EFFECT OF DIFFERENT HARVESTING TIMES ON QUALITY OF *JATROPHA ZEYHERI* INDIGENOUS TEA

ANNAH MANKUTU SEHLAPELO

MINI-DISSERTATION SUBMITTED FOR THE DEGREE MASTER OF SCIENCE IN HORTICULTURE, DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURE AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR : DR K.G. SHADUNG

CO-SUPERVISOR : PROF M.S. MPHOSI

2020

TABLE OF CONTENTS

DECLARATION

I, Annah Mankutu Sehlapelo, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Horticulture has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

Candidate: Annah Mankutu Sehlapelo _____ Signature _____ Date

DEDICATION

To my beloved mother, Mampaka Magdeline Sehlapelo.

ACKNOWLEDGMENTS

First and foremost, let me take this opportunity to thank the Almighty God for giving me strength, guidance, wisdom, knowledge and protection throughout my studies. My dream would not have been realized had it not been for the continued guidance and advice of my supervisory team Dr K.G. Shadung and Prof M.S. Mphosi. Your encouragements, patience, support and effort has provided me with the motivation needed most to successfully complete this research and reach my goal. I am humbled with gratitude to say thank you with an open heart and I honour your efforts and guidance for enabling me to reach my ultimate goal of completing this project.

To my beloved mother (Ms M.M. Sehlapelo), words alone are not enough to express my gratitude to you. You have been my pillar of strength and gave me the support I needed in all aspects throughout my academic years. I humbly thank God for your life. My appreciation and gratitude goes to my grandmothers (Mrs M.A. Sehlapelo and Ms M.M. Maredi), my aunt (Ms M.V. Sehlapelo), my siblings (Madene, Tshepo, Ouma and Shila) and my family at most for giving me the support and courage throughout my study. My gratitude goes to the Limpopo Agro-Food Technology Station (LATS) for providing funding which covered for phytochemicals, mineral elements analysis and my conference trip to Free State, where I presented two papers at the Combined Congress in January 2019. Special thanks also go to Ms L.K. Mamabolo and Mr H. Bango for helping me with samples analyses and other aspects of my research project. My sincere gratitude is directed to the National Research Foundation (NRF) for providing financial assistance. My gratitude goes to the Faculty of Science and Agriculture, for giving me an opportunity to present my two papers at the Faculty Research Day in 2018 and 2019.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Tea is globally regarded as the second most consumed in the world after water. It is associated with various health benefits such as anti-cancer, anti-inflammation, anti-obesity and reduction in cholesterol blood levels. *Jatropha zeyheri* indigenous tea has medicinal and nutritional properties, therefore knowledge of its chemical compositions is essential for increasing its quality. This indigenous tea is currently harvested in rural areas when the leaves are already dry, which is in contrary to what is practised in most tea industries. Therefore, the determination of harvesting time has an opportunity to contribute towards increasing the quality of *J. zeyheri* indigenous tea. The study investigated whether harvesting times has an effect on essential and non-essential mineral elements and phytochemicals and antioxidant activity of *J. zeyheri* leaves. The study materials were collected in the wild at Khureng village, Lepelle-Nkumpi Municipality, Limpopo Province, South Africa. Five treatments constituting of harvesting times (February, March, April, May and June) were arranged in a randomised complete block design, with 10 replications. Leaves were harvested, oven-dried at 60°C for 24 hrs and pulverised. A microwave digestion system (PerkinElmer, Titan MPS, United States) was used to prepare the samples prior analysis. After the preparations, mineral elements were determined using Inductively Coupled Plasma Emission Spectrometer-9000 (Shimadzu, Japan). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was used to quantify the antioxidant activity of the acetone extracts of plants. The total phenol and tannin contents in each plant extract were determined using the Folin-Ciocalteu assay method. The total flavonoid contents were determined using the Aluminium Chloride colorimetric assay. The absorbance for antioxidant activity and phytochemicals were recorded using UV/visible spectrophotometer (Beckman Coulter-DU730, USA).

