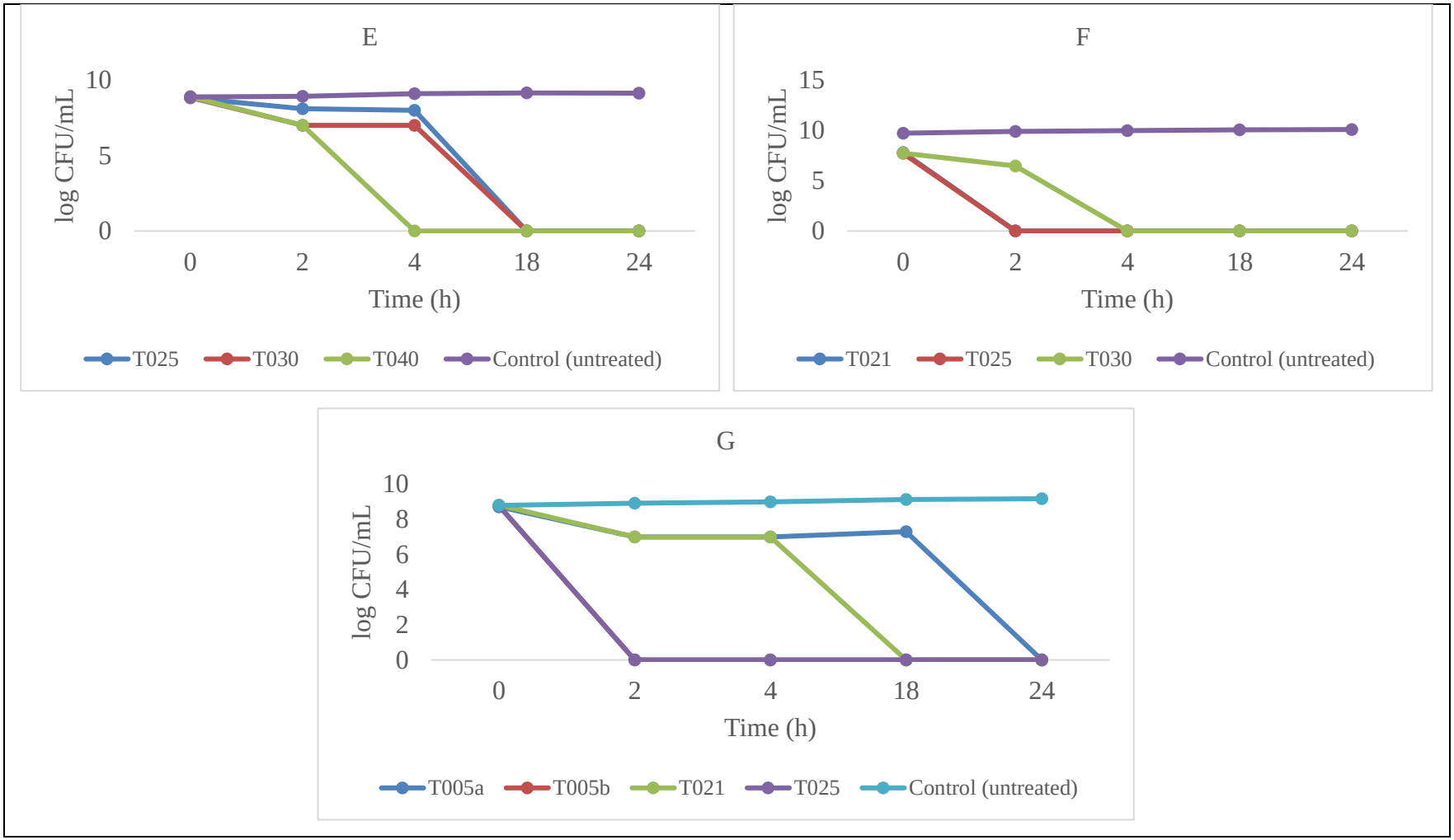
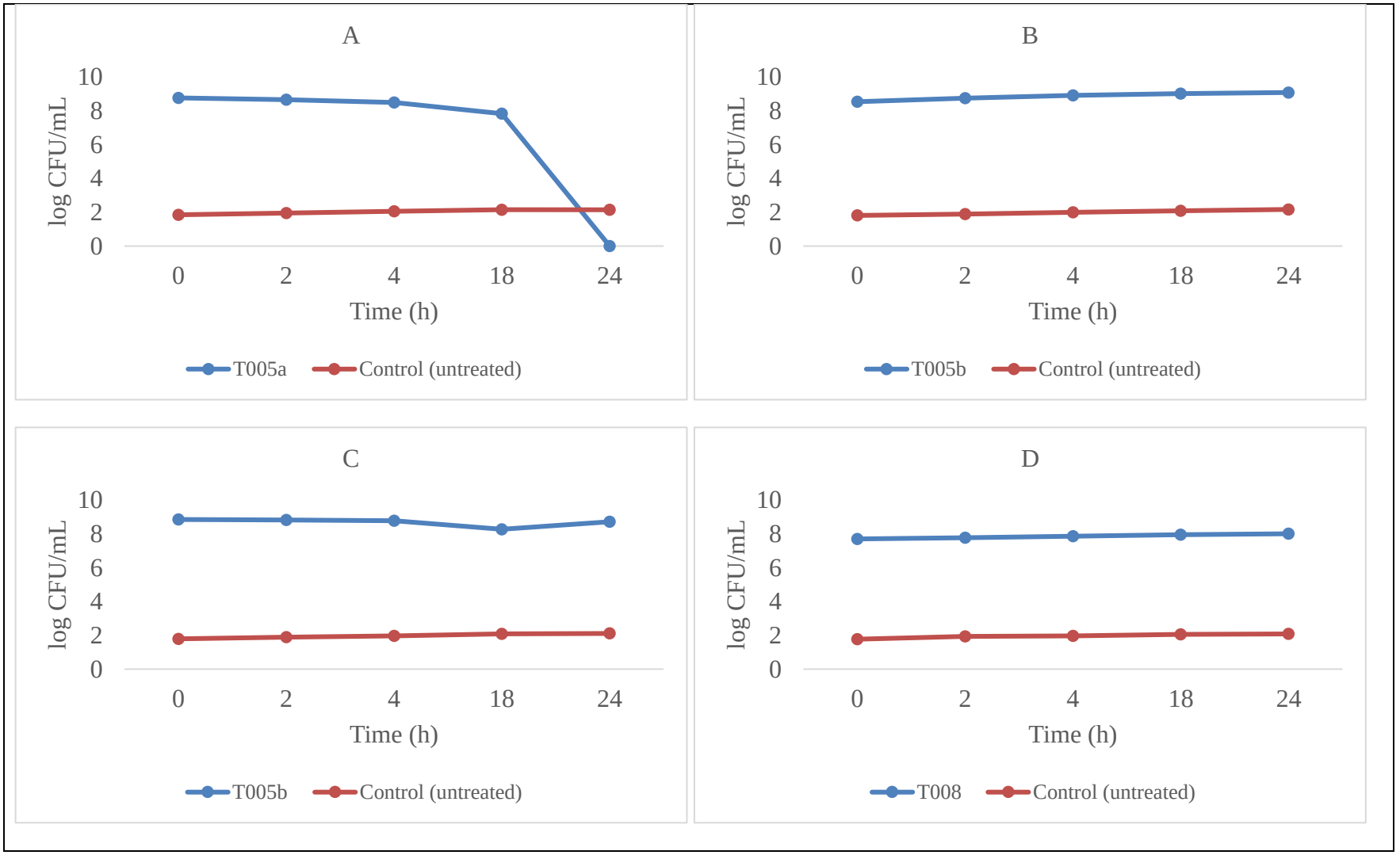


Figure 9: Effects of DCM:MeOH plants extracts used as herbs and spices on the viability of seven laboratory strains (*B. cereus* (A), *P. vulgaris* (B), *E. faecalis* (C), *S. sonnei* (D), *E. coli* (E), *C. albicans* (F), and *S. typhimurium* (G).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Time (h)
T005b
Control (untreated)
D
log CFU/mL
10
8
6
4
2
0
0
2
4
18
24
Time (h)
T008
Control (untreated)

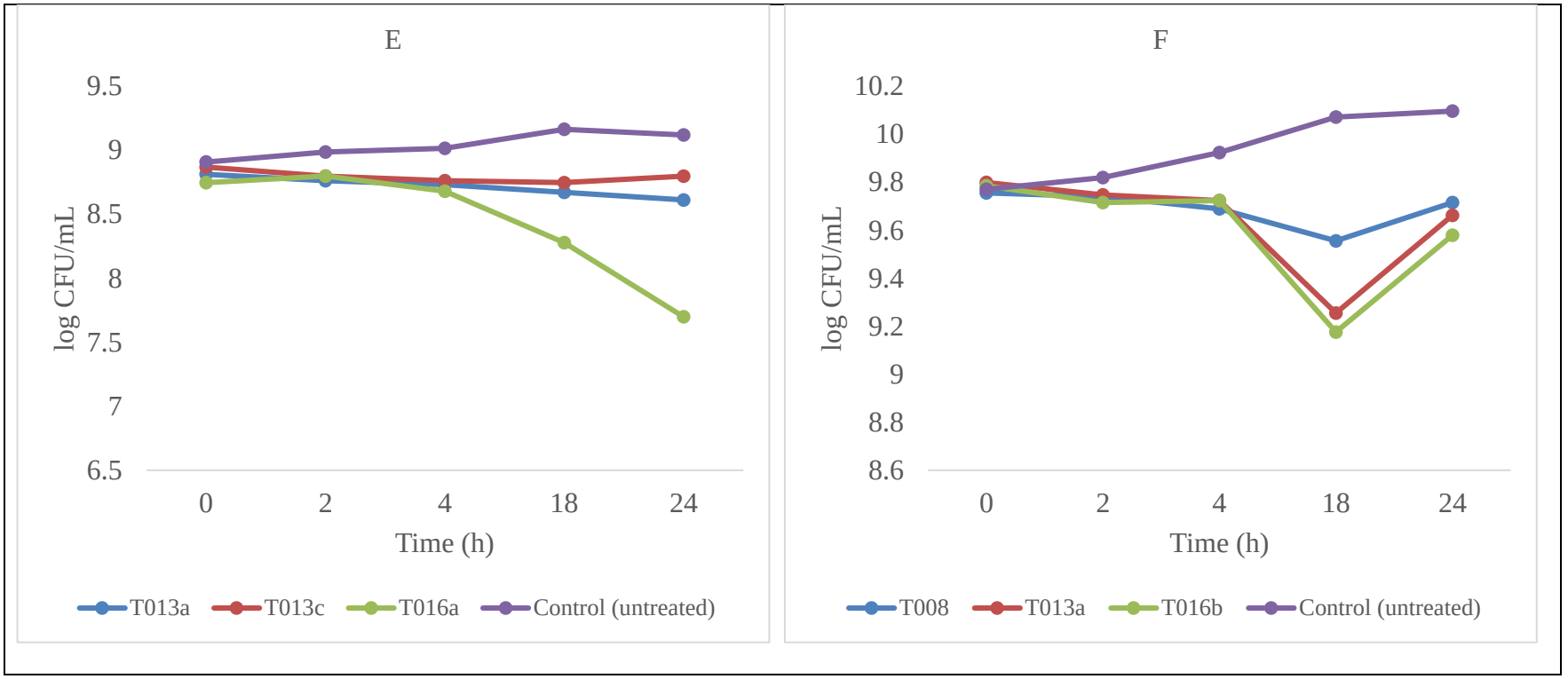

Figure 10: Effects of dd-H_2O plants extracts used as herbs and spices on the viability of six laboratory strains (*E. coli* (A), *S. typhimurium* (B), *P. vulgaris* (C), *C. albicans* (D), *S. aureus* (E), and *P. aeruginosa* (F)).

4.3.5 Time-Kill Synergy Studies

4.3.5.1 Single Plant Study

Only the leaves and barks of *Eucalyptus* sp. were microbicidal against all nine test microbial culture (*B. cereus, S. cerevisiae, C. albicans, S. typhimurium, S. aureus, P. vulgaris, P. aeruginosa, E. coli* and *C. perfringens*) (**Table 11**). They managed to decrease the numbers of colonies by ≥3 log10 in CFU/mL compared with the initial inocula after 24 hours of exposure with test microorganisms. The *Morus* sp. and *N. lotus* were both microbicidal against *C. albicans* and *S. typhimurium* after 4 hours of incubation. *Capsicum* sp. also had microbicidal against three test microorganisms in comparison with *C. gynandra* which had two microbicidal. None of the test microorganisms used in this study were completely killed by the *L. leptocarpa* (DCM:MeOH) extracts as a single plant extract at the concentration of 6.25 mg/mL.

Table 11: Microbicidal effects of single plant (DCM:MeOH plants extracts) against nine laboratory strains.

Test microorganisms strains	**Plant species**						
	Eucalyptus sp. leaves (T005A)	*Eucalyptus* sp. barks (T005B)	*Morus* sp. (T021)	*N. lotus* (T025)	*Capsicum* sp. (T030)	*C. gynandra* (T040)	*L. leptocarpa* (T043)
B. cereus	M	M	NM	NM	NM	M	NM
S. cerevisiae	M	M	NM	NM	NM	NM	NM
C. albicans	M	M	M	M	M	NM	NM
S. typhimurium	M	M	M	M	NM	NM	NM
S. aureus	M	M	NM	NM	NM	NM	NM
P. vulgaris	M	M	NM	NM	M	NM	NM
P. aeruginosa	M	M	NM	NM	NM	NM	NM
E. coli	M	M	NM	M	M	M	NM
C. perfringens	M	NM	NM	NM	NM	NM	NM
Normality test (Wilk-Shapiro test): $p = 0.000$, data are not normally distributed: $p < 0.05$							

*M= microbicidal; NM= non-microbicidal.

4.3.5.2 Synergy Study

Out of 189 combination tests conducted, synergistic (12.2 %), indifference (3.7%), and antagonistic (84.1%) activities were recorded (**Table 12**). No combination of plant extracts exerted synergistic effects on all nine test microbial culture (*B. cereus, S. cerevisiae, C. albicans, S. typhimurium, S. aureus, P. vulgaris, P. aeruginosa, E. coli,* and *C. perfringens*) after 24 hours of incubation. With *C. albicans* and *E. coli*, only the combination of *Eucalyptus* sp. leaves (T005A) and *Eucalyptus* sp. barks (T005B) demonstrated synergy and indifferent effects, respectively. Overall, twenty-three (23) synergistic effects were observed with *S. cerevisiae, S. typhimurium, S. aureus, P. vulgaris, P. aeruginosa, E. coli,* and *C. perfringens*. In addition, indifference effect only occurred in the combination of *Eucalyptus* sp. leaves and *Eucalyptus* sp. barks with the same microbes. Combinations of *Eucalyptus* sp. extracts with either *Morus* sp. (T021), *N. lotus* (T025) or *C. gynandra* (T040) were antagonistic for on all nine test microbial culture. Based on the test normality using the Shapiro-Wilk test, the mean $\log_{10}$ in CFU/mL of test microorganisms in different plant extracts were not normally distributed ($p < 0.05$). Therefore, the survivor curves for on all nine test microbial culture against combination of DCM:MeOH plant extracts was different ($p < 0.05$).

Table 12: Synergistic results of antimicrobial combinations against *B. cereus*, *S. cerevisiae*, *C. albicans*, *S. typhimurium*, *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, and *C. perfringens*.

Test microorganisms strains	**Synergistic effect**																			
	T005A + T005B	T005A + T021	T005A + T025	T005A + T030	T005A + T040	T005A + T043	T005B + T021	T005B + T025	T005B + T030	T005B + T040	T005B + T043	T021 + T025	T021 + T030	T021 + T040	T021 + T043	T025 + T030	T025 + T040	T025 + T043	T030 + T040	T030 + T043	T040 + T043
B. cereus	S	A	A	A	A	A	A	A	A	A	A	S	S	A	S	A	A	A	A	A	A
S. cerevisiae	I	A	A	A	A	A	A	A	A	A	A	S	A	S	A	A	S	A	A	A	A
C. albicans	S	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
S. typhimurium	I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	S	S	S
S. aureus	I	A	A	A	A	A	A	A	A	A	A	S	S	A	A	A	A	A	A	A	A
P. vulgaris	I	A	A	A	A	A	A	A	A	A	A	S	A	S	A	A	S	A	A	A	A
P. aeruginosa	I	A	A	A	A	A	A	A	A	A	A	A	S	S	A	A	S	A	A	A	A
E. coli	I	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
C. perfringens	I	A	A	A	A	A	A	A	S	A	S	S	S	A	A	A	A	A	A	A	A

Normality test (Wilk-Shapiro test): $p = 0.000$, data are not normally distributed: $p < 0.05$

* T005A= *Eucalyptus* sp. leaves; T005B= *Eucalyptus* sp. barks; T021= *Morus* sp.; T025= *N. lotus*; T030= *Capsicum* sp.; T040= *C. gynandra*; T043= *L. leptocarpa*; S= synergy; I= indifference; A= antagonistic.

4.3.6 Permeability of Cell Membrane

The effects of DCM:MeOH extracts of *C. gynandra*, *Eucalyptus* sp., *Morus* sp. and *N. lotus* on the permeability of cell membranes of *B. cereus*, *C. albicans*, *E. coli*, *E. faecalis* and *S. typhimurium* were reported (**Figures 11-13)**. Overall, the test normality using the Shapiro-Wilk test ($p < 0.05$) revealed that the mean relative electric conductivity results of plant extracts against test microorganisms were not normally distributed: $p= 0.024$. Therefore, the relative electric conductivity of each tested microorganisms was different in each plant extract at 1 × MIC and 2 × MIC treatments (Independent- Sample Kruskal-Wallis test: $p = 0.000$).

The relative electric conductivity for *Morus* sp. on the permeability of *C. albicans* (**Figure 11A**) decreased at 4 hours for control treatment but increased at 6 and 24 hours. However, the relative electric conductivity increased slightly at 4, 6 and 24 hours for 1 × MIC treatment. On the other hand, the relative electric conductivity decreased rapidly for 2 × MIC treatment at 4, 6 and 24 hours. The relative electric conductivity for the control treatment exhibited the highest values whereas the 1 × MIC showed the lowest values at 6 and 24 hours.

The relative electric conductivity for *N. lotus* on the permeability of *C. albicans* (**Figure 11B**) increased at 4, 6 and 24 hours for control and 1 × MIC treatments. However, the relative electric conductivity increased at 4 hours for 2 × MIC treatment but decreased at 6 hours and later increased at 24 hours. The relative electric conductivity for the control treatment exhibited the highest value whereas the 1 × MIC showed the lowest value at 24 hours.

For *Eucalyptus* sp. on the permeability of *C. albicans* (**Figure 11C**), the relative electric conductivity increased at 4, 6 and 24 hours for control and 1 × MIC treatments.

However, the relative electric conductivity slightly decreased at 4, 6 and 24 hours for 2 × MIC treatment. The relative electric conductivity differed among various treatments: the control exhibited the highest values whereas the 1 × MIC showed the lowest values at 2, 4, 6 and 24 hours.

The relative electric conductivity for *Morus* sp. on the permeability of *S. typhimurium* (**Figure 12A**) increased at 4, 6 and 24 hours for control treatment. However, the relative electric conductivity increased at 4 hours for 1 × MIC treatment but decreased at 6 and 24 hours. On the other hand, the relative electric conductivity decreased for 2 × MIC treatment at 4, 6 and 24 hours. The relative electric conductivity for the 1 × MIC treatment exhibited the highest values whereas the 2 × MIC showed the lowest values at 6 and 24 hours.

The relative electric conductivity for *N. lotus* on the permeability of *S. typhimurium* (**Figure 12B**) increased at 4, 6 and 24 hours for control treatment. However, the relative electric conductivity increased at 4 hours for 1 × MIC treatment but decreased at 6 hours and later increased at 24 hours. On the other hand, the relative electric conductivity decreased at 4 and 6 hours for 2 × MIC treatment but increased at 24 hours. The relative electric conductivity for the control treatment exhibited the highest value whereas the 1 × MIC showed the lowest value at 6 and 24 hours.

The relative electric conductivity of the *Eucalyptus* sp. leaves extracts on the permeability of *S. typhimurium* (**Figure 12C**) increased at 4, 6 and 24 hours for control treatment. However, the relative electric conductivity decreased at 4 hours for 1 × MIC and 2 × MIC treatments but increased at 6 and 24 hours. The relative electric conductivity differed among various treatments: the control exhibited the highest values whereas the 2 × MIC showed the lowest values at 2, 4, 6 and 24 hours.

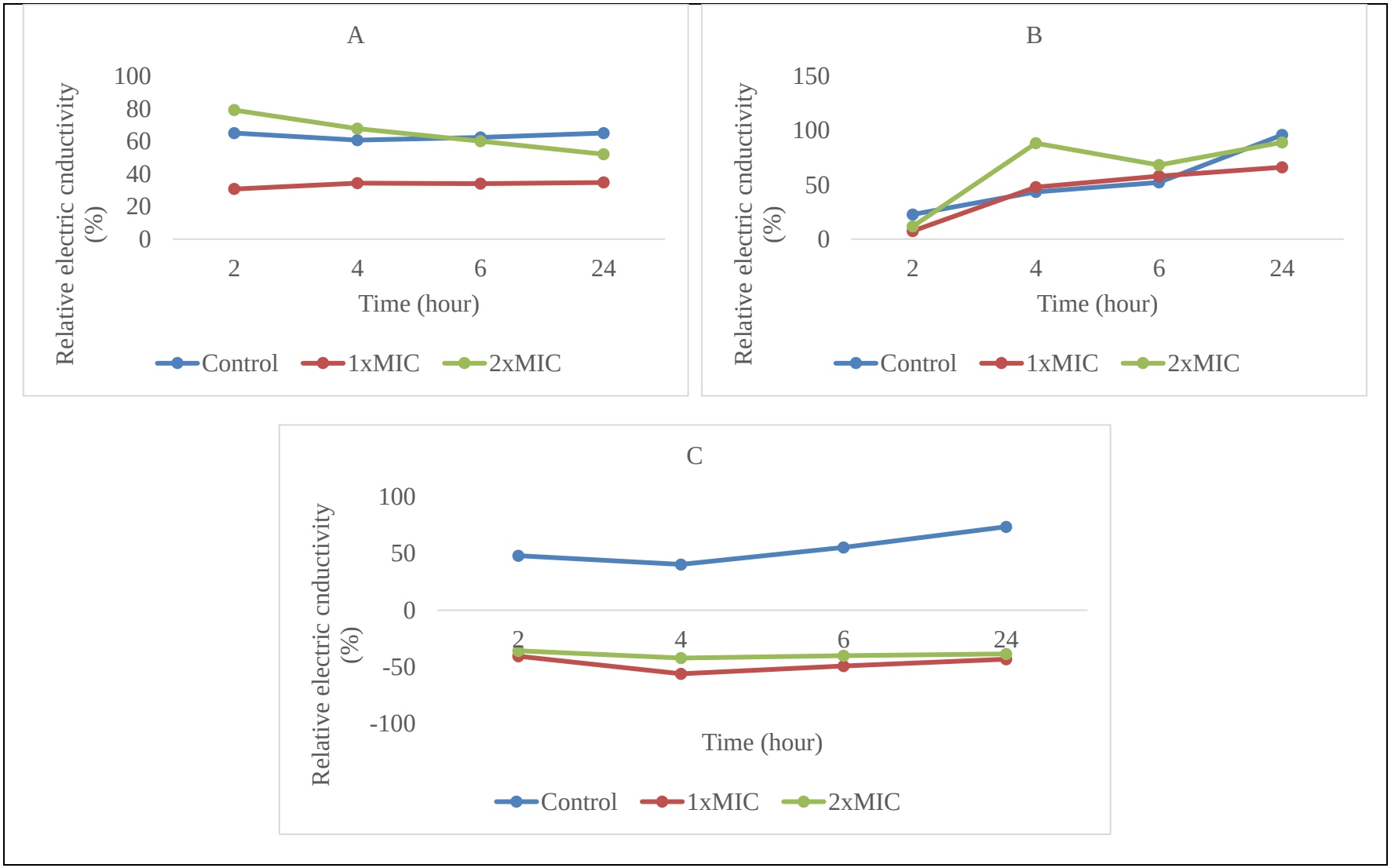

Figure 11: Effect of *Morus* sp. (A), *N. lotus* (B) and *Eucalyptus* sp. (C) on the permeability of *C. albicans*.

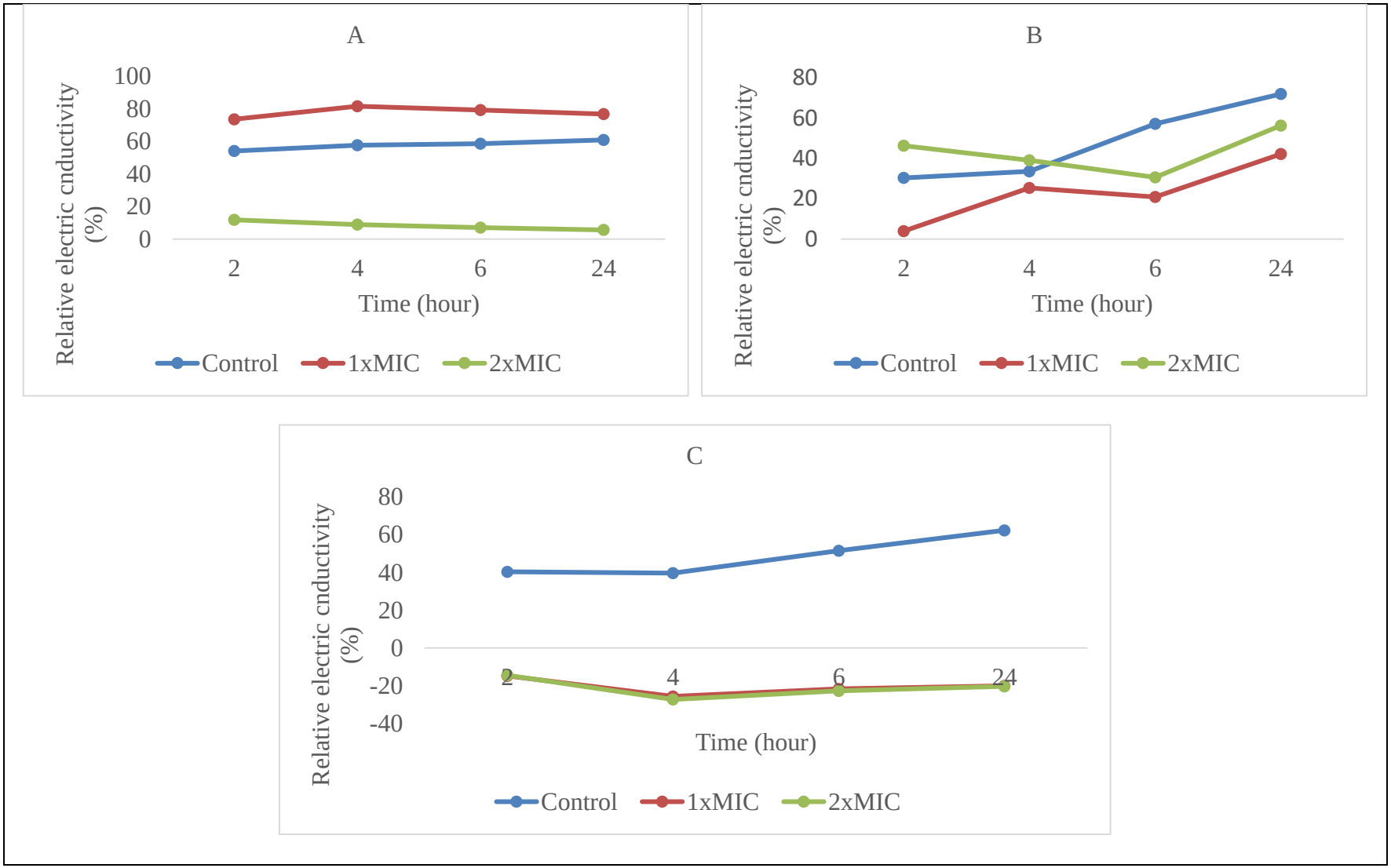

Figure 12: Effect of *Morus* sp. (A), *N. lotus* (B) and *Eucalyptus* sp. (C) on the permeability of *S. typhimurium*.

The relative electric conductivity for *C. gynandra* on the permeability of *E. coli* (**Figure 13A**) increased at 4, 6 and 24 hours for control and 1 × MIC treatments. However, the relative electric conductivity slightly decreased at 4, 6 and 24 hours for 2 × MIC treatment. The relative electric conductivity for the control treatment exhibited the highest value whereas the 2 × MIC showed the lowest value at 4, 6, and 24 hours. For *C. gynandra* on the permeability of *B. cereus* (**Figure 13B**), the relative electric conductivity increased at 4, 6 and 24 hours for control treatment. However, the relative electric conductivity decreased at 4 hours for 1 × MIC and 2 × MIC treatments but increased at 6 and 24 hours. The relative electric conductivity for the control treatment exhibited the highest value whereas the 1 × MIC showed the lowest value at 6 and 24 hours.

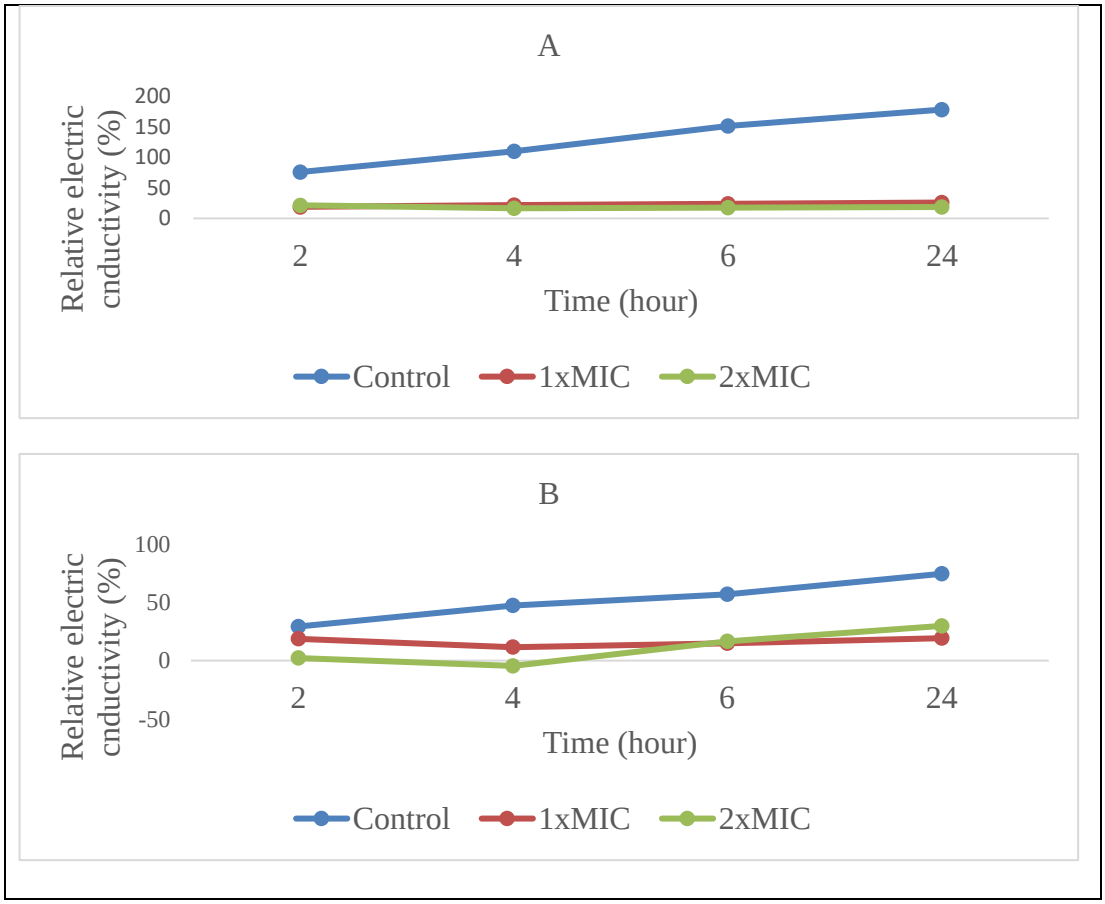

Figure 13: Effect of *C. gynandra* on the permeability of *E. coli* (A) and *B. cereus* (B).

4.3.7 Release of 260 nm Absorbing Materials and Proteins

The release of nucleic acids from the *B. cereus*, *C. albicans*, *E. coli*, and *S. typhimurium* after treated with plant extracts are shown in **Figure 14-16**. Overall, the test normality using the Shapiro-Wilk test ($p < 0.05$) revealed that the absorbance values at 260 nm results of test microorganisms in different plant extracts were not normally distributed: $p = 0.000$. Therefore, the absorbance values at 260 nm of each tested microorganisms was different in each plant extract at 1 × MIC and 2 × MIC treatments (Independent- Sample Kruskal-Wallis test: $p = 0.001$).

The absorbance values for nucleic acids of *C. albicans* after treated with *Eucalyptus* sp. slightly increased to unchanged at 8, 12 and 24 hours for 1 × MIC and 2 × MIC treatments (**Figure 14A**). The absorbance values for nucleic acids of *C. albicans* after treated with *N. lotus* slightly decreased at 8 hours for both 1 × MIC and 2 × MIC treatments but slightly increased at 24 hours (**Figure 14B**). The absorbance values for nucleic acids of *C. albicans* after treated with *Morus* sp. increased at 8 hours but decreased at 12 hours and slightly increased at 24 hours for 1 × MIC treatment (**Figure 14C**). For 2 × MIC treatment, the absorbance values for nucleic acids of *C. albicans* after treated with *Morus* sp. remain slightly unchanged at 4, 8, 12 and 24 hours. The OD_{260nm} values of 1 × MIC were slightly higher than that of 2 × MIC treatment for *C. albicans* treated with *Eucalyptus* sp. and *N. lotus*; while the OD_{260nm} values of 2 × MIC were slightly higher than that of 1 × MIC treatment at 4, 12 and 24 hours for *C. albicans* treated with *Morus* sp.. The OD_{260nm} values of control treatment had the lowest value at 4, 8, 12 and 24 hours for *C. albicans* treated with *Eucalyptus* sp. and *N. lotus*, and *Morus* sp..

The absorbance values for nucleic acids of *S. typhimurium* after treated with *Eucalyptus* sp. slightly decreased at 8 hours, but slightly increased at 12 and 24 hours

for 1 × MIC treatment (**Figure 15A**). For 2 × MIC treatment, the absorbance values for nucleic acids of *S. typhimurium* treated with *Eucalyptus* sp. slightly increased at 4, 8, 12 and 24 hours. The *S. typhimurium* treated with *N. lotus* slightly increased at 4, 8, 12 and 24 hours for both 1 × MIC and 2 × MIC treatments (**Figure 15B**). The absorbance values for nucleic acids of *S. typhimurium* after treated with *Morus* sp. increased at 8 hours but decreased at 12 hours and slightly increased at 24 hours for 1 × MIC treatment (**Figure 15C**). For 2 × MIC treatment, absorbance values for nucleic acids of *S. typhimurium* after treated with *Morus* sp. remain slightly unchanged at 4, 8, 12 and 24 hours. The OD_{260nm} values of 1 × MIC were slightly higher than that of 2 × MIC treatment for *S. typhimurium* treated with *Eucalyptus* sp. while the OD_{260nm} values of 2 × MIC were slightly higher than that of 1 × MIC treatment for *S. typhimurium* treated with *Morus* sp. and *N. lotus*. The OD_{260nm} values of control treatment had the lowest value at 4, 8, 12 and 24 hours for both *C. albicans* and *S. typhimurium* treated with *Eucalyptus* sp. and *N. lotus*, and *Morus* sp..

The absorbance values for nucleic acids of *E. coli* after treated with *C. gynandra* slightly decreased at 8 and 24 hours, although slight increase was observed at 12 hours for 1 × MIC treatment (**Figure 16A**). For 2 × MIC treatment, the absorbance values for nucleic acids of *E. coli* slightly increased at 8 and 12 hours but slightly decreased at 24 hours. The absorbance values of the *B. cereus* treated with *C. gynandra* slightly decreased at 8 hours, but slightly increased at 12 and 24 hours for 1 × MIC treatment (**Figure 16B**). On the other hand, the 2 × MIC treatment slightly remained unchanged at 8 hours but slightly decreased at 12 hours before slightly increased at 24 hours. The OD_{260nm} values of 2 × MIC were higher than that of 1 × MIC treatment at 4, 12 and 24 hours, and the OD_{260nm} values of control treatment had the lowest value at 4, 8, 12 and 24 hours for both *E. coli* and *B. cereus* treated with *C. gynandra*.