Harvesting times had highly significant effects on Cr, Fe, K, Mg, Ni, P and S contributing 58, 65, 73, 62, 55, 67 and 69%, respectively, in total treatment variation, but were significant on Ca, Cu, Mn, Al, Co and Na contributing 62, 58, 66, 53 and 57%, respectively, in total treatment variation (TTV). However, harvesting times did not influence Zn and Si amounts in *J. zeyheri* leaves. Harvesting times increased majority of essential and non-essential mineral elements, except for K which was gradually decreased. Essential and non-essential mineral elements over different harvesting times exhibited positive quadratic relations. Using the optimisation equation ($x = -b_1/2b_2$) from the quadratic equation, harvesting of *J. zeyheri* was optimised at 2.46 months. Harvesting times had highly significant effects on the antioxidant activity, total phenol and flavonoid contents contributing 62, 88 and 60% in total treatment variation, respectively, but was not significant on total tannin contents. The negative quadratic relationship models explained 51, 90 and 95% of the observed variation in antioxidant activity, total phenol and flavonoid contents, respectively. In conclusion, findings of this study suggested that harvesting of *J. zeyheri* leaves be done between April-May for improved accumulation of mineral elements, whereas, for phytochemicals and antioxidant activity the results suggested that further studies be conducted from early summer until winter to find the optimum harvesting time of *J. zeyheri* indigenous tea.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Jatropha zeyheri Sond is an indigenous densely perennial hairy plant that belongs to the Eurphorbiaceae family (Arnold *et al.,* 2002). Eurphorbiaceae family has approximately 300 genera and 7500 species (Rahman and Akter, 2013). *Jatropha zeyheri* is commonly known as Sefapabadia in Sepedi, Xidomeja in Xitsonga and Mafuredonga in Tshivenda among South African people (Van der Merwe *et al.,* 2001; Luseba and Van der Merwe, 2006; Mongalo *et al.,* 2013). *Jatropha zeyheri* is widely distributed throughout the Eastern and Northern parts of South Africa and neighbouring countries such as Botswana, Zimbabwe, Lesotho and Swaziland (Van Wyk and Gericke, 2003). Various plant parts of *J. zeyheri* are used in different ways for various purposes. For instance, the roots are used for the treatment of sexually transmitted, urinary tract infection and the treatment of women health-related issues such as menstrual pains and to ensure strong foetus development during pregnancy (Van Wyk and Gericke, 2007). The leaves of *J. zeyheri* are mainly used during winter when they are already dry to make tea beverage in rural communities.

Tea is regarded as a popular aromatic drink which is most widely consumed in the world after water (Zhen, 2002). Tea originated from South-east Asia and has been successfully cultivated in more than 30 countries worldwide (Hayat *et al.,* 2013; Sharma *et al.,* 2007). The demand and popularity of tea has increased due to the profusion of scientific data regarding the positive human health effect of tea (Yashin *et al.,* 2015). For instance, the antioxidant activities in tea leaves absorbs free radicals which help to prevent cancer (Mitra and Shashi, 2017). The tea phytochemicals are

related to properties such as anti-cancer, anti-carcinogenic as well as the reduction in cholesterol blood levels (Chen *et al.,* 2008; Pinto, 2013). Tea mineral elements help in the regulation of water-salt balance, immunity and metabolism (Mitra and Shashi, 2017).

Different teas vary in their chemical compositions such as mineral elements, alkaloids, proteins, carbohydrates, phytochemicals and amino acids which contribute to tea quality (Karori *et al.,* 2007). Tea quality is influenced by various factors including variety, climate, soil, manufacturing process and storage, with harvest season being the most crucial amongst these factors (Tounekti *et al.,* 2013; Jayasekera *et al.,* 2014). Harvesting times are characterised with different climatic conditions which pose a threat to the quality of tea. Yao *et al.* (2005) reported that the variations in climatic conditions during harvesting times affect the accumulation and synthesis of the tea shoot chemical compositions. Tea quality determines the price of the tea (Hajiboland, 2017). For instance, poor quality teas have a bitter taste, less aroma and health promoting properties and therefore lead to low production and profits of marketable tea products (Ahmed, 2011). Lack of knowledge on optimum harvest time of *J. zeyheri* leaves in most rural communities is unfavourable for production of high-quality tea. Therefore, the determination of optimum harvest time has an opportunity to contribute towards increasing the quality of *J. zeyheri* indigenous tea and thereby increasing its health and nutritional benefits.

1.2 Problem statement

Indigenous tea has gained global recognition due to its nutritional and medicinal benefits with the potential to be commercialised. However, the quality of indigenous

tea is greatly influenced by harvesting time. It is, therefore, necessary to investigate the appropriate harvesting time of *J. zeyheri* indigenous tea leaves for maintaining better quality tea. Therefore, this study investigated the effect of harvesting times on tea phytochemicals, antioxidants activity and mineral elements.