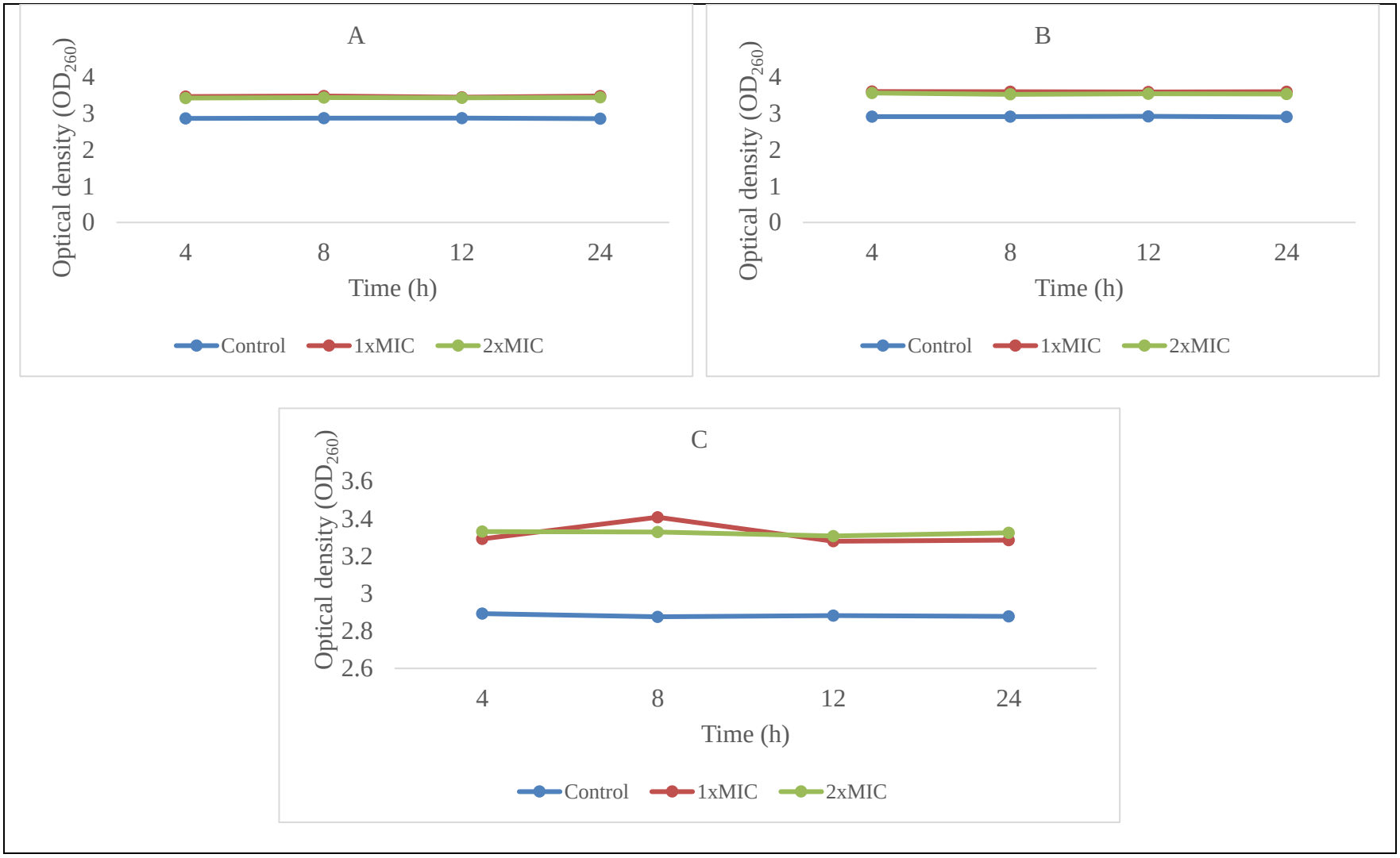

Figure 14: Release of 260 nm absorbing material from *C. albicans* treated with *Eucalyptus* sp. (A), *N. lotus* (B), and *Morus* sp. (C).

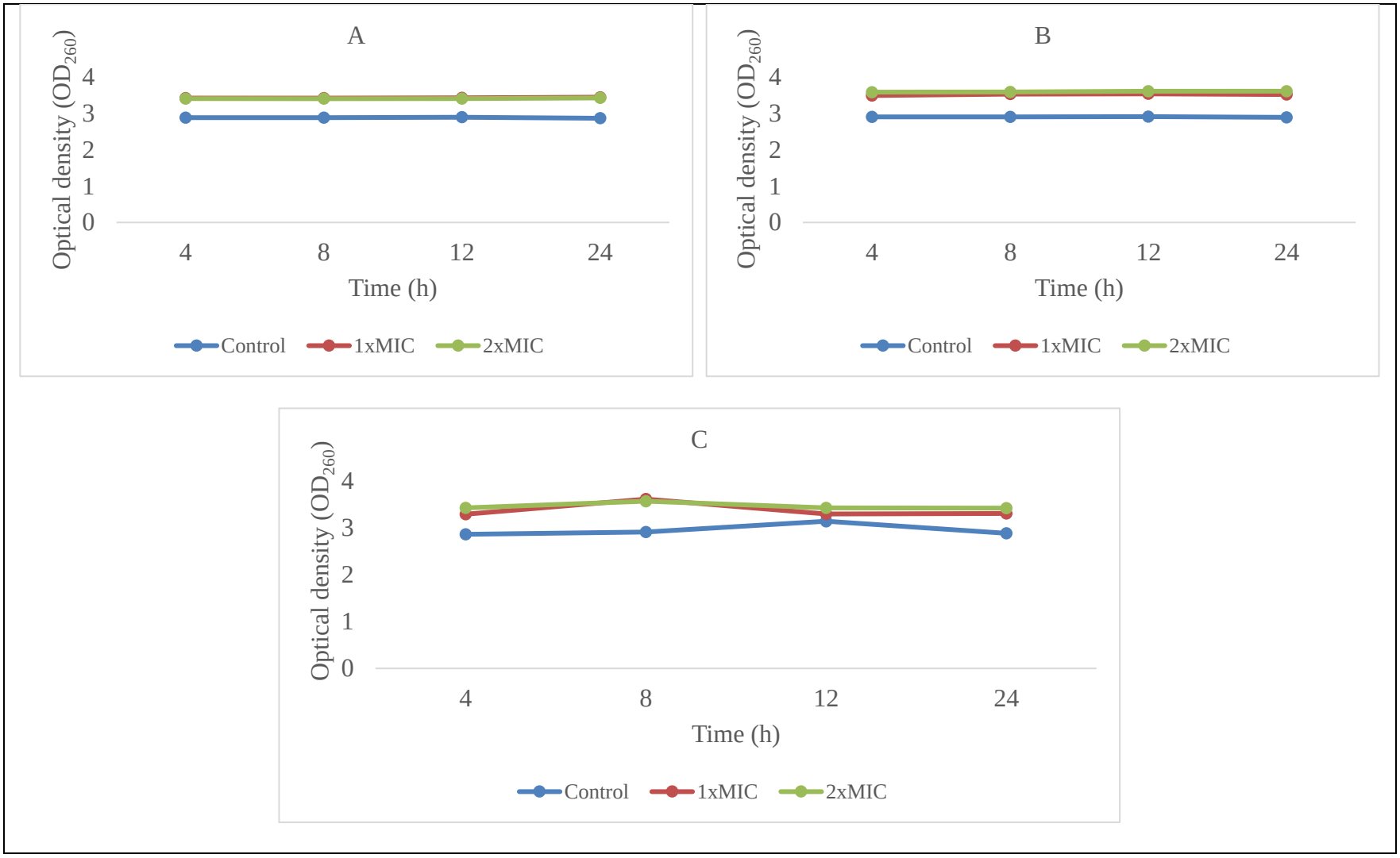

Figure 15: Release of 260 nm absorbing material from *S. typhimurium* treated with *Eucalyptus* sp. (A), *N. lotus* (B), and *Morus* sp. (C).

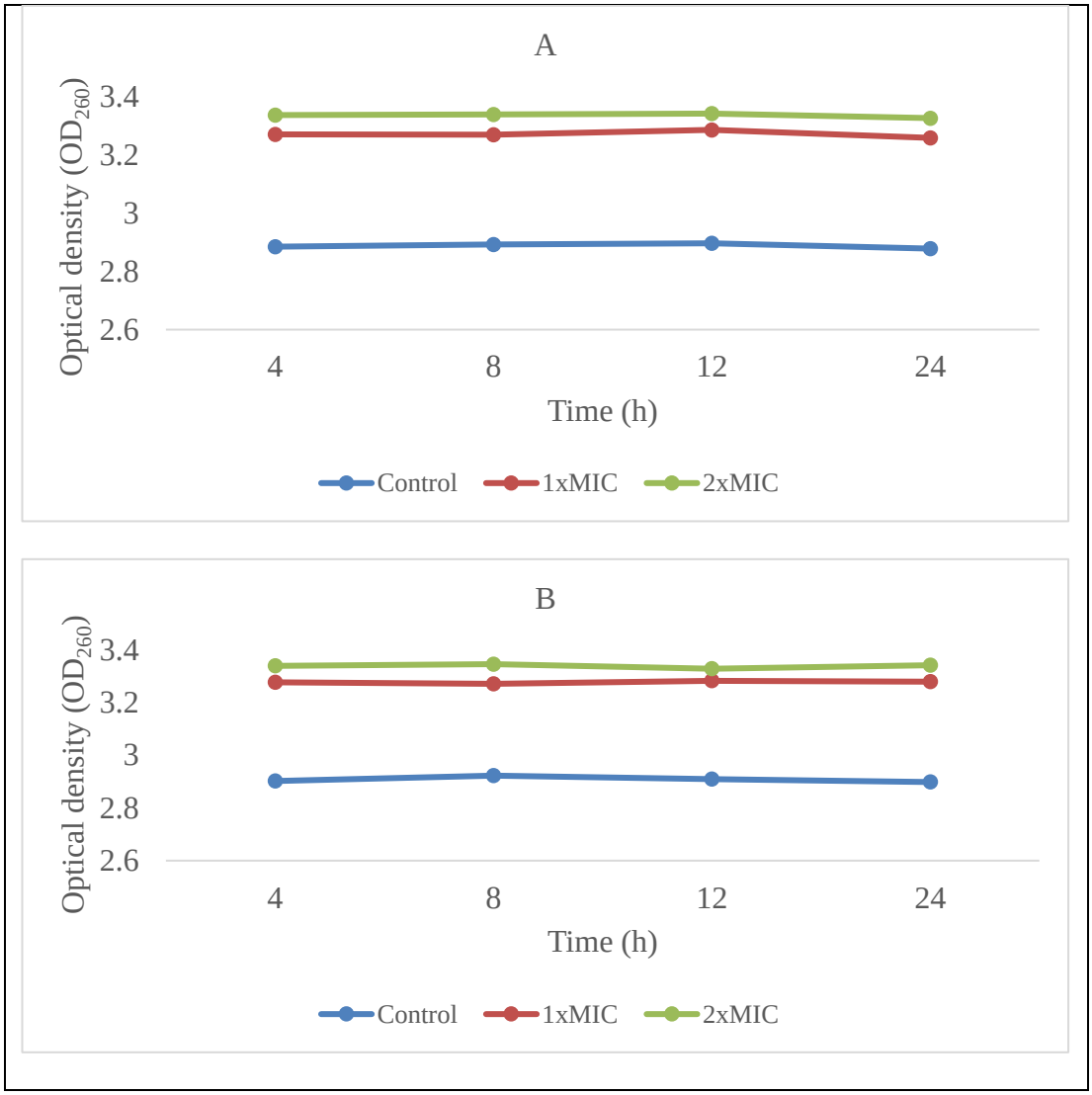

Figure 16: Release of 260 nm absorbing material from *E. coli* (A) and *B. cereus* (B) treated with *C. gynandra*.

The release of proteins from the *B. cereus*, *C. albicans*, *E. coli*, and *S. typhimurium* after treated with DCM:MeOH plant extracts are shown in **Figure 17-19**. Overall, the test normality using the Shapiro-Wilk test ($p < 0.05$) revealed that the absorbance values at 280 nm results of test microorganisms in different plant extracts were not normally distributed: $p= 0.000$. The Independent- Sample Kruskal-Wallis test indicated significant difference ($p = 0.003$). The values of proteins from *C. albicans* after treated with *Eucalyptus* sp. of 1 × MIC were slightly higher than that of 2 × MIC treatment (**Figure 17A**). The values of proteins from *C. albicans* after treated with *N. lotus* of 1 × MIC were slightly higher than that of 2 × MIC treatment (**Figure 17B**). On the other hand, the values of proteins from *C. albicans* after treated with *Morus* sp. of 2 × MIC were higher than that of 1 × MIC treatment at 4, 12 and 24 hours, although the protein value of 1 × MIC treatment was higher than 2 × MIC at 8 hours (**Figure 17C**).

The values of proteins from *S. typhimurium* after treated with *Eucalyptus* sp. of 1 × MIC were slightly higher than that of 2 × MIC treatment (**Figure 18A**). Moreover, the values of proteins from *S. typhimurium* of 2 × MIC were higher than that of 1 × MIC treatment after treated with *N. lotus* extract (**Figure 18B**). On the other hand, the values of proteins from *S. typhimurium* after treated with *Morus* sp. of 2 × MIC were higher than that of 1 × MIC treatment at 4, 12 and 24 hours, although the protein value of 1 × MIC treatment was higher than 2 × MIC at 8 hours (**Figure 18C**). Similarly, the values of proteins from *E. coli* and *B. cereus* after treated with *C. gynandra* of 2 × MIC were slightly higher than that of 1 × MIC treatment (**Figure 19A, B**). All in all, the values of proteins from *B. cereus*, *C. albicans*, *E. coli*, and *S. typhimurium* of all control treatment had the lowest value at 4, 8, 12 and 24 hours after treated with plant extracts.

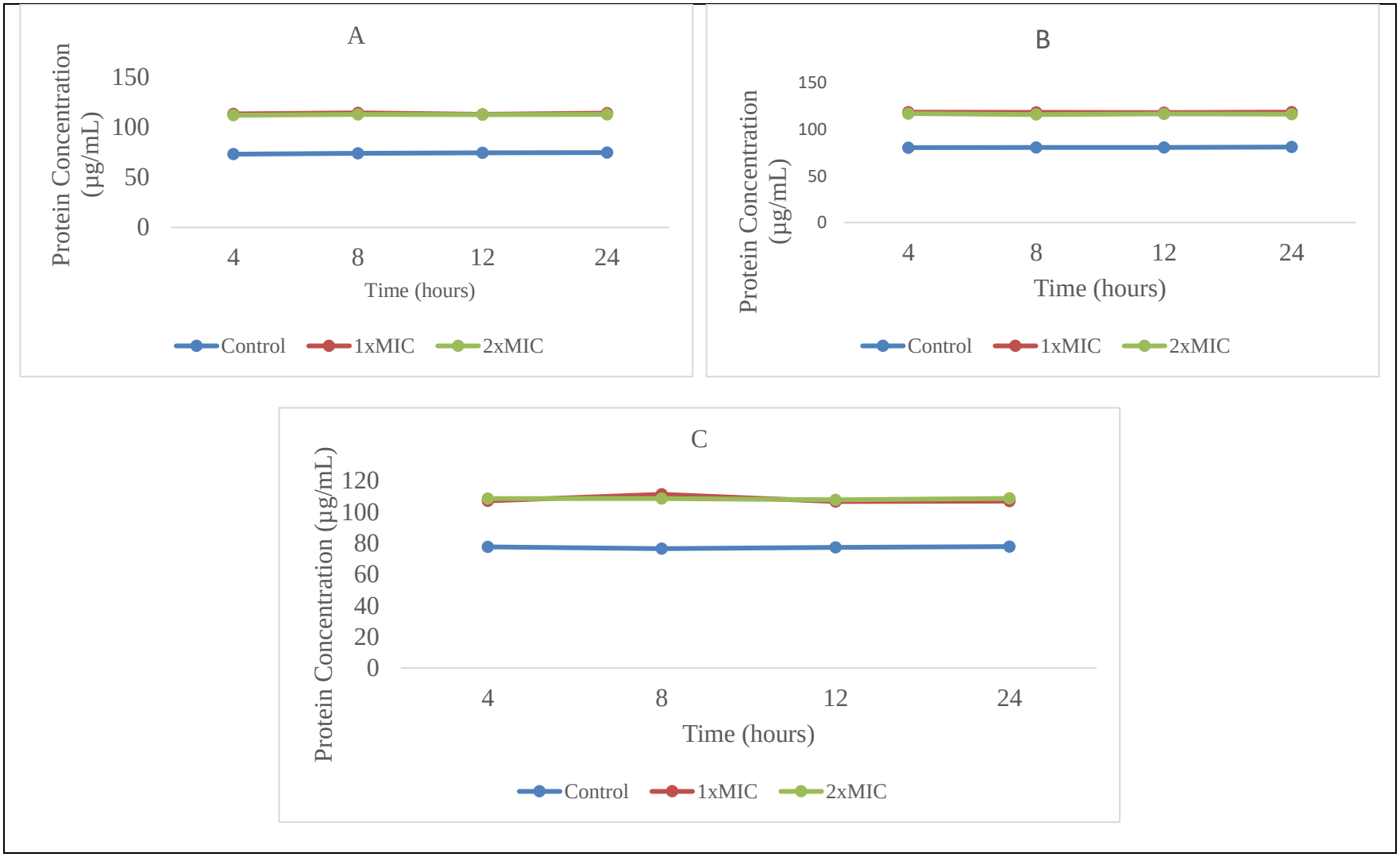

Figure 17: Release of protein from *C. albicans* treated with *Eucalyptus* sp. (A), *N. lotus* (B), and *Morus* sp. (C).

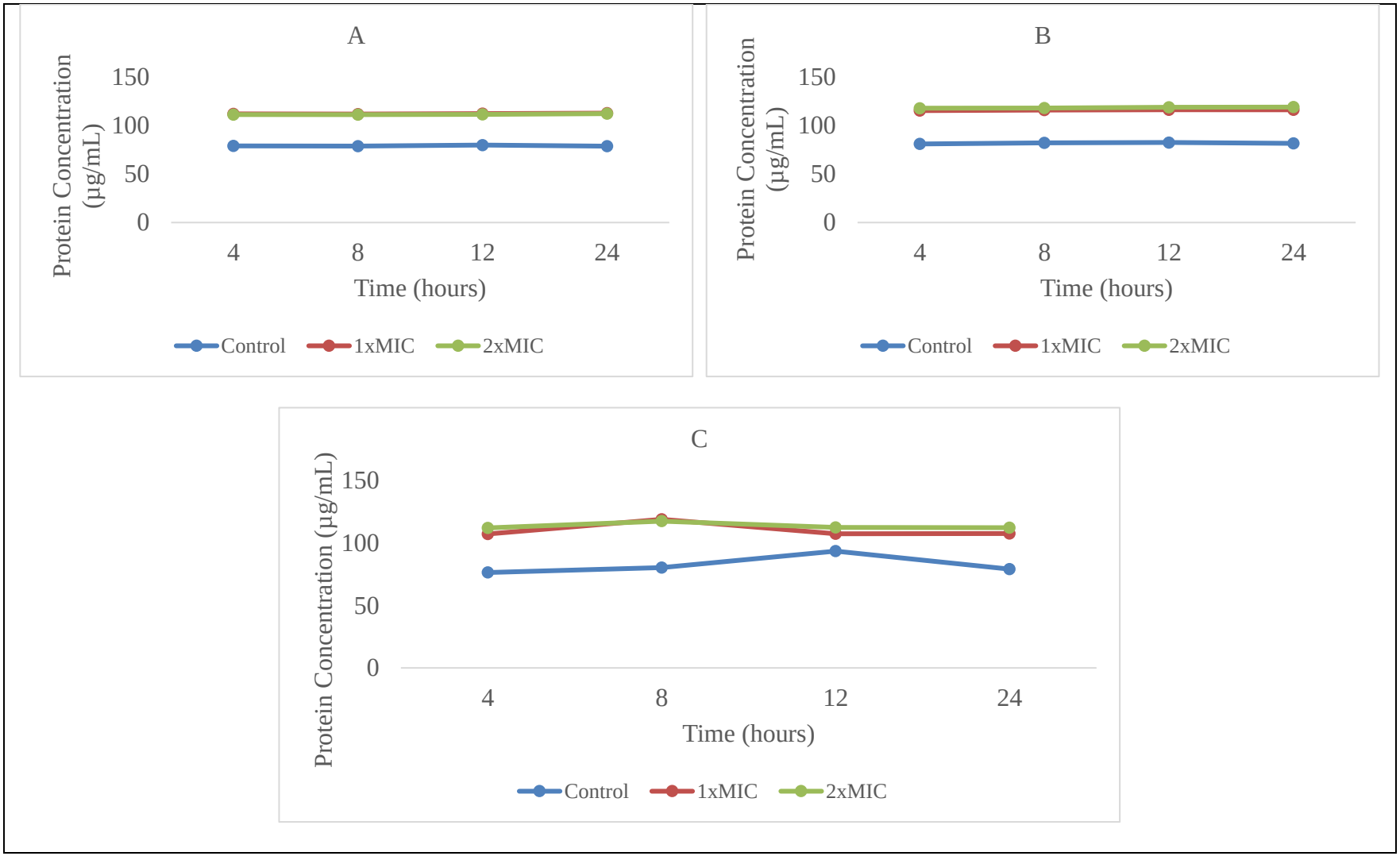

Figure 18: Release of protein from *S. typhimurium* treated with *Eucalyptus* sp. (A), *N. lotus* (B), and *Morus* sp. (C).

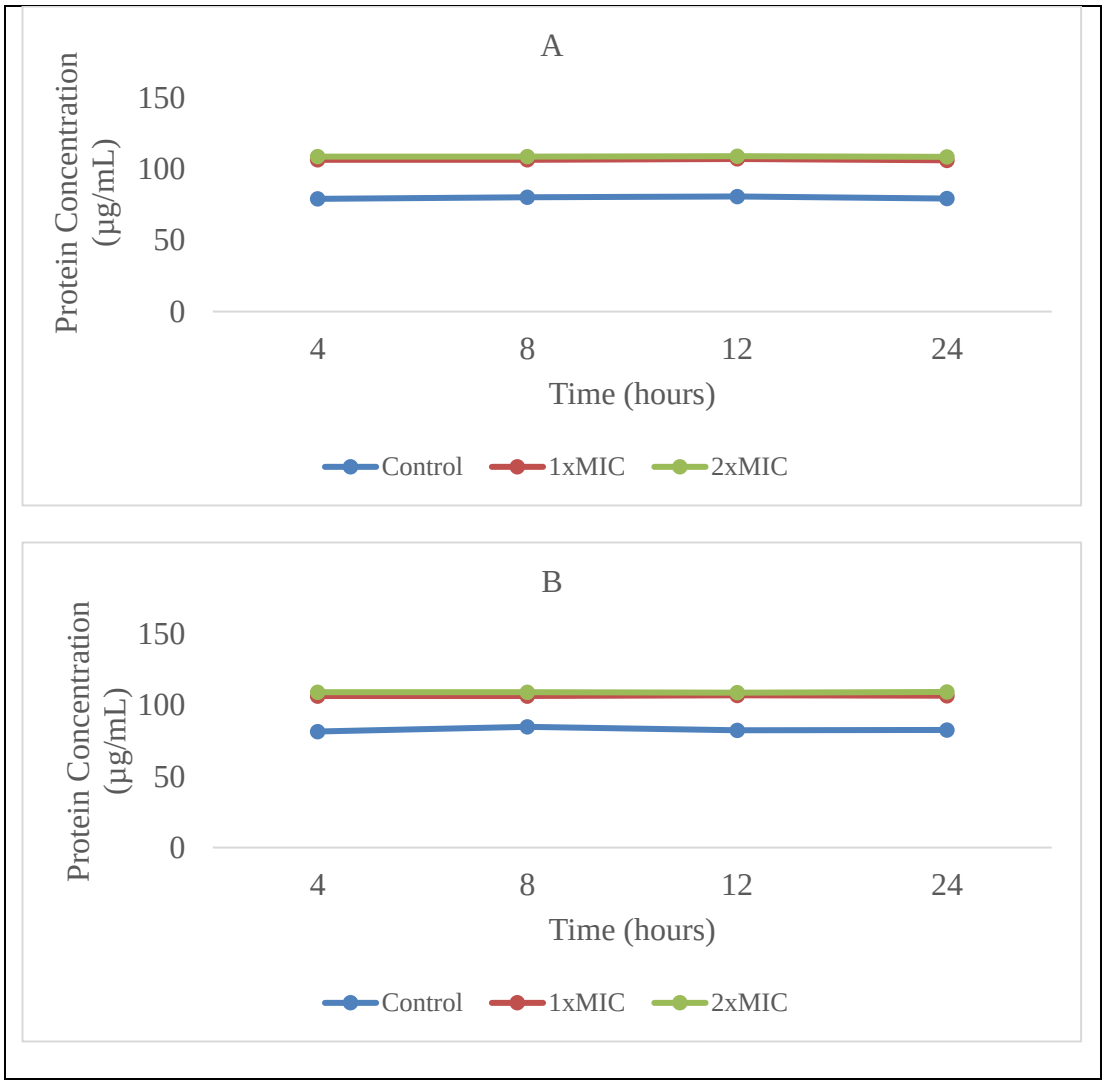

Figure 19: Release of protein from *E. coli* (A) and *B. cereus* (B) treated with *C. gynandra*.

4.4 Total Phenolic and Total Flavonoids Contents

The total phenolic content data for the extracts displaying strong antimicrobial activity are summarized in **Table 13**. Overall, the test normality using the Shapiro-Wilk test ($p < 0.05$) revealed that the mean total phenolic content of different plant extracts were not normally distributed: $p = 0.044$. Therefore, the mg Gallic acid equivalent per gram (GAE/g) of each extract was different. The Independent-Sample Kruskal-Wallis test revealed that there was significant difference between total phenolic content values and plant extract ($p = 0.423$). The highest total phenolic content was observed for

DCM:MeOH extracts of *Eucalyptus* sp. (leaves) with 598.4±0.04 mg GAE/g extract, followed by *Morus* sp. with 352±0.57 mg GAE/g extract. The lowest content was noted for DCM:MeOH extract of *Capsicum* sp. (213.7±0.06 mg GAE/g extract).

On the other hand, the test normality using the Shapiro-Wilk test revealed that the mean total flavonoids content of different plant extracts were normally distributed: p= 0.105. Therefore, the mg CAE/g of each extract was different (One-Way ANOVA p= 0.004). The highest total flavonoids content was observed for DCM:MeOH leaves and barks extracts of *Eucalyptus* sp. with 9865.7±0.00 mg CAE/g (**Table 13**). The lowest flavonoids content was recorded for DCM:MeOH extract of *N. lotus* with 2106.8±0.04 mg CAE/g.

Table 13: Phytochemicals of DCM:MeOH extracts of plants traditional used as herbs and spices (mean ± standard error).

Plant species	**Plant part**	**Total phenolic content (mg GAE/g)±SE**	**Total flavonoid content (mg CAE/g)±SE**
Eucalyptus sp.	Leaves	598.4±0.04	9865.7±0.00
	Barks	241.8±0.20	9865.7±0.00
Morus sp.	Leaves	351.5±0.57	5746.8±0.12
N. lotus	Flowers	302.5±0.03	2106.8±0.04
Capsicum sp.	Fruits	213.7±0.06	2529±0.04
C. gynandra	Leaves	268.6±0.10	2702.3±0.01
L. leptocarpa	Leaves	325.7±0.12	5014.6±0.05
Normality test (Wilk-Shapiro test)		p= 0.044, p < 0.05	p= 0.105, p >0.05
One way ANOVA		-	p= 0.004, p < 0.05
Independent-Sample Kruskal-Wallis test		p = 0.423, p < 0.05	-

*SE= Standard Error. Values are mean ± SE, n=3

5. CHAPTER FIVE: DISCUSSION

5.1 Socio-Demographic Information

The 23-39 years age group mentioned more plant species (44.0 %) in comparison with other age groups (**Table 2**). This indicated that the number of knowledge holders was gradually decreasing as the age groups increased in the study areas. This is not in agreement with several studies which reported the opposite (Eyong, 2011; Kangalawe, et al., 2014). Therefore, it is important to document the indigenous knowledge in the elderly groups, so that it can be maintained and sustained for the young and future generations to learn from it (Negi, et al., 2010). If not, these knowledge holders will pass and the knowledge will be lost. The traditional ethnobotanical knowledge (TEK) from elderly people is underlying threats of disappearing (Eyong, 2011). This is due to ignorance and disbelieve in young generation in learning tradition and indigenous knowledge from the elderly (McCarter & Gavin, 2011), as well as Western influences including formal education, medicine, political systems, religion and technology (Haselmair, et al., 2014). According to Shisanya (2017), TEK is often conferred between individuals of a particular indigenous group during social interaction and/or by oral transmission through storytelling.

During the ethnobotanical survey, more female informants (64.7 %) participated in the present study in comparison to male informants (**Table 2**). Asowata-Ayodele et al. (2016) and Reicks et al. (2014) suggested that women are known to take care of most of the cooking, culinary activities, traditional health care and cultivating gardens in most African traditional families. Female have therefore the potential to contribute toward conserving the environment, developing sustainable agriculture and ensuring food security (Ahmed, et al., 2013). Therefore, the indigenous knowledge on plants

that are traditionally used as herbs and/or spices must be documented and protected to encourage the maintenance of traditional practices and lifestyles in rural communities (Eyong, 2011).

The Masubia and Mafwe people were the dominant ethnic groups reported during the ethnobotanical survey in Kabbe constituencies (**Figure 4**). However, there is a lack of data about the distribution of these ethnic groups within the Zambezi region. This study noted that the Masubia people were dominant in Kabbe constituencies because of their traditional authority (*kuta*) which is based in Bukalo, a village in Kabbe North constituency. The Masubia and Mafwe people live off farming and fishing, where women in villages are more responsible for farming activities while men deal more with the hunting and fishing (Colpaert, et al., 2013). Because of this, more female informants in Kabbe constituencies participated in the ethnobotanical survey and overall mentioned more plant species, in comparison with male informants.

5.2 Botanical Description of Collected Herbs and Spices

The 16 plant families indicated a great diversity of plants used as culinary herbs and/or spices (**Figure 5**). The largest proportion of plants documented belong to the family Malvaceae which includes plant species like *A. digitate*, *C. tridens*, *H. fuscus*, *H. mechowii* and *Hibiscus* sp.. The Malvaceae family, also known as the mallows, consist of flowering plants with an estimation of about 244 genera and 4225 known species (Christenhusz & Byng, 2016). Among the 244 genera, *Hibiscus* and *Adansonia* are some of the commonly studied for their traditional uses as sources of edible oil, relish, soup, and water (Braca, et al., 2018; Mariod, et al., 2017). Therefore, Malvaceae family is one of the important plant family used as herbs and spices.

On the other hand, the Amaranthaceae, Cucurbitaceae, Fabaceae and Moraceae family were each represented by two plant species. The Amaranthaceae family contains about 165 genera and 2,040 species (Christenhusz & Byng, 2016). The Amaranthaceae family consist of species which are commonly used as vegetables (*Spinacia oleracea*), pseudocereals (*Chenopodium berlandieri*), and medicinal herbs (*Dysphania anthelmintica*) (Kadereit, et al., 2003). This is in agreement with the study by Asowata-Ayodele et al. (2016) that reported the culinary and therapeutic uses of *A. caudatus* and *A. hybridus*. Equally, the Cucurbitaceae family, also known as the gourd family, contains 98 genera and about 975 species of food and ornamental plants (Paris, et al., 2017). Its members includes five vegetable crops of worldwide importance, such as cucumbers, gourds, melons, squashes, and pumpkins (Patela & Rauf, 2017). These plants have been grown for food and medicinal purposes for thousands of years, and they have long been part of the Mediterranean diet (Aronson, 2006). Likewise, the Fabaceae family consist of flowering plants, which have been staple human food since ancient times (Silva, et al., 2018). Plant species such as soybean (*Glycine max*), beans (*Phaseolus*), pea (*Pisum sativum*), and peanut (*Arachis hypogaea*) are members of the Fabaceae family, which are not important only in agriculture but in food sector as well (Hedimbi & Chinsembu, 2012). Plant species in the Fabaceae family are not only known for their favouring and nutritional functions, but also for their contribution in cosmetic and primary health sectors (Sachdeva, et al., 2018).

Among different plant parts reported, leaves were the most commonly used plant parts after roots (**Figure 6**). In agreement with this, a similar study by Chinsembu et al. (2014) reported that harvesting of leaves, unlike roots, is not labour-intensive and may not permanently damage the plants. Therefore, leaves are commonly preferred than any other plant parts used. Furthermore, leaves possess chemical constituents in larger

quantities (compared to most plant parts used) which might be responsible for medicinal, flavouring, preservative, and seasoning properties (Rashid, et al., 2018). On the contrary, the results obtained in the present study were not in agreement with some literatures. Few studies had reported that roots were more preferred than any other plant parts (Cheikhyoussef, et al., 2011; Teklehaymanot, 2009). A study by Tolossa et al. (2013) reported that this could be because roots are usually available throughout the year, even during the long drought seasons. However, care must be considered when harvesting plant roots to avoid loss of biodiversity due to permanent damage of the plants, which might lead to extinction of plant species (Chapin, 2003).

5.3 Culinary and/or Therapeutic Plant Uses

The culinary and/or therapeutic uses of twenty-three plants species revealed that most households in Kabbe constituencies use plant parts to prevent food from spoilage (preservation) as well as enhance food flavor (seasoning) (**Figure 7**). In addition, some plant parts were used to treat and manage illnesses such as diarrhoea, malaria, low blood pressure, tuberculosis symptoms, stomachache, joint pain, connective tissues, swelling, mouth and throat sores, kidney infections, expel parasitic worms and other internal parasites (**Table 4**).

Leaves of *A. hybridus* were reported collected from the wild, consumed as leafy vegetable (relish) and/or as traditional medicine. This is in agreement with , a study by Akubugwo et al. (2007) who reported similar uses of *A. hybridus* as soup, green vegetables or sometimes boiled and mixed with a groundnut sauce to make salad. Furthermore, another study by Nana et al. (2012) is in line with the medicinal uses of *A. hybridus* recorded in this study. The *A. hybridus* is also used in traditional medicine to treat liver infections, knee pain, stomachaches, diarrhoea and dysentery. Other *Amaranthus* spp. are known to treat several conditions including urinary infections,

gynecological conditions, diarrhoea, pain, respiratory disorders, diabetes and as diuretic (Cheikhyoussef, et al., 2011). These culinary and therapeutic uses could be due to presence of phytochemical compounds such as polyphenols, tannins, flavonoids, steroids, terpenoids, saponins and betalains found in the leaves of the *A. hybridus* (Okunlola, et al., 2017).

The *B. massaiensis* roots were recorded used to prepare herbal tea or used as chewing sticks (toothbrush) in Kabbe constituencies. However, literatures have indicated that the *B. massaiensis* is widely used as a source of food, traditional medicine and other household uses (Siangulube, 2007). In traditional medicine, the *B. massaiensis* is used to treat impotence, infertility, wounds, sores, body measles, dizziness, and haemorrhages during pregnancy and birth (Keroletswea, et al., 2018). A study by Kapingu and Magadula (2008) has analysed phytochemicals on the genus *Baphia* and revealed the presence of terpenoids, iminosugars (iminosaccharide), flavonoids, flavonoid glycosides, isoflavonoid glycosides, isoflavonoids, pterocarpans, pigments, daidzein, phenolics, xanthones, and benzofuran. These phytochemicals possessed wound healing, antipyretic, antimicrobial and anti-inflammatory properties (Keroletswea, et al., 2018).