1.3 Rationale

The leaves of *J. zeyheri* are often harvested when they are already dry and are utilized to make tea beverages. This is in contrary to what is practised in the tea industry, as the old tea leaves are not used in tea manufacture and are considered to be of agricultural wastes (Farhoosh *et al.,* 2007). The choice of harvesting time is important for optimum contents of mineral elements, phytochemicals and antioxidant activity of tea. The information on the effect of harvesting times on the quality of *J. zeyheri* is lacking. Therefore, research focused on finding suitable harvesting time for maximizing the quality of *J. zeyheri* tea is warranted.

1.4 Purpose of the study

1.4.1 Aim

The aim of this study was to assess the quality of *J. zeyheri* indigenous tea as affected by different harvesting times.

1.4.2 Objectives

The objectives of this study were to:

(i) Investigate whether harvesting times would have an effect on essential and non-essential mineral elements of *J. zeyheri* leaves.

(ii) Determine whether harvesting times would have an effect on phytochemicals and antioxidant activity of *J. zeyheri* leaves.

1.4.3 Hypotheses

(i) Harvesting times would have an effect on essential and non-essential mineral elements of *J. zeyheri* leaves.

(ii) Harvesting times would have an effect on phytochemicals and antioxidant activity of *J. zeyheri* leaves.

1.5 Reliability, validity and objectivity

In this study, reliability was based on a statistical analysis of data at the probability level of 5%. Validity was achieved by repeating the experiments in time. Objectivity was achieved by ensuring that the findings are discussed on the basis of empirical evidence, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through adequate replications. Also, treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Significance of the study

The study would provide rural people and farmers with a suitable harvesting time for *J. zeyheri* leaves, which would effectively increase the quality of *J. zeyheri* indigenous tea. The study intends to find the chemical composition (mineral elements, phytochemicals and antioxidant activity) in tea, which are beneficial for human health

and plant quality at optimum levels. The results of the study might assist in the eventual commercialization of *J. zeyheri* indigenous tea.

1.8 Structure of the mini dissertation

Following the description and detailed outlining of the general introduction (Chapter 1), the work done and not yet done on the research problem were reviewed (Chapter 2). Then, the two subsequent chapters (Chapters 3 and 4) addressed each of the two objectives, sequentially. In the final chapter (Chapter 5), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in conclusions which tied together with the entire study. The citations in text and references used in the study were as in the Harvard style as prescribed by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Tea quality is a term that is used to indicate the presence of desirable attributes in tea such as aroma, flavour, colour and taste. The quality is determined by the compounds which are synthesized during the growth and development of tea (Obanda *et al.,* 1997). Good tea quality is rated according to the presence of chemical compositions such as phytochemicals, antioxidant activity and mineral elements. These chemical compositions are of importance as they dictate the taste, flavour and colour of tea (Hara *et al.,* 1995). Mineral elements of tea have also been used as a parameter of different tea varieties due to their relation to the taste of tea (Alcázar *et al.,* 2007; Chaturvedula and Prakash, 2001). Tea quality is important for determining the production, market and profits. For instance, good quality tea catches a good price in the market resulting in increased profits and economic viability of tea production estate (Wright, 2005).

2.2 Chemical compositions of tea

2.2.1 Phytochemicals and antioxidant activity

Total phenol contents: Phenols are chemical compounds which are responsible for the derived beneficial effects from the consumption of teas, fruits and vegetables (Han *et al.,* 2007). For instance, phenol contents are beneficial for weight loss, reduced risk of cardiovascular diseases and inflammatory bowel (Carloni *et al.,* 2013). Phenolic compounds such as theaflavins and thearubigins are responsible for tea colour, flavour and brightness which are important for its intrinsic quality (Khanum *et al.,* 2017). The total phenol contents of tea leaves have also been used as tea quality

indicator. For instance, green tea has high quality as compared to oolong tea and black tea due to its high total phenol contents (Chan *et al.,* 2007). Low total phenol contents produced a black tea of lower quality (Obanda *et al.,* 1997). Contrarily, Ertuk *et al.* (2010) reported that the high total phenol contents have the potential to produce high quality of black tea.

Total flavonoid contents: Flavonoids are polyphenolic in nature and contain an aromatic ring bearing one or more hydroxyl groups. The most common flavonoid in tea is flavan-3-ol, which provides the tea with its typical flavonoid pattern (Kaur *et al.,* 2014). Flavonoids are one of the potential quality indicators which are naturally found in tea. Flavonoids also contribute significantly to tea colour, thickness, brightness and taste by contributing to the formation of theaflavins and thearubigins (Maung and He, 2013; Kaur *et al.,* 2014). Catechins, important flavonoid compound, is responsible for the bitterness and astringency in tea, therefore decreased contents of catechins in tea lead to the improved flavour and tea quality (Kallithraka *et al.,* 1997; Ninomiya *et al.,* 1997). Feng *et al.* (2014) suggested that the total flavonoid contents were responsible for the reduction of bitterness in albino tea, therefore improving the taste of the tea. In rooibos tea, the presence of flavonoids indicates the potential of the health-benefiting properties of this tea (De Wet, 2015).