The *C. gynandra* is another wild vegetable documented mainly used as a leafy vegetable in Kabbe constituencies. In a similar study carried out in South Africa, the *C. gynandra* was reported used in herbal medicine to treat diseases such as rheumatism, piles, thread worm infection, conjunctivitis, convulsions, malaria, tumour, fever, headaches, and prevent sepsis when applied on the surface of wounds (Sowunmi & Afolayan, 2015). Moreover, *C. gynandra* was reported also in India as a well-known medicinal plant with anti-inflammatory, antioxidant, analgesic, disinfectant, and antiseptic properties (DeepaShree & Gopal, 2014). According to

Anbazhagi et al. (2009), the *C. gynandra* have insecticidal, antifeedant, and repellent properties. This can be due to the presence of phytochemicals such as flavonoids, alkaloids, steroids, terpenoids, phenols, cardiac glycosides, tannins and saponins (Harborne & Williams, 2000; Olufunmiso & Afolayan, 2011). Another different wild leafy vegetable reported was *T. arnotii*. Fritts et al. (2018) also reported the culinary uses of *T. arnotii*. It is commonly eaten raw to treat thirst since it contain high concentration of water and it can be used as ingredients to make green salad.

The leaves and barks of *Eucalyptus* sp. were reported to be added to food during cooking as food supplement or as herbal medicine to treat symptoms of coughs, colds, and congestion. Vecchio et al. (2006) reported similar ethnobotanical uses. According to this study, the *Eucalyptus* sp. has been used as herbal plant as well as seasoning for thousands of years, due to essential oil found in leaves, fruits, buds and bark. The *Eucalyptus* sp. was also reported used to control algae proliferation in an aquatic milieu, in addition to pharmacological and medicinal properties such as antiperiodic, antiphlogistic, antiseptic, astringent, deodorant, anthelmintic, diaphoretic, expectorant, inhalant, insect repellant, rubefacient, sedative yet stimulant, suppurative, and vermifuge (Dixit, et al., 2012; Zhao, et al., 2019). The study by Ghisalberti (1996) also reported the traditional uses of *Eucalyptus* spp. in food preservative and treatment of respiratory tract infections.

The *H. fuscus* was another leafy vegetable documented in Kabbe constituencies used to treat diarrhoea, mouth and throat sores in addition to being eaten as relish. Studies by Bunalema et al. (2014) and Gumisiriza et al. (2019) also documented the therapeutic uses of *H. fuscus* in traditional medicine. The *H. fuscus* was reported in these studies used to treat tuberculosis, splenomegaly, kidney disease, obstructed labour, febrile seizures, pleurisy and fracture in Uganda. Another study by Kakudidi

(2004) also reported additional ethnobotanical uses of *H. fuscus* during childbirth, court cases and in religion/shrines in the Western Uganda. Similarly, in Tamil Nadu of India, *H. fuscus* was reported used to boost low blood pressure, treat mental illness as well as headache (Prabhu, et al., 2014).

The culinary and therapeutic uses of *L. leptocarpa* recorded in the present study were unique to the Kabbe constituencies and to the best of our knowledge; this was the first report in this aspect. However, the medicinal and ornamental uses of other plant species in the *Ludwigia* genus were reported before (Hernández & Walsh, 2014). A study by Oyedeji et al. (2011) reported numerous compounds, such as saponins, tannins, polyphenols, alkaloids, linoleic acids, flavonoids, starch grains, and calcium oxalate crystals, responsible for the genus culinary and therapeutic properties.

The *M. ovalifolia* has exceptional medicinal and nutritional properties in Kabbe constituencies. Similar culinary and therapeutic uses were reported in the studies by Ananias (2015) and Saini et al. (2016). These studies assessed the traditional uses of *M. ovalifolia* with the phytochemical compounds found in its plant parts. The common phytochemical compounds found in *M. ovalifolia* are alkaloids, carotenoids, tannins, anthraquinones, anthocyanins, proanthocyanidns, phenolic acids, flavonoids, phytosterols and organic acids (Singh, et al., 2019). These phytochemicals provide several health benefits including antiproliferation, hepatoprotective, anti-inflammatory, antinociceptive, antiatherosclerotic, antioxidant, antiperoxidative, cardioprotective, anti-aging, detoxifying agent against snake and scorpion bites, antimicrobial and coagulant properties (Ma, et al., 2018). Moreover, the *M. ovalifolia* was also recorded rich in vitamin A, vitamin C, calcium, potassium, iron, as well as protein (Ananias, 2015). These essential nutrients help to prevent diseases. Most of all,

additional uses of *M. ovalifolia* in water purification and biodiesel production were reported (Jee & Jee, 2018).

The *Morus* sp., *Cucurbita* sp. and *Capsicum* sp. were reported used in culinary to enhance tastes, preserve food and prevent diseases. To support the uses of *Morus* sp. documented, the genus *Morus* is considered a potential source for curing debilitating diseases and phytochemicals (Ramesh, et al., 2014). The *Cucurbita* sp. are generally considered rich in essential nutrients such as protein, fibres, minerals, polyunsaturated fatty acids and phytosterols (Mujaffar & Ramsumair, 2019). Therefore, they are regarded as valuable nutraceutical, with beneficial effects on blood glucose level, immunity, cholesterol, liver, prostate gland, bladder, depression, learning disabilities and parasite inhibition (Patel, 2013). The culinary uses of *Capsicum* sp. are in agreement with the study by Gurnania et al. (2016). The study discussed its agricultural and economic importance in terms of food flavouring, colouring, nutritional and medicinal value. These uses are due to phytochemicals such as capsaicinoids, carotenoids, and phenolic compounds, which give *Capsicum* sp. hot sensory and spicy taste (El-Massry, et al., 2008).

5.4 Methods of Preparation

This study reported the traditional methods utilized for the preparation of plants for culinary and/or therapeutic uses (**Figure 8**). Most plants were reported traditionally prepared by infusion only (52 %) followed by either decoction or infusion (39 %). Similar results were also reported in a study conducted in the Eastern Cape, South Africa (Asowata-Ayodele, et al., 2016). According to literature, plant parts prepared by infusion are usually immersed in an amount of boiling water for a short period and then filtered through a filter or allowed to remain suspended in the solvent over time (Thakur, et al., 2011). This is typically used for delicate herbs, leaves and fresh tender

plants to prevent evaporation of chemical compounds or flavours from plant material due to high temperature (Raghavan, 2006).

On the other hand, some studies reported decoction as the common and preferred method of preparation for culinary and/or therapeutic uses (Grønhaug, et al., 2008; Simbo, 2010). Recent study has reported that decoction method is used to extract chemical compounds (water soluble) from tougher and more fibrous plants, barks and roots by boiling the harder woody material longer to soften and release its active constituents (Daswani, et al., 2011). Therefore, the decoction method is used for active ingredients that does not modify with temperature (Wyllie & Ryabchikov, 2000).

5.5 Consensus Factor among Informants

5.5.1 The Use Value (UV) and Frequency Index (FI)

The UV and FI of plant species were considered by using the proportion of positive mentions of plant species for a particular use over the total number of interviews. The highest calculated use value (UV) and frequency index (FI) recorded in this study demonstrated that the leaves of *C. gynandry* in the Kabbe constituencies has the widest potential for its culinary and therapeutic uses (**Table 3**). These indexes demonstrated the relative importance of each plant species in Kabbe constituencies as a reflection of its potential uses either as a culinary herb and/or spice (Sargin, 2015). Other plant species reported with higher UV and FI (above 0.600 and 60.000 respectively) were *A. thunbergii*, *Cucurbita sp.*, *H. mechowii*, and *M. esculenta*. These plant species were reported with exceptional medicinal and nutritional values in Kabbe constituencies.

5.5.2 The Informant Consensus Factor (ICF)

The diarrheal group had the lowest ICF score of 0.62 in comparison with other categories (**Table 4**). This illustrated that diarrheal was less frequently experienced in

comparison to joint/connective tissues pain, kidney infections or stomach-ache that have higher ICF scored. Based on that, the illnesses recorded with high number of ICF were most ordinarily observed in the Kabbe constituencies (Hedimbi & Chinsembu, 2012). For instances, the high ICF scored in joint/connective tissues pain, kidney infections, and stomach-ache categories indicate high occurrence of such diseases. These could be due to microbial infections. The study areas are situated is a tropical area, with high temperatures and much rainfall during the December-to-March rainy season, and the terrain is mostly made up of swamps, floodplains, wetlands and woodland making it easier for microbial to spread and cause infections (Chinsembu, et al., 2014). The ICF was used to determine the relative homogeneity of the informants' knowledge in terms of categories of diseases for which the plant species are traditionally used for (Asowata-Ayodele, et al., 2016).

5.5.3 The Fidelity level (FL)

The *A. digitata*, *A. thunbergii*, *A. senegalensis*, *Capsicum* sp., *C. tridens*, *Cucurbita* sp., *Eucalyptus* sp., *F. exasperate*, and *M. ovalifolia* had highest FL values above 70.0 % for being used against given culinary and/or therapeutic categories (**Table 5**). Based on the results of the present and other studies, folk knowledge of plants traditionally used as herbs and spices is still alive in the studied areas (Ugulu & Baslar, 2010). Therefore, the present study encourage the documentation of traditional practices and lifestyles of the Kabbe community, so that it can be sustained for the young and future generations to learn from it (Negi, et al., 2010). If not, this knowledge will pass and lost.

5.6 Disc Diffusion, MIC and IC_{50}

The DCM:MeOH barks (T013b) and roots (T013c) extracts of the *A. digitata* showed strong inhibition strength at the concentration of 200 mg/mL against *C. albicans*.

Braca, et al. (2018) implied that this could be due to the presence of phytochemicals such as tannins, flavonoids, alkaloids and saponins in *A. digitata* leaf, bark and root extracts. In agreement with this study, similar findings were observed by Masola et al. (2009) broad-spectrum bacterial and fungal activity. The same study reported that the *A. digitata* stem and root extracts had significant antibacterial activity and their MICs ranged from 6 to 1.5 mg/mL. Moreover, the stem and root barks of *A. digitata* has shown to contain bioactive constituents such as tannins, phlobatannins, terpenoids, cardiac glycosides and saponins which are responsible for antimicrobial activity (Wickens, 2010). Therefore, there is a strong relationship between the phytochemical compounds found in the *A. digitata* with the ethno-medicinal uses in traditional systems of medicine, antimicrobial, antioxidant, hepatoprotective effect, cardioprotective, antidiabetic, as well as antitumor actions (Rahul, et al., 2015).

The alcohol extracts of the *A. thunbergii* showed negative inhibition strength at 200 mg/mL against *E. faecalis* and *E. coli*. Al-Mamun et al. (2016) previously reported similar results against *S. aureus, B. subtilis, E. coli, P. aeruginosa,* and *S. typhimurium* for methanolic extracts from *A. lividus* and *A. hybridus* at 50 μg/disc. The study reported that none of these bacterial species showed susceptibility to the extracts of *Amaranthus* spp. (*A. lividus* and *A. hybridus*), despite having strong antioxidant, lectin and anti-proliferative activity on Ehrlich's ascites carcinoma (EAC) cells. Another study reported poor inhibition strength ranging between 129 and 755 mg/mL exhibited by *A. hybridus*, *A. spinosus,* and *A. caudatus* extracts against *S. aureus, Bacillus* spp., *E. coli, S. typhi, P. aeruginosae, P. mirabillis, K. pneumonia* and *C. albicans* (Maiyo, et al., 2010). Peter and Gandhi (2019) reported that the chloroform, ethanolic, and aqueous leaf extracts of *A. viridis* displayed the same antimicrobial activity against *Bordetella bronchisseptica, Micrococcus flavus, Sarcina lutea, B. pumilus, S. aureus,*

P. vulgaris, *B. subtilis*, *B. cereus*, *E. coli*, and *P. vulgaris*. Data on the antimicrobial activity of other *Amaranthus* spp. was acquired to provide understanding of negative inhibition strength for DCM:MeOH *A. thunbergii* extracts at 200 mg/mL against test microorganisms. Since the antimicrobial activity of *A. thunbergii* is poorly documented, reports on antimicrobial activity of other *Amaranthus* spp. have indicated that most possess antimicrobial activity above 129 mg/mL (Al-Mamun, et al., 2016). Because of these, *A. thunbergii* could be could be concluded to have poor antimicrobial activity.

The aqueous extracts of *B. massaiensis* at 200 mg/mL showed strong antimicrobial activity (inhibition zones ranging between 18.7±0.471-19.7±2.494 mm) against *E. coli*, *B. cereus* and *P. aeruginosa* in comparison to alcohol extracts. Keroletswe et al. (2017) reported similar findings. They documented the antimicrobial activity of fatty acids methyl esters in the seed oil of *B. massaiensis*. They were ranging between 10-16 mm inhibition zone against *E. coli*, *S. aureus* and *B. subtilis*. They argued this could be due to the presence of terpenoids, imino sugars, flavonoids, flavonoid glycosides, isoflavonoid glycosides, isoflavonoids, pterocarpans, pigments, daidzein, phenolics, xanthones, benzofuran, linoleic acid and linolenic methyl esters in *B. massaiensis*. In comparison with other species of *Baphia*, the antimicrobial activity of *B. nitida* dyes was evaluated against *B. cereus*, *S. aureus*, *E. coli*, *P. vulgaris* and *P. aeruginosa* (Agwa, et al., 2012). Their results authenticated *B. massaiensis* plant extract activity against *B. cereus* (18.60-19.20 mm zones of inhibition) and *S. aureus* (16.3-18.00 mm), *E. coli* (14.00-15.60 mm), *P. vulgaris* (12.2-14.00 mm) and *P. aeruginosa* (10.40-12.80 mm). This can be due to the resistance pattern and mechanism of microbial strains, which has been associated with foodborne diseases outbreak across the globe (Amrita, et al., 2009).

On another distinction, the *Morus* sp. aqueous extracts showed negative inhibition strength on average at 200 mg/mL against *S. aureus*, *B. cereus*, *L. monocytogenes*, *E. faecalis* and *S. typhimurium*. These results are in agreement with other studies on *Morus* sp. (Fukai, et al., 2005; Niratker, et al., 2015; Wasoh, et al., 2017). The aqueous extract of *M. indica* showed antibacterial activity against *E. coli* (16 mm) and *B. anthracis* (12 mm) (Jagtap & Chavan, 2016). The results suggested that the Gram-positive bacteria were more susceptible than the Gram-negative bacteria to the *Morus* sp. aqueous extracts.

Above all, the inhibition zone values of organic extracts were more effective in comparison with those of aqueous extracts (Independent-Sample Mann-Whitney U Test: $p = 0.000$) (**Table 8**). This is because the combination of DCM and MeOH has higher polarity than water. Therefore, it concentrates and extracts more bioactive compounds than water, which mostly disperse active compounds that are responsible for antibacterial activity (Barchan, et al., 2014). Although this might be true, the agar disc diffusion methods alone cannot be reliable. This is because they are strongly influenced by agar depth, diffusion rate of the antimicrobial agent and growth rate of the specific microorganisms (Lehtopolku, et al., 2012).

The alcohol extracts of *C. gynandra* had the lowest MIC and IC_{50} values against *B. cereus, E. faecalis, E. coli, S. typhimurium, P. vulgaris,* and *C. albicans*. Previous studies have discussed the relationship between the antimicrobial activities and phytochemicals in *C. gynandra* (Anbazhagi, et al., 2009; Olufunmiso & Afolayan, 2011). It was supported that *C. gynandra* has high concentrations of polyphenolic, flavonoids, proanthocyanidin, flavonols, alkaloids, steroids, terpenoids, phenols, cardiac glycosides, tannins, saponins, and phenols contents. These phytochemicals might be responsible for antimicrobial activity of *C. gynandra*.

The aqueous extracts of *Eucalyptus* sp., *B. massaiensis* and *A. digitata* showed the lowest MIC and IC_{50} values against *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *P. vulgaris*, and *C. albicans*. Our results are in agreement with recent scientific studies which explained the contributions of phytochemical compounds such as terpenoids, tannins, flavonoids, phloroglucinol derivatives, imino sugars, flavonoid glycosides, isoflavonoid glycosides, isoflavonoids, pterocarpans, pigments, daidzein, phenolics, xanthones, and benzofuran to antimicrobial properties of these plants (Agwa, et al., 2012; Sabo & Knezevic, 2019). According to these studies, these compounds have moderate activities against both Gram positive and Gram bacteria, and good activity against *C. albicans*. In comparison with literatures, other antimicrobial studies have reported the alcohol extracts of *A. digitata* root, bark and leaves to have strong antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis, Bacillus subtilis*, *Escherichia coli* and *Mycobacterium phlei* than water extracts (Ananil, et al., 2000). The methanolic root extracts of *A. digitata* have also been reported with antitrypanosomal activity against *Trypanosoma congolense* and *T. brucei* (Atawodi, et al., 2002). This could be due to extraction method and polarity of solvent used.

5.7 Kill-Time Study

The kill-time studies were performed to get a better understanding on the microbistatic and microbicidal effects of the plant extracts exhibiting the strongest antimicrobial activity (6.25 mg/mL). In the present study, a standardised *in vitro* kill-time curve assays were developed (**Figure 9-10**). The resulting data were analysed using a pharmacodynamic model that describes the relationship between the concentration of antimicrobials and the bacterial growth rate (Foerster, et al., 2016). Based on pharmacodynamic model, our results showed that Gram-negative bacteria were killed in shorter period in comparison with Gram-positive bacteria (Independent-Sample

Mann-Whitney U Test: $p = 0.000$). The DCM:MeOH extracts of *Eucalyptus* sp., *Morus* sp. and *N. lotus* showed microbicidal effects for *C. albicans*, *S. typhimurium*, *E. faecalis* and *S. sonnei* after 2 hours of exposure and counts remained undetectable until 24 hours of exposure. In accordance with our results, previous studies have indicated that the alcohol extracts of *Eucalyptus* species (*E. bicolor*, *E. griffithsii*, *E. camaldulensis*, *E. incrassate*, *E. torquata* and *E. microtheca*) and *Morus* spp. (*M. alba* and *M. nigra*) exerted its maximum microbicidal effect after 10-15 and 110 minutes of exposure, respectively (Ambrosio, et al., 2018; Hendry, 2011; Minhas, et al., 2016; Naz, et al., 2018). These effects were maintained throughout 24 hours of incubation. The *Capsicum* sp., *C. gynandra* and *Eucalyptus* sp. extracts displayed moderate bactericidal effects against *E. faecalis*, *P. vulgaris*, *E. coli*, and *S. sonnei*. On the other side, the aqueous extracts of *A. digitata* and *A. senegalensis* showed microbistatic effects against *S. aureus*. The *Eucalyptus* sp. barks and *B. massaiensis* roots extracts had no effects on the numbers of viable *C. albicans*, and *S. typhimurium* cells after 24 hours of treatment. These results confirmed the evidence in previous studies that alcoholic solvents such as DCM:MeOH are more suitable than aqueous solvents in extracting components of plants that are responsible for antimicrobial activities (Gberikon, et al., 2015).

5.8 Time-Kill Synergy Study

The time-kill synergy study was performed to assess the *in vitro* interaction of plant extract combinations and determine whether their combination activities was greater than the sum of their individual (Doern, 2014). This combinational approach is preferred over monotherapy, especially in multiple life-threatening infectious diseases due to its ability to target multiple facets of a disease and to curb resistance (Cheesman, et al., 2017). Antimicrobial combinations can act either as additively, synergistically

or antagonistically (Bonapace, et al., 2000). They act additively when the cumulative antimicrobial effect is simply the sum total of the two antimicrobials acting together; synergistically when the combined activity is greater than the sum of their activities when used individually; or antagonistically when the combined activity is less than the sum of their activities when used individually (Sopirala, et al., 2010).

The present study did not report any combination of plant extracts that exerted synergistic effects on all nine test microbial culture (**Table 14**). However, twenty-three synergy effects were observed in some combinations against *S. cerevisiae, S. typhimurium, S. aureus*, *P. vulgaris*, *P. aeruginosa*, *E. coli,* and *C. perfringens*. The time-kill method is regarded to be a reliable predictor of *in vivo* synergy (Bae, et al., 2016). However, it possesses some challenges such as lack of accepted standards for synergy testing, time-consuming and labour-intensive laboratory procedures (Kumar, et al., 2017). Also, the time-kill method has shortcomings with the effect of inoculum size, the difficulties in interpretation of results due to insufficient literature on antimicrobial synergy, and the reliance on the reading at one-time point (usually 24 hour) as the sole determinant of the interaction (White, et al., 1996).

5.9 Evaluation of Mechanism of Action

The antimicrobial mode of action of *C. gynandra*, *Eucalyptus* sp., *Morus* sp. and *N. lotus* were confirmed based on leakage of the electrolytes and absorption of material cell at 260 and 280 nm from *B. cereus*, *C. albicans, E. coli*, and *S. typhimurium* cells. The microbial cells were exposed to DCM:MeOH extracts at the concentration of 6.25 mg/mL (1 × MIC) and 12.5 mg/mL (2 × MIC). The calculated relative electric conductivity values of both *C. albicans* and *S. typhimurium* when treated with *Eucalyptus* sp. were negative for both 1 × MIC and 2 × MIC concentrations in contrast to control treatment (**Figure 12C**). In much the same way, the relative electric

conductivity values (2 × MIC concentration) for *B. cereus* was negative after 4 hours in comparison to the control treatment when treated with *C. gynandra* (**Figure 13B**). This could be due to hyperpolarization and repolarization, which cause the cell's internal charge become more positive and outside more negative (Watari, et al., 2013). They are often caused by efflux of potassium ion (K^+) out of cells through K^+ channels, or by influx of chloride ion (Cl^-) from the extracellular space into the intracellular space through Cl^- channels (Lutz, et al., 1998).

On the contrary, the relative electric conductivity values of 1 × MIC and 2 × MIC concentrations for *C. albicans* (**Figure 11B**) and *S. typhimurium* (**Figure 12B**) treated with *N. lotus* extracts underwent a shift between 4 and 24 hours. During the first 4 hours, the relative electric conductivity values of concentrations with plant extracts were high in comparison with the control. This could be due to the fact that the tested microbial cells were depolarized by the plant extracts, causing changes within cells which resulted in less negative charge (more positive) inside the cell and more electric conductivity outside (Bortner, et al., 2001). After 4 hours, the *C. albicans* and *S. typhimurium* cells repolarized, and the internal charge returns to a more negative value in comparison to the control treatment. The results observed in present study are similar to those reported by Miksusanti et al. (2008) and Zhang et al. (2017). These studies both explained the ability of antimicrobial agents, such as *Kaempferia pandurata* essential oil, to disrupt the permeability barrier of microbial membrane structures. The disrupted microbial cell membrane affect membrane's permeability and ability to osmoregulate the cell adequately or to exclude toxic materials (Tagousop, et al., 2018).

The absorption of material cell at 260 (**Figure 14-16**) and 280 nm (**Figure 17-19**) from *B. cereus*, *C. albicans*, *E. coli*, and *S. typhimurium* after treated with plant extracts

indicated that there were cell materials that leakage from the tested microbial cells. The 260 nm absorbance was used to measure nucleic acids while 280 nm was used to absorb proteins (Miksusanti, et al., 2008). Both nucleic acids and proteins are key structural components, which reside throughout the interior of the cell and cytoplasm (Wal, et al., 1995). These structural components can be release from the microbial cells if there is an irreversible damage to the cytoplasmic membranes (Bajpai, et al., 2013). Therefore, the more absorption value, the more material cell loss from the cell. The leakage of nucleic acids and proteins cause the disorder of function in the synthesis of proteins and DNA materials as well as inhibition of microbial growth (Field & Field, 2010). This is most likely the cause of microbial death. The exposure of *B. cereus, C. albicans, E. coli,* and *S. typhimurium* to *C. gynandra, Eucalyptus* sp., *Morus* sp., and *N. lotus* suggested that nucleic acids and certain protein were lost through a damaged cytoplasmic membrane. This was observed when the OD_{260nm} and protein concentration values of 1 × MIC and 2 × MIC treatments were higher than that of control treatments.

Previous studies have reported many antimicrobial compounds such as chlorohexidine, hexachlorophene, phenetyl alcohol, tetracycline, and lemongrass oil, which act on the microbial cytoplasmic membrane. They induce the loss of 260 nm absorbing material (nucleic acid) and 280 nm absorbing material (protein) (Miksusanti, et al., 2008; Yamada, et al., 1979). These antimicrobial compounds change the intracellular material, enhance membrane permeability and leakage of the intracellular material (Tagousop, et al., 2018). As a result, more antimicrobial compounds cross the cell membranes into the interior of the cell and interact with intracellular sites, which is critical for antibacterial activity (Zhang, et al., 2017). This

mode of action was reported similar to that of broad-spectrum antibiotics, membrane-active disinfectants and preservatives (Wal, et al., 1995).

5.10 Quantitative Determination of Phytochemical Contents

The phenolic compounds are class of plant secondary metabolites characterized by the presence of several phenol groups (Song, et al., 2010). They have a large diversity of structures, such as simple molecules (Gallic acid), polyphenols (flavonoids) and polymers derived from these various groups (Cheynier, 2012). Moreover, the diversity in structures of phenolic compounds is associated to variety of properties, specific roles and distribution in plants (Minatel, et al., 2017). Phenolic compounds are widely known for their contribution to quality and nutritional value of food (colour, taste, aroma, and flavour) as well as health properties (OliveiraI, et al., 2014). Also, they provide plant defence mechanisms to microorganisms, insects, and herbivores (War, et al., 2012). In humans, phenolic compounds play major roles as antioxidant, anti-inflammatory effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism, antibacterial, and antiviral effects (Maiyo, et al., 2010). For example, anthocyanins are a type of flavonoid responsible for pigments such as red, purple, and blue in plants (Nyambe, 2018). They are responsible for antioxidant activity, fighting free radicals, and may offer anti-inflammatory, antiviral, and anticancer benefits (Wong, 2019).

The leaves extracts of *Eucalyptus* sp. and *Morus* sp. had the highest total phenolic and total flavonoid content (**Table 15**). These results are in agreement with studies by Boulekbache-Makhlouf et al. (2013) as well as Gungor and Sengul (2008) which documented high content of total phenolic and total flavonoid in the extract of *E. globulus* and *M. alba*. The lowest total phenolic and total flavonoid content in the present study was noted for *Capsicum* sp.. In comparison with previous studies, the

total phenolic content and total flavonoid content in *C. annuum* and *C. frutescens* ranged between 7.95–26.15 mg GAE/g) of dry weight of extract (Gurnania, et al., 2016; Salima, et al., 2014). Based on the literature, there is a strong correlation between the total phenolic and flavonoids contents and MIC (Skerget, et al., 2005). In other words, the higher the total phenolic and flavonoids contents, the lower the MIC values.

6. CHAPTER SIX: CONCLUSIONS

This study contributed scientific information about the ethnobotanical usage of plants as herbs and spices in Kabbe constituencies of the Zambezi region. Although there was poor rainfall experienced during the November 2018/March 2019 rainy season, 23 plants were collected for taxonomic identification of which 17 collected and tested for antimicrobial activity. Based on antimicrobial activity observations of this study, some plant parts from *C. gynandra* (Sishungwa), *Eucalyptus* sp. (Kapulanga), *Morus* sp. (Murobeni) and *N. lotus* (Lisoto) had effective antimicrobial agents against food pathogens in individual and combined forms with MIC and IC_{50} range between 5-6.25 mg/mL ($p < 0.05$). This was in agreement with the time-kill synergy study, which indicated 12.2 % synergy activity. Also, the antimicrobial mode of action of *C. gynandra, Eucalyptus* sp., *Morus* sp. and *N. lotus* confirmed leakage of relative electric conductivity, nucleic acids and proteins through an irreversible damage to the cytoplasmic membrane of tested foodborne pathogens at 4, 8, 12 and 24 hours of treatment ($p < 0.05$), similar to those of broad-spectrum antibiotics. Quantitative analysis of phytochemical contents revealed that the higher the total phenolic and total flavonoids contents, the stronger the strength antimicrobial activity ($p < 0.05$). Therefore, this highlighted strengths in using natural antimicrobial compounds, including extracts of herbs and spices, to replace synthetic chemical compounds used in food preservation and modern medicine. The documented results in the present study indicate that plant extracts from Kabbe constituencies can serve as natural preservatives to control foodborne pathogens. This important because these plant extracts can be used as substrates in the formation of new antimicrobial agents, functional foods or nutraceuticals.

7. CHAPTER SEVEN: RECOMMENDATIONS

In future, more ethnobotanical surveys are recommended to document and evaluate the antimicrobial activity of edible herbs and spices used in different constituencies of Zambezi region, and the rest of Namibia at large. Detailed evaluation of mode of actions of the collected plant species against foodborne pathogens using scanning and transmission electron microscopes are needed, in order to understand better the full mechanisms of the antimicrobial activity observed and effect on microbial cell organelles. In addition, further studies are needed to determine and characterize the bioactive compounds of edible herbs and spices documented from this study. Toxicity studies are also needed to support the safe usage of these plants as food additives and natural preservatives for food safety applications. Last but not least, the community in Kabbe constituencies are recommended to increase cultivation, marketing and usage of these plants extracts, in order to improve the primary health, socio-economic and employment creation in Zambezi region.

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9. APPENDICES

Appendix 1: Prior Informed Consent Agreement Form

PRIOR INFORMED CONSENT AGREEMENT

ETHNOBOTANICAL SURVEY AND *IN VITRO* EVALUATION OF ANTIMICROBIAL ACTIVITY OF SELECTED TRADITIONAL HERBS AND SPICES FROM KABBE CONSTITUENCIES IN ZAMBEZI REGION

Researcher: Abner Tomas, Master of Science student, Department of Biological Sciences, Faculty of Science, University of Namibia, Cell: 0812075522, Email: option356@yahoo.com

Supervisor: Dr. Ahmad Cheikhyoussef, Science and Technology Division, Multidisciplinary Research Centre, University of Namibia, Tel. 0612063283, Cell: 0813516357, Email: acheikhyoussef@unam.na

Co-Supervisor: Prof. Kazhila C. Chinsembu, Department of Biological Sciences, University of Namibia, Tel. 0612063426, Cell: 0814393517, Email: kchinsembu@unam.na

Dear Participant

We would appreciate your assistance with this research project on the Ethnobotanical Survey and *in vitro* Evaluation of Antimicrobial Activity of Selected Traditional Herbs and Spices from Kabbe Constituencies Zambezi Region.

The objectives of this study are:

1. To conduct an ethnobotanical survey of indigenous knowledge of traditional herbs and spices used in Kabbe constituencies of Zambezi region.
2. To screen *in vitro* antimicrobial activity of different traditional herb and spice extracts used in Kabbe constituencies from Zambezi region against selected food-borne pathogens.
3. To determine whether any synergistic effects existed for various combinations of traditional herb and spice extracts used in Kabbe constituencies from Zambezi region against selected food-borne pathogens.

Therefore, we would like to suggest a financial protection agreement for all Zambezi region herb and spice extracts studied to declare that there are no financial gains will come from this project. The project and results thereof, should only be used for educational and academic purposes only.

Confidentiality will be maintained throughout the research stages. The results will be provided to all parties upon request to supervisors of this project.

Consent

I have read and I understand the provided information and have had the opportunity to ask questions and get all clarification needed. I also understand that I will get a copy of this consent form and agreed to take part in this study.

Participant's Name and Address:

_____
_____
_____
_____
_____
_____

Participant's Signature: _____ Date: _____

Researcher's Signature: _____ Date: _____

Supervisor's Signature: _____ Date: _____

Appendix 2: Ethical Confidentiality Agreement Form

CONFIDENTIALITY AGREEMENT

Title of Research Project: Ethnobotanical survey and *in vitro* evaluation of antimicrobial activity of selected traditional herbs and spices from Kabbe constituency in Zambezi Region

I, _____, Identity number: _____
hereby take this oath of confidentiality for the above mentioned research study at the University of Namibia in the Department of Biological Sciences as Master of Science research project of the Master of Science in Microbiology.

By signing this agreement I, the undersigned, acknowledge, understand and agree to adhere to the following conditions:

- I will maintain the privacy and confidentiality of all accessible project data, which will only be accessed and utilized for the purpose for which I am authorised.
- Plants identity will be protected by use of a number and letter combination so as to prevent identification of subject.
- I will store subject information under lock and key of which I will have sole access.
- I will not disclose data or information to anyone other than those to whom I am authorised to do so.

Signature: ----------

Date: ----------

Appendix 3: Open-Ended Questionnaire

DATA COLLECTION FORM

BACKGROUND INFORMATION

This data acquisition questionnaire form is for the **Ethnobotanical survey and *in vitro* evaluation of antimicrobial activity of selected traditional herbs and spices from Kabbe constituencies in Zambezi Region** research project in fulfilment of the requirements for the degree of Master of Science in Microbiology of the University of Namibia.

PART 1: INFORMANTS DETAILS

Name of Interviewee		Date (DD/MM/YY)	
Gender (*Male/Female*)		Location/Residence	
Age in Years (*Optional*)		Ethnic group	
Level of education		Occupation (*Optional*)	

PART 2: DESCRIPTION OF HERBS AND/OR SPICES

Local name of plant	**Plant parts used**	**Voucher Number**	**Photo Number**	**Dosage use – Quantity, How often?** (*If medicinal*)	**Use(s) of the Herb/Spice**	**Preparation method(s)** (*dry or fresh parts*)	**Mode of Administration** (*e.g. rubbing, drinking*)	**Occurrence** (*e.g. common*)

PART 3: RESEARCHER DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual property of the Zambezi region community.
2. The respondents will be informed of the intended project elaborately prior to questionnaire administration and in confidential to eliminate any degree of conspiracy.
3. The information collected will be used for the described research purpose and not any undisclosed intentions.

Appendix 4: Research Permit from National Commission on Research, Science and Technology (NCRST)

NCRST
NATIONAL COMMISSION ON RESEARCH SCIENCE & TECHNOLOGY

AUTHORIZATION OF RESEARCH PROJECTS

Authorization is hereby granted in terms of section 21 of the RST Act No. 23 of 2004, to:

Name: UNIVERSITY OF NAMIBIA (UNAM)

Address: 340 Mandume Ndemufayo Avenue, Pionerspark
Windhoek,
Namibia

Coworkers: Abner Tomas, Dr. Ahmad Cheikhyoussef, and Prof. Kazhila C. Chinsembu.

Certificate Number (if applicable): RCIV00022018 **Authorization No:** AN20181109

Type of research

Non Commercial research and the use of the resources be limited to what is specified in the proposal

Title of Research authorized:

Ethnobotanical survey and in vitro evaluation of antimicrobial activity of selected traditional herbs and spices from Kabbe constituency in Zambezi region

Locality:

Kabbe Constituency of Zambezi region

Duration: 15 February 2019- 28 February 2020

Research/Sample collection conditions:

Prior informed consent must be obtained in writing from community representatives in the areas of the research and acknowledge that the knowledge remains the property of the community and it will not be shared and used without the consent of the community.

Yours sincerely,

National Commission on Research Science & Technology
15 Feb 2019
P/Bag 13253
Windhoek, Namibia
CEO Office

Ms. Enid Keramen
Acting Chief Executive Officer

Head Office:
ERF 490, Platinum Street - Prosperita, Windhoek
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+ 246 61 235 758

RESEARCH/COLLECTING PERMIT CONDITIONS

****

Specific Conditions

1. Prior informed consent must be obtained in writing from community representatives in the areas of the research.
2. The specimens and their derivatives may be used for the purposes of this study only and may not be patented, commercialised, donated or sold to a third party without the written consent of the Ministry of Environment and Tourism.
3. The traditional knowledge accessed remains the property of the community and shall not be shared and used without the consent of the community. Where benefits are derived from knowledge of the communities, these benefits are to be shared in a fair and equitable way with the communities.
4. For *Sesamothamnus guerichii*, a protected species, permission is granted to collect leaves not exceeding 500g.
5. For export of biological and genetic resources and their derivatives, an export permit should be obtained from the Ministry of Environment and Tourism.
6. Digital sequencing of genetic information of plants and their derivatives is strictly prohibited under this project.
7. All applicants and institutions cited in the applications are subjected to these terms and conditions.