Total tannin contents: Tannins are phytochemicals which are typically astringent and bitter (Ashok and Upadhyaya, 2012). The astringency from the tannins is that which causes the dry and pucker feeling after consumption of strong tea. The total tannin contents are potential indicators of medicinal properties. For instance, the content of tannins was reported to be essential in preventing cancer and heart problems by

reducing the ability of blood platelets to stick together (Stensveld *et al.,* 1992). Khasnabis *et al.* (2015) reported that black tea contained the highest total tannin contents. High total tannin contents in green tea indicated the high quality of tea (Bizuayehu *et al.,* 2016). Tabasum *et al.* (2001) reported that green tea had the lowest tannin contents.

Antioxidant activity: Antioxidant activity is a biological activity affected by the presence of phenols, catechins, flavonoids, theaflavins and tannins in tea leaves. For instance, the strength of antioxidant activity depends largely on the total phenol contents. It has been reported that tea leaves are high in antioxidant activity (Hara *et al.,* 1995). For instance, *Monsonia burkeana* was reported to contain a significantly higher content of antioxidant activities which could be related to the presence of polyphenols (Tshivhandekano *et al.,* 2015). Langley-Evans (2000) also reported that the high antioxidant activity in green tea may be correlated to the polyphenol content in tea. Mate tea had high antioxidant activity as compared to black tea (Bravo *et al.,* 2007). High antioxidant activity indicated high tea quality of green tea (Armoskaite *et al.,* 2011).

2.2.2 Mineral elements

Mineral elements are inorganic substances which originate in the soil and are absorbed by plants. The presence of minerals such as Mg and K within tea leaves improve the quality of tea, although Na has a very little impact on tea quality (Adnan *et al.,* 2013). Inadequate supply and uptake of mineral elements to the tea plant is a key factor which leads to poor tea quality and productivity (Han *et al.,* 2002). Knowledge of mineral elements in tea leaves is important because of the nutritional

requirements and intoxication risks related to its consumption (Salahinejad and Aflaki, 2010). Maedza (2015) reported that the presence of Zn and Cu contents increased the levels of polyphenols in green tea, therefore increasing tea quality. However, the tea plant has an ability to accumulate Cu and Zn only to a lesser extent (Saud and Oud, 2003; Street *et al.,* 2006). Higher contents of Mg and Ca were reported in herbal teas (Nookabkaew *et al.,* 2006). Soliman (2016) also reported that Fe contents were predominant in black tea. Muller *et al.* (1998) reported that black tea leaves contained high Al contents.

2.3 Factors affecting tea quality

2.3.1 Harvesting time

Tea harvesting time is one of the key factors which influence the physiological, physical and chemical qualities of tea (Kim *et al.,* 2016). Harvesting time is an important factor to consider in tea production for maintaining a better and consistent quality of tea. Generally, tea is traditionally harvested in early autumn or late spring, depending on the flowering period of the tea species (DAFF, 2016a). Alternatively, harvesting time can be prolonged to late summer due to the increasing demand for teas (DAFF, 2016b). Climatic changes in different seasons lead to changes in the volume of aromatics and metabolites in tea plants and therefore affect tea flavour.

Mudau *et al.* (2007a) recommended that bush tea should be harvested between early autumn and mid-winter for improved sweetness. Ahmed and Stepp (2012) reported that the spring harvest gave the highest quality in green tea. According to Kim *et al.* (2016), green tea leaves harvested in mid-April were of the highest quality due to their rarity, characteristic taste and softness, whereas those harvested after mid-May are

believed to be of the lowest quality. Zhao and Zhao (2019) postulated that tea leaves harvested in spring had a less bitter taste and increased flavour complexity, whereas those harvested in summer and autumn are more bitter and astringent. Lower polyphenol contents of bush tea leaves harvested in summer months were an indicator of low-quality tea (Chiu, 1990). Contrarily, harvesting in July had high phenolic contents and antioxidant activity in green tea leaves, indicating high-quality tea (Ercisli *et al.,* 2008).