The research permit does not exempt the holder(s) from complying with any other requirements under the Access to Biological and Genetic Resources and Associated Traditional Knowledge Act (Act No. 2 of 2017), its Regulations or any other legislation

General Conditions

1. You must report to the Park Chief warden and / or Regional Office of the Ministry of Environment and Tourism prior to arrival in fieldwork area, and must present your permit.
2. This permit does NOT entitle the holder to free entry to the protected areas or state land outside protected areas.
3. For Field work in National Parks you have to make arrangement with park management in advance prior to arrival in fieldwork area.
4. Voucher specimens should be deposited with National Museum of Namibia.
5. If you would like to export samples of specimens you must loan them from the National Museum of Namibia.
6. To conduct research work in the rhinos and elephants range all persons listed on the permit must be in possession of a police clearance certificate.
7. The permission of the land owner / communal Authority is required to work/collect on private lands/communal areas.
8. The permission of the concession holder is required to work/collect in concession areas.
9. No commercial filming will be permitted without prior approval by the Ministry of Environment and Tourism under this permit.
10. Duplicates of publications and / or final report should be made available to the Ministry of Environment and Tourism and also the final report.
11. The specimens and their derivatives may be used for the purposes of this study only and may not be patented, commercialised, donated or sold to a third party without the written consent of the Ministry of Environment and Tourism.
12. All results (raw materials) or technology derived directly or indirectly from this research must be made available free of charge without reservations to the Ministry of Environment and Tourism.
13. A report on the work conducted under this permit must be submitted to the Ministry of Environment and Tourism not later than one month after the expiry of this permit as well as to regional office in whose area research was conducted.
14. Applications for renewal of this permit must reach this office at least three months prior to the expiry of this permit.
15. Habitat destructive collecting methods must not to be used.
16. Veterinary restriction may apply in the case of movement of samples and it is the applicants' responsibility to obtain such permits.
17. Foreign (or destination) wildlife import, and veterinary import permits may be required.
18. CITES import permit from the country of the destination is required for the application of export permit for CITES – listed species.
19. All field teams must be in possession of the permit and permit copy must accompany the transport of specimens.
20. You are subject to all conditions listed on the entry permit to any of the protected areas, unless specifically exempted.
21. Failure to adhere to the conditions will lead to cancellation of the research permit.
22. It is your responsibility to make the necessary contacts and arrangements as specified above.

Appendix 5: Authorization to Conduct Research in Kabbe North Constituency

Republic of Namibia
Zambezi Regional Council

Tel: + 264-66-261700
Fax: + 264-66-254579

Ngoma Street
Govt Building

Private Bag 5002
Katima Mulilo

Enquiries:Ms R.M.SIMATAA

14 March 2019

Kabbe North Constituency

TO: WHOM IT MAY CONCERN

This is to certify that Mr. Abner Tomas, Ms. Bianca Masule and Mr. Nawa were conducting an ethnobotanical survey on traditional herbs and spices used by the Community of Kabbe North Constituency, on the 14th march 2019. In fulfilment of the requirement for the degree of Master of Science in microbiology of the university of Namibia.

We look forward to work together as usual. For any queries do not hesitate to contact the above office.

Yours truly,

ZAMBEZI REGIONAL COUNCIL
Kabbe North Constituency
Office of the Councilor
2019 -03- 14
Private Bag 5002
Katima Mulilo
REPUBLIC OF NAMIBIA

HON.MWALA PETER MWALA
KABBE NORTH CONSTITUENCY
ZAMBEZI REGION

Appendix 6: Plants Identification Reports from National Herbarium of Namibia

Ministry of Agriculture, Water and Forestry

National Herbarium of Namibia (WIND)

Identification Report

Report No.: 2019/400

29 January 2019

Collector/s: Mr. A. Tomas
Address: University of Namibia
P O box 787
Ondangwa

Number	ID cat.	Identification
T 020	1	Amaranthus thunbergii Moq.
T 014	1	Ficus sycomorus L.
T 017	1	Ximenia americana L.
T 013	1	Adansonia digitata L.
T 012	1	Diospyros lycioides Desf. subsp. sericea (Bernh.) De Winter
T 001	1	Combretum imberbe Wawra
T 018	1	Diospyros mespiliformis Hochst. ex A.DC.
T 016	1	Diospyros chamaethamnus Dinter ex Mildbr.
T 002	1	Sclerocarya birrea (A.Rich.) Hochst. subsp. caffra (Sond.) Kokwaro
T 009	1	Dichrostachys cinerea (L.) Wight & Arn. subsp. africana Brenan & Brummitt
T 003	1	Kigelia africana (Lam.) Benth.
T 006	1	Schinziophyton rautanenii (Schinz) Radcl.-Sm.
T 010	1	Baikiaea plurijuga Harms
T 004	1	Acacia erioloba E.Mey.
T 008	1	Baphia massaiensis Taub.
T 011	1	Commiphora glandulosa Schinz
T 019	2	Hibiscus mechowii Garcke
T 007	1	Terminalia sericea Burch. ex DC.
T 015	1	Pterocarpus angolensis DC.
T 021	3	Morus
T 005	3	Eucalyptus

Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify

Private Bag 13184, Windhoek Tel: +264 - 61 - 202 - 2021 Fax: +264 - 61 - 259 - 153 e-mail: Frances.Chase@mawf.gov.na 1

Ministry of Agriculture, Water and Forestry

National Herbarium of Namibia (WIND)

Identification Report

Report No.: 402

10 May 2019

Collector/s: Thomas, A.
Address: UNAM
P O Box 787
Ondangwe

Number	ID cat.	Identification
TO 22	1	Moringa ovalifolia Dinter & A.Berger
TO 23	1	Amaranthus hybridus L. var. hybridus
TO 24	1	Corchorus tridens L.
TO 25	1	Nymphaea lotus L.
TO 26	2	Ipomoea batatas (L.) Lam.
TO 27	2	Cucurbita sp.
TO 28	1	Manihot esculenta Crantz
TO 29	1	Amaranthus thunbergii Moq.
TO 30	1	Capsicum sp.
TO 31	1	Corchorus tridens L.
TO 32	2	Vigna unguiculata
TO 33	1	Amaranthus thunbergii Moq.
TO 34	4	
TO 35	4	
TO 36	2	Morus sp.
TO 37	1	Ficus sycomorus L.
TO 38	1	Manihot esculenta Crantz
TO 39	1	Amaranthus thunbergii Moq.
TO 40	1	Cleome gynandra L.
TO 41	2	Ipomoea batatas (L.) Lam.
TO 42	1	Hibiscus mechowii Garcke
TO 43	1	Ludwigia leptocarpa (Nutt.) Hara
TO 44	1	Talinum arnotii Hook.f.

Identification categories: 1. Certain identification 2. Closest to 3. Certain to genus only 4. Unable to identify

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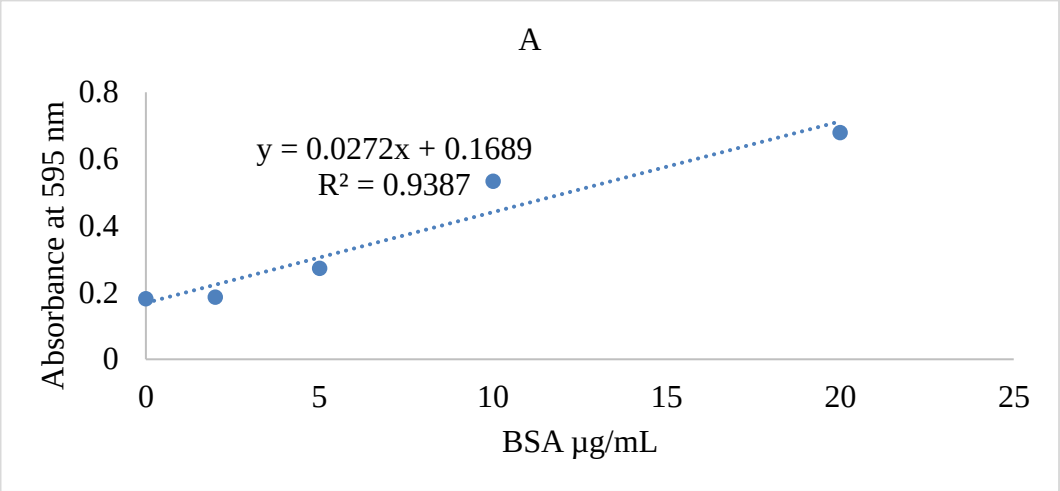
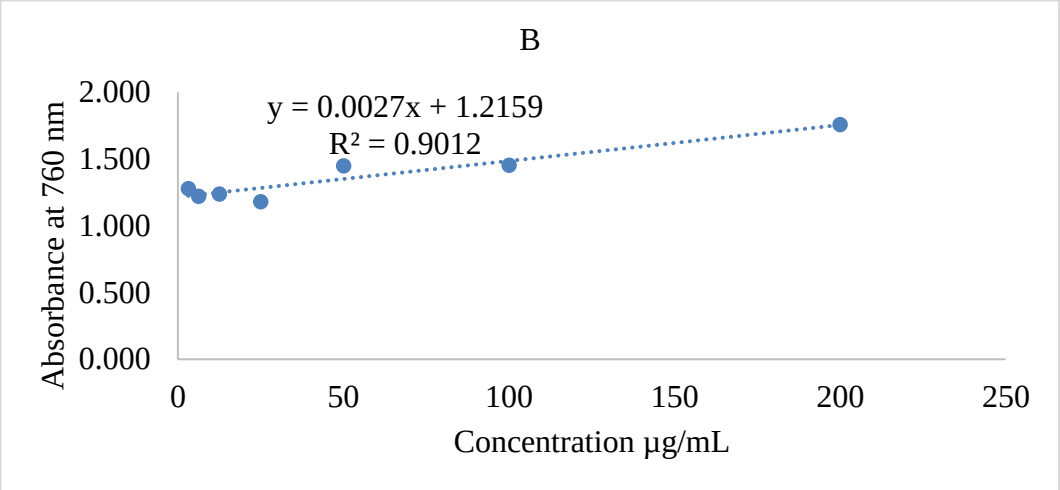
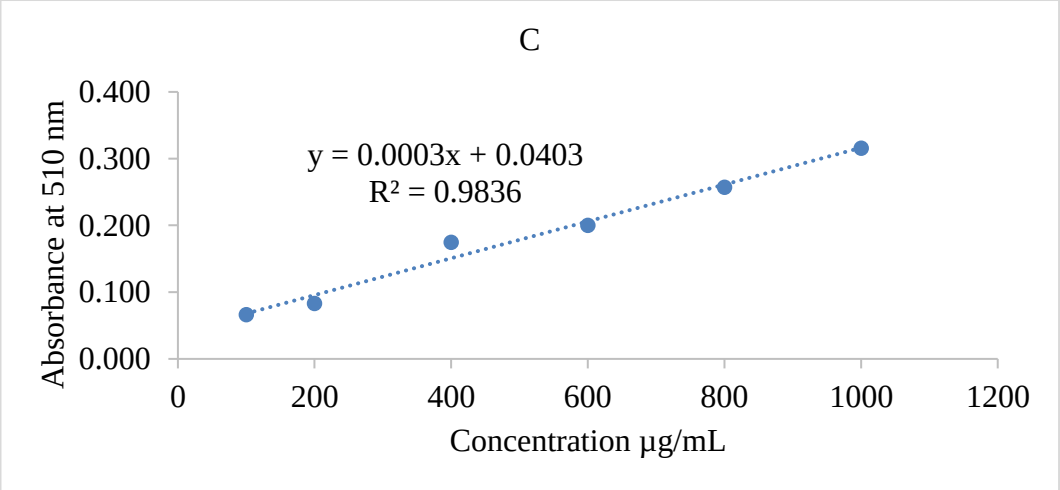
Appendix 7: Standard curves of Gallic acid (A), Catechin hydrate (B), and Bovine Serum Albumin (C)







Appendix 8: Disc diffusion data

8.1 Mean of inhibition zones (mm ± Standard Error) for 1:1 Dichloromethane:methanol extracts

Inhibition zones (mm ± Standard Error)												
Plants Species	*S. aureus*	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
T005a	16.3 ±1.886	18.3 ±2.055	15.0 ±1.414	16.0 ±2.160	15.3 ±2.625	17.7 ±7.409	16.0 ±3.559	18.3 ±3.300	13.0 ±2.828	17.7 ±4.028	20.0 ±5.888	21.7 ±7.364
T005b	18.3 ±1.247	15.7 ±3.091	16.7 ±3.859	20.7 ±2.357	13.7 ±2.357	17.7 ±2.055	19.3 ±1.247	19.0 ±0.816	18.0 ±1.633	20.3 ±3.399	20.7 ±3.091	20.7 ±0.943
T008	10.0 ±2.160	13.7 ±1.247	10.3 ±0.471	12.3 ±3.859	11.7 ±0.943	7 .7 ±1.247	12.3 ±2.625	12.7 ±1.247	10.3 ±2.357	11.7 ±1.700	10.7 ±0.471	12.7 ±0.471
T013a	7.3 ±1.247	11.3 ±5.558	9.7 ±4.497	7.7 ±1.247	10.0 ±2.160	8.7 ±2.055	11.7 ±4.190	9.0 ±3.559	6.7 ±0.943	12.0 ±7.789	10.0 ±2.160	18.0 ±2.944
T013b	18.0 ±2.160	12.3 ±3.682	11.7 ±8.014	7.0 ±1.414	15.3 ±1.700	13.7 ±1.247	16.7 ±1.700	8.3 ±1.700	17.7 ±1.247	13.7 ±1.700	24.0 ±3.559	21.7 ±5.907
T013c	15.0 ±3.559	11.3 ±3.091	11.0 ±6.377	8.3 ±0.471	8.3 ±0.471	10.0 ±2.944	15.7 ±4.190	11.0 ±1.633	15.3 ±2.625	11.7 ±1.886	23.0 ±6.976	18.7 ±5.312
T016a	13.3 ±2.625	12.3 ±0.943	11.3 ±3.091	9.0 ±0.816	9.7 ±0.943	11.7 ±1.886	10.7 ±1.247	17.0 ±5.354	14.3 ±2.625	12.7 ±0.471	13.7 ±2.055	13.7 ±0.943
T016b	11.3 ±2.357	17.3 ±3.771	11.3 ±1.247	15.3 ±3.399	18.7 ±5.437	7.7 ±1.247	11.7 ±4.497	12.3 ±4.497	13.3 ±1.886	14.7 ±2.867	13.3 ±3.399	14.7 ±1.886
T019	8.0 ±0.816	12.7 ±4.922	12.0 ±3.559	9.7 ±2.055	6.7 ±0.471	8.7 ±1.247	10.0 ±1.414	17.7 ±4.784	14.0 ±6.683	15.3 ±4.190	14.3 ±5.437	14.0 ±3.559
T020	9.7 ±2.055	10.7 ±4.497	10.0 ±2.944	8.3 ±0.943	6.0 ±0.000	7.0 ±0.000	8.7 ±1.700	15.3 ±3.682	12.7 ±6.650	14.3 ±0.943	12.0 ±2.160	8.7 ±1.700
T021	11.3 ±1.247	11.0 ±1.414	14.0 ±4.320	9.7 ±1.247	7.7 ±1.247	7.0 ±0.816	12.0 ±2.944	13.7 ±3.682	11.0 ±2.160	11.3 ±0.943	20.3 ±6.018	12.7 ±0.471
T022	15.3 ±0.816	12.7 ±0.816	13.3 ±0.943	14.0 ±0.943	8.0 ±0.471	20.0 ±0.943	18.0 ±0.943	17.0 ±0.943	9.0 ±0.471	18.0 ±0.471	16.0 ±0.471	14.0 ±0.943
T024	15.7 ±0.816	8.7 ±0.816	15.3 ±0.816	9.0 ±0.471	9.0 ±0.471	22.0 ±0.471	22.0 ±0.943	22.0 ±0.943	12.0 ±0.943	17.0 ±0.471	18.0 ±0.471	12.0 ±0.943
T025	13.7 ±0.816	17.3 ±0.943	16.7 ±0.816	13.0 ±0.943	18.0 ±0.471	17.0 ±0.943	14.0 ±0.943	19.0 ±0.943	17.0 ±0.943	16.0 ±0.471	18.0 ±0.943	16.0 ±0.471
T027	16.0 ±1.247	15.3 ±1.633	12.7 ±0.816	17.0 ±0.471	18.0 ±0.943	18.0 ±0.943	15.0 ±0.943	15.0 ±0.943	16.0 ±0.943	20.0 ±0.943	14.0 ±0.471	20.0 ±0.943
T030	13.3 ±0.471	10.3 ±5.437	11.3 ±0.471	13.7 ±1.247	8.3 ±0.471	12.3 ±0.816	16.3 ±0.816	11.3 ±0.471	13.3 ±0.816	13.7 ±1.247	18.3 ±0.816	13.3 ±0.816
T035	12.7 ±0.816	15.7 ±1.700	12.3 ±0.943	11.0 ±0.471	10.0 ±0.471	12.0 ±0.943	12.0 ±0.000	14.0 ±0.943	13.0 ±0.471	14.0 ±0.943	16.0 ±0.471	12.0 ±0.943

T040	13.3 ±0.816	11.3 ±1.247	10.7 ±0.471	13.0 ±0.471	11.0 ±0.471	22.0 ±0.943	8.0 ±0.471	8.0 ±0.471	16.0 ±0.943	19.0 ±0.471	16.0 ±0.943	12.0 ±0.943
T041	15.0 ±1.247	17.0 ±1.247	8.7 ±0.816	12.0 ±0.943	14.0 ±1.414	18.0 ±0.943	15.0 ±0.471	15.0 ±0.471	9.0 ±0.471	17.0 ±0.943	9.0 ±0.000	10.0 ±0.471
T043	14.7 ±1.247	11.3 ±0.943	13.3 ±0.943	13.0 ±0.943	18.0 ±0.943	14.0 ±0.943	12.0 ±0.000	13.0 ±0.471	16.0 ±0.471	14.0 ±0.943	14.0 ±0.471	12.0 ±0.943
Ampicillin	26.30.943	24.3 ±0.943	26.3 ±0.816	26.0 ±0.471	28.0 ±0.943	25.0 ±0.943	26.0 ±0.943	26.0 ±0.943	23.0 ±0.943	27.0 ±0.471	18.0 ±0.943	25.0 ±0.471
DCM:MeOH	6.0 ±0.000	6.0 ±0.000	6.3 ±0.471	6.0 ±0.000	6.7 ±0.471	6.0 ±0.000	7.3 ±0.943	6.0 ±0.000	6.0 ±0.000	6.7 ±0.471	6.7 ±0.471	6.0 ±0.000