2.3.2 Climatic conditions

2.3.2.1 Temperature

Temperature is one of the climatic factors which affect plant growth and development (Lesfrud *et al.,* 2005). Tea plant grows well within an air temperature range of about 18-25 ^{o}C (Hajiboland, 2017). Han *et al.* (2018) reported that low temperature ranges are associated with nitrogen metabolism and the biosynthesis of amino acids. Sud *et al.* (1995) suggested that high temperatures induce the uptake of calcium contents in tea leaves. Alternatively, high temperatures decrease the quality of tea by reducing the antioxidant activity and total phenol contents (Lee *et al.,* 2010; Wang *et al.,* 2011a). In contrast, high temperatures decrease the biosynthesis of polyphenols in tea leaves (Han *et al.,* 2018).

2.3.2.2. Light intensity

Sunlight synthesis affects the tea plant growth and production through photosynthesis and photoperiodic reactions. Sunlight is required for the biosynthesis of total phenol and flavonoid contents, and the formation of total flavonoid contents (Graham, 1998). Adequate sunlight induces the chlorophyll content which plays a major role in colour

formation of tea leaves. However, low light intensity effectively enhances the biosynthesis of the total flavonoid contents (Ghasemzadeh *et al.,* 2010). Zhang *et al.* (2014) reported that stronger light increased the total phenol contents in green tea. The application of shade treatment for the reduction of direct sunlight is effective in improving the quality of tea beverages (Zhang *et al.,* 2014).

2.3.2.3 Rainfall

The quantity and quality of chemical compositions in tea depend upon the prevailing climatic conditions where tea production occurs (McKay and Blumberg, 2002; Lin *et al.,* 2003). Rainfall changes during the growing season may affect the quality of tea such that inadequate rainfall will lead to moisture stress, and therefore decreasing total phenol contents (Chakraborty *et al.,* 2002). Furthermore, Gulati and Ravindranath (1996) suggested that low rainfall leads to reduced accumulation abilities of phytochemicals by tea leaves. In contrast, excess rainfall to the soil may lead to leaching of important mineral elements thereby reducing the quality and growth of tea plant (Boehm *et al.,* 2016). Sud *et al.* (1995) reported that high rainfall reduced the uptake of essential mineral element especially calcium. Ahmed *et al.* (2014) suggested that high rainfall may also lead to lower concentrations of total flavonoid contents.

2.3.2.4 Soil types

Different soil properties and types determine the availability and uptake of essential mineral elements by tea plants (Chintala *et al.,* 2012; Wang *et al.,* 2010). For instance, tea plants thrive well in acidic soils with a pH ranging between 4 and 4.5 (Jayasinghe *et al.,* 2019). Also, well-drained, deep well-aerated soils, sandy loam to clay and clay loam textures are optimal conducive for tea growth and quality (Chen *et al.,* 2015).

The content of phosphorus in soil also plays a role in soil fertility and tea quality. For instance, the higher the average level of available phosphorus, the more conducive to tea growth and quality (Dong *et al.,* 2016). Furthermore, the availability of P contents in the soil is closely related to the total phenol contents in plant leaves (Chen *et al.,* 2015).

2.3.3 Cultural practices

Plucking is a practise of harvesting tea leaves from the plant using different methods, frequencies and intervals (Wijeratne *et al.,* 2002). Plucking is an important practise which affects the quality of tea. Plucking intervals may be short or long, where shorter plucking interval increases the quality of green tea and the longer plucking interval reduces the quality of black tea (Habimana *et al.,* 2014). Extended plucking frequencies have also been reported to reduce the quality of tea (Wijeratne *et al.,* 2002). Plucking standards has an effect on the quality of tea and can be described as fine, medium and coarse. With fine plucking, only the first two leaves and a bud are harvested whereas with coarse plucking three to four leaves and a bud are harvested (Wright, 2005). The methods of plucking include hand, mechanical and shear plucking. For harvesting of tea leaves, hand plucking is recommended as mechanical plucking may damage the leaves and affect tea quality (UNCTAD, 2016). Caffin *et al.* (2004) explained that hand plucking increases the phenolic contents in tea leaves. Owuor and Odhiambo (2006) reported that hand plucking increased tea quality attributes such as brightness, theaflavins, caffeine and flavour in green tea. Owuor *et al.* (2000) reported that hand plucking increased the contents of polyphenols and antioxidants in *C. sinensis* (green tea) leaves.

Pruning is regarded as an operation of removing the leaf-bearing branches which is done to rejuvenate the tea plants and to increase the quality of tea (Yilmaz *et al.,* 2004; Nissanka *et al.,* 2004). In tea production, pruning takes place after plucking. Pruning of tea leaves directly after plucking may lead to low polyphenol contents (Kaur *et al.*, 2014). Maudu *et al.* (2010) reported that frequent pruning reduced the quality of tea, as compared to unpruned tea plants. Contrarily, frequent pruning increased the total phenolic contents in black tea of *C. sinensis* (Mahanta and Baruah, 2006). Medhi *et al.* (2006) also reported that the quality attributes (pigment contents) of black tea were increased by pruning. Kaur *et al.* (2014) suggested that the shorter pruning cycle may lead to better colour and strength of the tea. Sequentially, pruning adds important mineral elements N, P and K to the soil through the decomposition of the pruned material, which will later be absorbed by the leaves (Kamau *et al.,* 2008).

2.3.4 Processing

Processing is one of the methods that is used to transform the tea leaves to dried leaves by withering, steaming, rolling, fermenting and drying the tea leaves for brewing tea, maintaining and increasing the quality of the final product tea. The steps which are taken during the processing of tea leaves influence tea quality attributes and chemical compositions such as antioxidant activity, phenol contents, colour and aroma development (Chong and Lim, 2012). The amount of phenol contents and antioxidant activity in the final tea products can be changed during processing, consequently the final tea products contain highly varied amounts of antioxidant activity depending on the type of tea (Kosińska and Andlauer, 2014). Processing is also important for preventing the colour change of leaves and reducing the undesirable grassy taste in tea (Liang *et al.,* 2005). The steps involved in tea processing are the determinant of

the resultant kind of tea which will be obtained (Kosińska and Andlauer, 2014). The main tea processing steps are *viz.* withering, steaming, rolling, fermentation and drying.

Withering is the first step taken after the harvesting of tea leaves to reduce the humidity content of the leaves. During withering, there are certain chemical changes which affect the fermentation process and thereby the quality of tea (Sarkar *et al.,* 2016). This is followed by steaming which is done to retain the green colour of the leaves by reducing or stopping the enzymatic reaction in green leaves (Singh *et al.,* 2014). Rolling is performed to crush the tea leaves into smaller particles and press out the juice which is then coated on the leaf particles surface to enhance the chemical changes (Javed, 2015). However, heavy rolling can result in excessive loss of tea sap which will lead to poor tea quality (Zhu and Ning, 2016). The correct fermentation is crucial for tea quality. It is done to lose the green colour of the leaves, attain the copper colour and to allow the formation of the colour (Javed, 2015). Temple *et al.* (2001) suggested that during processing, drying of tea leaves which is the final step is also important because the enzyme reactions in earlier phases are terminated by moisture loss and heat; hence new compounds are produced by the heat action. However, the longer the drying period, the more detrimental the quality and flavour (Javed, 2015).

2.4 Work not done on the problem statement

The effect of harvesting times on the quality of *J. zeyheri* indigenous tea has not been yet documented. Therefore, the researcher intended to evaluate the effect of harvesting times on essential and non-essential mineral elements, phytochemicals and antioxidant activity of *J. zeyheri* indigenous leaves.

CHAPTER 3
EFFECT OF HARVESTING TIMES ON ESSENTIAL AND NON-ESSENTIAL MINERAL ELEMENTS OF *JATROPHA ZEYHERI* LEAVES

3.1 Introduction

Tea has historically gained international recognition due to its beneficial medicinal, nutritional and health effects that are associated with its regular consumption (Johnson *et al.,* 2012). Generally, different type of teas may vary in mineral compositions depending on various factors such as climate, soil types, processing, storage conditions, plant development stages and seasonal changes. There are 28 mineral elements in tea leaves but potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S) are the most important minerals (Zhen, 2002). The available mineral elements at optimum levels serve for medicinal purpose for example, reducing blood cholesterol levels. Although, tea may contain heavy metals such as aluminium (Al), arsenic (As), cobalt (Co), chromium (Cr), nickel (Ni) and lead (Pb) which may be toxic to human health (Ercisli *et al*., 2008; Zhao *et al.,* 2017). Also, there are numerous health benefits which are associated with the presence of mineral elements within tea leaves.