*_Eucalyptus_ sp. (T005a), _Eucalyptus_ sp. (T005b), _B. massaiensis_ (T008), _A. digitata_ (T013a), _A. digitata_ (T013b), _A. digitata_ (T013c), _A. senegalensis_ (T016a), _A. senegalensis_ (T016b), _H. fuscus_ (T019), _A. thunbergii_ (T020), _Morus_ sp. (T021), _M. ovalifolia_ (T022), _C. tridens_ (T024), _N. lotus_ (T025), _Cucurbita_ sp. (T027), _Capsicum_ sp. (T030), _Cucurbita_ sp. (T035), _C. gynandra_ (T040), _I. batatas_ (T041), L. leptocarpa (T043)

8.2 Mean of inhibition zones (mm ± Standard Error) for double distilled water extracts

Inhibition zones (mm ± Standard Error)												
Plants Species	*S. aureus*	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
T005a	11.0 ±0.816	11.3 ±1.700	13.0 ±1.414	11.0 ±1.414	9.0 ±2.160	11.3 ±0.943	10.7 ±0.471	11.7 ±0.471	13.3 ±0.471	12.7 ±1.247	14.3 ±3.399	12.3 ±2.494
T005b	13.3 ±3.399	12.0 ±0.816	12.7 ±0.471	10.7 ±2.055	10.7 ±3.091	11.7 ±0.943	12.0 ±0.816	11.3 ±1.700	13.0 ±0.816	13.0 ±1.414	9.0 ±0.816	11.3 ±1.247
T008	6.3 ±0.471	18.7 ±0.471	15.0 ±4.546	13.7 ±5.437	10.0 ±4.320	19.7 ±2.494	18.3 ±0.471	17.3 ±0.943	18.3 ±0.943	18.0 ±1.633	12.0 ±1.414	10.0 ±0.816
T013a	9.3 ±4.028	7.0 ±0.816	8.0 ±1.414	7.0 ±1.414	6.3 ±0.471	7.7 ±0.471	8.0 ±0.816	9.0 ±0.816	10.3 ±2.055	8.3 ±0.943	9.7 ±1.700	9.0 ±1.633
T013b	6.7 ±0.943	7.0 ±0.816	7.3 ±0.943	6.0 ±0.000	6.0 ±0.000	7.7 ±1.247	11.0 ±3.559	11.0 ±2.160	10.3 ±0.471	7.7 ±0.943	9.3 ±0.471	8.0 ±0.816
T013c	9.3 ±2.357	10.0 ±1.414	8.3 ±1.700	7.3 ±1.247	6.0 ±0.000	7.0 ±0.816	6.3 ±0.471	7.7 ±1.247	7.3 ±0.471	8.0 ±1.414	9.7 ±0.471	8.7 ±0.943
T016a	9.3 ±1.247	7.3 ±1.247	6.3 ±0.471	13.0 ±2.160	7.0 ±1.414	9.0 ±2.160	11.3 ±4.110	10.7 ±2.055	10.3 ±0.471	9.3 ±0.471	14.3 ±0.471	14.0 ±2.449
T016b	7.0 ±0.816	7.3 ±0.943	7.0 ±0.816	16.3 ±5.437	6.7 ±0.943	9.3 ±1.700	16.0 ±7.789	9.0 ±0.816	9.0 ±1.414	9.3 ±1.886	13.0 ±0.816	10.0 ±0.816
T019	7.7 ±0.943	6.0 ±0.000	6.7 ±0.943	14.0 ±4.899	7.0 ±0.816	11.3 ±4.110	8.0 ±0.816	9.7 ±2.625	10.7 ±2.055	8.0 ±0.816	11.0 ±0.816	9.7 ±1.700
T020	7.7 ±1.700	6.0 ±0.000	7.3 ±0.943	10.0 ±2.160	6.7 ±0.943	10.0 ±1.414	9.7 ±1.700	7.3 ±0.471	11.3 ±0.943	10.0 ±2.160	11.3 ±0.471	10.0 ±0.816
T021	6.3 ±0.471	6.3 ±0.471	6.3 ±0.471	10.0 ±2.449	6.0 ±0.000	8.3 ±1.700	7.0 ±0.816	7.3 ±0.471	10.0 ±2.160	9.0 ±0.816	16.3 ±3.091	11.7 ±0.471

T022	15.7 ±2.625	12.0 ±0.816	11.7 ±0.943	12.3 ±1.247	14.0 ±1.414	12.3 ±0.943	15.3 ±2.357	13.0 ±3.266	10.0 ±2.449	14.3 ±0.471	12.0 ±0.816	14.3 ±0.471
T024	11.7 ±2.625	12.3 ±0.943	10.3 ±0.943	11.3 ±0.943	11.7 ±1.886	9.0 ±0.816	13.0 ±1.414	12.3 ±2.867	8.3 ±0.471	12.7 ±2.055	11.7 ±0.471	11.7 ±1.886
T025	10.3 ±1.886	8.0 ±2.160	9.3 ±2.357	9.3 ±0.943	9.0 ±1.414	12.3 ±0.943	10.3 ±0.471	10.7 ±0.943	10.3 ±1.247	11.3 ±1.700	11.7 ±1.886	9.3 ±0.943
T027	10.0 ±0.816	7.7 ±0.943	12.0 ±0.816	10.0 ±1.414	7.3 ±0.471	7.0 ±0.816	8.7 ±1.247	10.0 ±0.816	8.7 ±0.471	7.3 ±0.471	8.7 ±0.471	8.7 ±1.247
T030	11.0 ±1.414	15.7 ±4.028	10.0 ±0.816	12.3 ±	7.7 ±0.471	11.7 ±0.471	15.7 ±3.682	11.3 ±0.471	11.7 ±1.886	12.3 ±1.700	17.7 ±0.471	12.0 ±0.816
T035	9.7 ±1.247	9.7 ±0.471	12.0 ±0.816	9.3 ±1.700	9.0 ±1.633	9.0 ±1.414	11.0 ±0.816	11.7 ±0.471	8.7 ±0.471	7.7 ±0.471	11.3 ±0.943	9.7 ±1.247
T040	16.0 ±4.546	12.3 ±2.867	15.0 ±0.816	14.7 ±0.471	8.7 ±0.943	10.7 ±0.943	14.0 ±2.449	14.3 ±0.471	9.7 ±0.471	12.7 ±1.247	12.7 ±1.700	14.0 ±0.816
T041	7.3 ±0.471	9.7 ±2.055	12.0 ±0.816	9.0 ±2.494	8.0 ±0.816	9.3 ±0.943	11.3 ±1.247	12.3 ±0.471	8.0 ±0.816	10.7 ±1.700	11.3 ±1.247	12.7 ±0.471
T043	10.0 ±0.816	11.3 ±1.700	13.0 ±0.816	9.3 ±1.414	13.3 ±2.867	11.3 ±1.700	9.3 ±1.700	12.7 ±1.247	9.7 ±1.247	10.7 ±0.471	11.7 ±0.471	10.3 ±0.943
Ampicillin	17.0 ±0.816	23.0 ±0.816	21.3 ±4.497	34.0 ±0.816	15.0 ±0.816	18.3 ±1.247	25.0 ±0.816	23.3 ±1.700	23.7 ±0.471	28.0 ±0.816	15.0 ±0.816	17.7 ±1.247
dd-H2O	6.0 ±0.000	6.0 ±0.000	6.7 ±0.943	6.0 ±0.000	6.3 ±0.471	6.0 ±0.000	6.0 ±0.000	7.0 ±0.816	6.0 ±0.000	6.3 ±0.471	6.3 ±0.471	6.0 ±0.000

**Eucalyptus* sp. (T005a), *Eucalyptus* sp. (T005b), *B. massaiensis* (T008), *A. digitata* (T013a), *A. digitata* (T013b), *A. digitata* (T013c), *A. senegalensis* (T016a), *A. senegalensis* (T016b), *H. fuscus* (T019), *A. thunbergii* (T020), *Morus* sp. (T021), *M. ovalifolia* (T022), *C. tridens* (T024), *N. lotus* (T025), *Cucurbita* sp. (T027), *Capsicum* sp. (T030), *Cucurbita* sp. (T035), *C. gynandra* (T040), *I. batatas* (T041), L. leptocarpa (T043)

Appendix 9: Minimum Inhibitory Concentration (MIC) Data

9.1 MIC of DCM:MeOH extracts against test microorganisms (mg/mL)

Plant species	Plant part tested	Test microorganisms strains											
		S. aureus	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
Eucalyptus sp.	Leaves	25	>100	>100	100	>100	100	6.25	100	25	12.5	100	>100
	Barks	50	>100	>100	25	100	100	6.25	6.25	50	25	6.25	25
B. massaiensis	Roots	12.5	100	>100	100	>100	>100	100	100	12.5	50	50	>100
A. digitata	Leaves	>100	>100	>100	100	>100	>100	100	50	12.5	>100	>100	50
	Barks	>100	25	>100	>100	>100	>100	>100	100	50	>100	>100	100
	Roots	>100	>100	>100	100	>100	>100	25	>100	50	100	100	>100
A. senegalensis	Fruits	>100	>100	>100	50	>100	>100	>100	>100	100	50	>100	>100
	Roots	>100	>100	>100	>100	>100	>100	>100	>100	12.5	100	>100	>100
H. fuscus	Leaves	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	25	>100
A. thunbergii	Leaves	>100	>100	>100	>100	>100	>100	>100	100	>100	>100	25	>100
Morus sp.	Leaves	>100	>100	>100	100	12.5	50	6.25	>100	100	>100	6.25	50
M. ovalifolia	Leaves	>100	>100	50	>100	>100	100	>100	>100	100	>100	>100	>100
C. tridens	Leaves	>100	>100	100	>100	>100	>100	>100	>100	>100	>100	>100	>100
N. lotus	Flowers	>100	>100	100	50	6.25	6.25	6.25	>100	>100	50	6.25	>100
Cucurbita sp. (T027)	Leaves	>100	>100	>100	>100	100	>100	>100	>100	>100	100	>100	>100
Capsicum sp. (T030)	Fruits	>100	>100	100	>100	>100	6.25	>100	>100	>100	6.25	6.25	100
Cucurbita sp. (T035)	Leaves	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	100	>100
C. gynandra	Leaves	100	6.25	100	>100	6.25	6.25	6.25	>100	>100	6.25	6.25	>100
I. batatas	Leaves	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
L. leptocarpa	Leaves	>100	25	>100	>100	100	6.25	25	>100	>100	>100	12.5	12.5

* >100= No MIC observed at the highest crude extract concentration (100 mg/mL) used in the present study. The results were the overall of three replicate experiments.

9.2 MIC of dd-H_2O extracts against test microorganisms (mg/mL)

Plant species	Plant part tested	Test microorganisms strains											
		S. aureus	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
Eucalyptus sp.	Leaves	>100	50	>100	12.5	>100	6.25	25	50	12.5	12.5	25	>100
	Barks	25	50	>100	100	>100	25	6.25	25	50	6.25	25	25
B. massaiensis	Roots	12.5	100	>100	50	50	50	12.5	25	6.25	50	6.25	12.5
A. digitata	Leaves	6.25	>100	>100	100	50	>100	>100	>100	6.25	>100	100	100
	Barks	12.5	>100	>100	25	>100	>100	>100	>100	50	>100	>100	>100
	Roots	6.25	>100	>100	>100	>100	>100	>100	>100	100	50	>100	100
A. senegalensis	Fruits	6.25	>100	>100	50	>100	100	100	100	100	25	100	25
	Roots	100	>100	>100	50	50	>100	100	100	6.25	50	>100	100
H. fuscus	Leaves	>100	50	>100	50	100	>100	>100	>100	>100	>100	>100	>100
A. thunbergii	Leaves	100	12.5	>100	50	>100	>100	>100	>100	>100	>100	>100	>100
Morus sp.	Leaves	25	100	>100	100	100	100	>100	>100	100	>100	>100	>100
M. ovalifolia	Leaves	50	>100	>100	>100	50	>100	>100	>100	100	>100	50	100
C. tridens	Leaves	50	>100	>100	25	50	100	>100	>100	>100	>100	25	>100
N. lotus	Flowers	>100	50	>100	>100	100	50	100	>100	>100	50	25	>100
Cucurbita sp. (T027)	Leaves	>100	100	>100	>100	>100	>100	25	>100	>100	100	>100	>100
Capsicum sp. (T030)	Fruits	100	100	100	>100	>100	>100	100	>100	100	50	100	>100
Cucurbita sp. (T035)	Leaves	>100	>100	50	>100	>100	100	100	>100	>100	>100	>100	>100
C. gynandra	Leaves	>100	>100	>100	>100	25	12.5	100	>100	>100	25	25	>100
I. batatas	Leaves	50	>100	>100	>100	25	>100	100	>100	>100	>100	25	>100
L. leptocarpa	Leaves	50	>100	>100	>100	100	100	>100	>100	>100	>100	12.5	50

* >100= No MIC observed at the highest crude extract concentration (100 mg/mL) used in the present study. The results were the overall of three replicate experiments.

Appendix 10: 50 % growth reduction concentrations (IC_{50}) Data

10.1 IC_{50} of DCM:MeOH extracts against test microorganisms (mg/mL)

Plant species	Plant part tested	Test microorganisms strains											
		S. aureus	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
Eucalyptus sp.	Leaves	6.25	100	>100	25	100	50	5	50	12.5	6.25	50	100
	Barks	6.25	>100	>100	6.25	25	12.5	5	5	25	12.5	12.5	12.5
B. massaiensis	Roots	6.25	25	100	25	50	100	25	25	6.25	25	25	>100
A. digitata	Leaves	>100	>100	100	25	100	100	6.25	50	6.25	100	100	25
	Barks	>100	12.5	>100	50	50	100	100	50	25	100	100	50
	Roots	>100	50	>100	12.5	>100	50	6.25	100	25	50	50	100
A. senegalensis	Fruits	>100	>100	>100	12.5	>100	50	>100	100	50	25	100	50
	Roots	>100	100	>100	>100	100	50	>100	100	6.25	50	100	>100
H. fuscus	Leaves	50	>100	>100	>100	>100	100	100	100	100	>100	6.25	>100
A. thunbergii	Leaves	50	>100	>100	100	100	100	>100	25	100	100	12.5	>100
Morus sp.	Leaves	50	100	100	25	6.25	25	6.25	100	50	100	6.25	25
M. ovalifolia	Leaves	>100	>100	25	>100	>100	50	>100	>100	50	50	>100	>100
C. tridens	Leaves	100	>100	50	>100	>100	100	100	100	100	100	>100	>100
N. lotus	Flowers	100	>100	50	25	6.25	6.25	6.25	100	100	25	6.25	100
Cucurbita sp. (T027)	Leaves	>100	>100	100	>100	50	>100	>100	100	100	50	>100	100
Capsicum sp. (T030)	Fruits	100	>100	50	>100	>100	6.25	100	>100	50	6.25	6.25	50
Cucurbita sp. (T035)	Leaves	100	>100	>100	100	100	>100	>100	>100	>100	>100	100	>100
C. gynandra	Leaves	50	5	50	100	5	5	5	>100	100	5	6.25	100
I. batatas	Leaves	100	100	>100	>100	>100	>100	>100	>100	>100	100	>100	>100
L. leptocarpa	Leaves	100	12.5	50	>100	50	6.25	12.5	100	>100	50	12.5	6.25

* >100= No IC_{50} observed at the highest crude extract concentration (100 mg/mL) used in the present study. The results were the overall of three replicate experiments.

10.2 IC_{50} of dd-H_2O extracts against test microorganisms (mg/mL)

Plant species	Plant part tested	Test microorganisms strains											
		S. aureus	*B. cereus*	*L. monocytogenes*	*C. perfringens*	*E. faecalis*	*E. coli*	*S. typhimurium*	*S. sonnei*	*P. aeruginosa*	*P. vulgaris*	*C. albicans*	*S. cerevisiae*
Eucalyptus sp.	Leaves	>100	25	>100	6.25	50	5	6.25	12.5	6.25	6.25	6.25	25
	Barks	12.5	25	>100	25	12.5	12.5	5	6.25	12.5	5	6.25	6.25
B. massaiensis	Roots	6.25	50	100	6.25	12.5	12.5	6.25	6.25	5	12.5	5	6.25
A. digitata	Leaves	5	>100	100	25	12.5	50	100	100	5	100	12.5	25
	Barks	6.25	>100	>100	6.25	25	50	50	100	12.5	100	50	25
	Roots	5	>100	50	100	50	12.5	25	50	6.25	12.5	50	25
A. senegalensis	Fruits	5	>100	>100	12.5	25	12.5	25	25	6.25	12.5	12.5	6.25
	Roots	50	>100	>100	12.5	6.25	6.25	12.5	25	5	6.25	50	12.5
H. fuscus	Leaves	50	25	12.5	12.5	12.5	100	100	100	50	>100	100	>100
A. thunbergii	Leaves	25	6.25	25	25	12.5	100	100	100	100	>100	>100	>100
Morus sp.	Leaves	12.5	50	25	50	12.5	25	100	100	25	>100	100	>100
M. ovalifolia	Leaves	12.5	100	>100	>100	25	100	100	50	50	50	25	50
C. tridens	Leaves	12.5	50	100	12.5	25	50	50	50	100	100	12.5	100
N. lotus	Flowers	50	12.5	>100	>100	50	25	25	>100	100	25	6.25	>100
Cucurbita sp. (T027)	Leaves	50	50	100	>100	100	50	12.5	>100	100	50	50	100
Capsicum sp. (T030)	Fruits	50	25	50	>100	50	100	25	100	50	6.25	50	50
Cucurbita sp. (T035)	Leaves	100	>100	25	100	>100	25	12.5	50	>100	>100	50	100
C. gynandra	Leaves	>100	50	>100	>100	6.25	6.25	50	>100	100	25	12.5	>100
I. batatas	Leaves	12.5	>100	>100	>100	12.5	100	50	>100	>100	100	12.5	>100
L. leptocarpa	Leaves	25	100	100	100	100	50	25	>100	>100	50	6.25	25

* >100= No IC_{50} observed at the highest crude extract concentration (100 mg/mL) used in the present study. The results were the overall of three replicate experiments.