Tea quality is rated according to the presence of mineral elements which has various medicinal properties essential to human health (Chaturvedula and Prakash, 2001). The presence of manganese (Mn), sodium (Na) and P in tea leaves is beneficial for hypertensive patients while Mg protects against incidence of chronic diseases such as diabetes (Bo and Pisu, 2008; Xie *et al.,* 1998). Iron (Fe), zinc (Zn), copper (Cu), Cr and Ni have been studied in many diseases including auto-immune, neurological, and psychiatric disorders (Yanik *et al.*, 2004). Potassium is important for lowering blood pressure in patients with high blood pressure (He and MacGregor, 2008). Aluminum

is important for the protection of mucous membranes and gastro-intestinal duodenum whereas silicon (Si) is important for contributing to the structure and resiliency of connective tissue (Hays and Swenson, 1985). Also, Co is normally used in medical application, such as the fighting of cancer and Ca is important for bone strength and regulation of cell and tissue functions such as reduction of obesity (Bronner and Pansu, 1998).

In most tea plants, the quality of the final product is affected by harvest time, plant age, topography, soil factors and climatic conditions (Zhao and Zhao, 2019; Chen *et al.,* 2009). Due to the increased consumption and interest in indigenous teas, empirically derived information on essential and non-essential mineral elements is crucial. Therefore, the objective of this study was to investigate the effect of harvesting times on essential and non-essential mineral elements of *Jatropha zeyheri* leaves.

3.2 Materials and Methods

3.2.1 Description of the study area

Jatropha zeyheri plant material was collected at Khureng village, Lepelle-Nkumpi Municipality (24°33’53” S, 29°23’4” E) in Limpopo Province, South Africa between February and June 2018. Khureng village is characterised by semi-arid climate with maximum/minimum temperature that averages 30/10°C and an average rainfall of less than 400 mm per annum (Shadung *et al.,* 2012). The soil is predominately clay with bushveld vegetation (Shadung *et al.,* 2012). The leaves of *J. zeyheri* were harvested and transported to Limpopo Agro-Food Technology Station (LATS) laboratory in brown paper bags for further analysis.

3.2.2 Research design, treatments and procedures

Five harvesting times namely, February, March, April, May and June (Figure 3.1) were used as treatments arranged in a randomised complete block design with 10 replications. A 5×5 (25 m^2) area was demarcated and the plant leaves were harvested randomly within the demarcated area. Leaves were harvested on a monthly basis, cleaned by dusting and brushing between the leaves for soil particles and dirt. Harvested leaves were further dried at 60°C in an air-forced oven for 24 hrs (Kissinger *et al*., 2005). The dried leaves were ground using an electric grinder to pass through 1 mm sieve (MF 10 basic, IKA WERKE, United States) prior to analysis.

3.2.3 Data collection

A microwave digestion system (PerkinElmer, Titan MPS, United States) was used to prepare the samples. Approximately, 0.5 g of each sample was weighed, transferred into the digestion vessels and 10 ml of HNO_3 was added. The mixture was cooled for 10 min prior to closing the vessels and inserted into the microwave digester to run for 48 min. The microwave digester vessels were cooled down to room temperature for 20 min. The solution was transferred into 50 ml centrifuge tubes and was diluted with deionized water to top up to 50 ml. The extracts were stored in the cold room at 5°C prior to mineral elements analysis. Essential mineral (Fe, P, K, Mg, S, Ca, Cu, Mn, Ni, and Zn) and non-essential mineral elements (Al, Co, Cr, Na and Si) were determined using Inductively Coupled Plasma Emission Spectrometer (ICPE-9000 Shimadzu, Japan).

Figure 3.1 *Jatropha zeyheri* plant at A) February, B) March, C) April, D) May and E) June harvesting period.

3.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using the Statistix 10.0. When the treatments were significant at the probability level of 5%, the degree of freedom and their associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fisher's Least Significant Difference Test ($P \leq 0.05$). The variable with significant ($P \leq 0.05$) treatment means were further subjected to lines of the best fit using mineral elements responses to different harvesting times, resulting in a quadratic equation: $y = ax^2 + bx = c$ where y = mineral elements and x = harvest time with $x = -b_1/2b_2$ being the value for the optimum harvest time. Unless otherwise stated, only treatment means significant at the probability level of 5% were discussed.

3.3 Results

Harvesting times had highly significant effects ($P \leq 0.01$) on essential mineral elements Fe, K, P and S contributing 65, 73, 67 and 69% in TTV, respectively (Table 3.1), whereas Ca, Cu, Mg, Mn and Ni were significant ($P \leq 0.05$) contributing 62, 58, 62, 66 and 55% in TTV, respectively (Table 3.1-3.2). However, harvesting times did not influence Zn amount in *J. zeyheri* leaves (Table 3.2). Harvesting times had significant effects ($P \leq 0.05$) on non-essential mineral elements Al, Co, Cr and Na contributing 60, 53, 58 and 57% in TTV of the respective variables (Table 3.3), whereas it had no significant effects on Si (Table 3.3).

Relative to harvesting in February, harvesting times increased essential mineral elements Ca, Mg, S, Ni, P, Cu, Fe and Mn by 11-44, 1-32, 7-25, 11-25, 10-33, 7-9, 1-

37 and 17-46%, respectively (Table 3.4-3.6). However, relative to February, K increased by 10% in March, followed by a gradual decrease of 5-20% in April-June (Table 3.4). Relative to harvesting in February, harvesting times increased non-essential mineral elements Al, Co, Cr and Na by 18-24, 15-24 8-38 and 12-26%, respectively (Table 3.7).

Jatropha zeyheri leaves had higher contents of S and K as compared to rooibos tea (*Aspalathus linearis*) *and* honeybush tea (*Cyclopia intermedia*), but it was lower than all the other compared teas contributing 1036 and 7150 mg/kg, respectively (Table 3.9). They also had higher Mg contents than all the other compared teas but was lower as compared to mate tea (*Ilex paraguareinsis*) by 4096 mg/kg (Table 3.9). The leaves of *J. zeyheri* had higher contents of Ca as compared to the other teas, however, was lower than in special tea (*Monsonia burkuena*) contributing 10993 mg/kg. The contents of P in *J. zeyheri* leaves were higher than in *C. intermedia* by 607.5 mg/kg but was lower than all the other compared teas (Table 3.9).

Non-essential mineral elements over different harvest times exhibited positive quadratic relations. The relationship models were explained by 81, 72, 72 and 57% of Al, Co, Cr and Na, respectively (Figure 3.2). Essential mineral elements over different harvesting times of *J. zeyheri* leaves exhibited quadratic relations. The relationship model explained by 90, 88, 63, 65, 80, 70, 63, 77 and 85% of P, Mn, Cu, Ca, Fe, S, Ni, Mg and K, respectively (Figure 3.3-3.5). The essential mineral elements over the harvesting times were increasing displayed existence of positive quadratic relations, which enhanced the use of $x = -b_1/2b_2$ for calculated harvest times of Ca, K, Mg, P and S which were 2.96, 1.55, 2.67, 2.67 and 2.47 months, respectively, with the

optimum harvest time being at 2.46 months, which translated to harvesting approximately between April and May months (Table 3.8).

Table 3.1 Partitioning mean sum of squares for manganese (Mn), calcium (Ca), phosphorus (P), sulphur (S), cupper (Cu), iron (Fe), potassium (K) and magnesium (Mg) to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

		Mn (mg/kg)		Ca (mg/kg)		P (mg/kg)		S (mg/kg)	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	41137	14	1.048E+07	18	146951	21	153662	19
Treatment	4	194338	66**	3.703E +07	62**	472401	67***	544929	69***
Error	36	58793	20	1.199E +07	20	81799	12	96257	12
Total	49	294268	100	5.95E +07	100	701151	100	794848	100

		Cu (mg/kg)		Fe (mg/kg)		K (mg/kg)		Mg (mg/kg)	
Source	Df	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	85.039	25	67059	25	4461136	14	2239560	21
Treatment	4	198.07	58**	173540	65***	2.25E +07	73***	6552792	62**
Error	36	57.564	17	27361	10	3894981	13	1748525	12
Total	49	340.673	100	267960	100	308560	100	10540877	100

** = significant at P ≤ 0.05; *** = highly significant at P ≤ 0.01.

Table 3.2 Partitioning mean sum of squares for nickel (Ni) and zinc (Zn) to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

		Ni (mg/kg)		Zn (mg/kg)	
Source	Df	MMS	TTV (%)	MMS	TTV (%)
Block	9	1929.54	30	735.183	37
Treatment	4	3503.86	55**	797.117	41^{ns}
Error	36	965.85	15	421.588	22
Total	49	6399.24	100	1953.888	100

^{ns} = non-significant at P ≥ 0.05; ** = significant at P ≤ 0.05.

Table 3.3 Partitioning mean sum of squares for cobalt (Co), sodium (Na), chromium (Cr), aluminum (Al) and silicon (Si) to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

		Co (mg/kg)		Na (mg/kg)		Cr (mg/kg)		Al (mg/kg)		Si (mg/kg)	
Source	Df	MMS	TTV (%)	MMS	TTV (%)	MMS	TTV (%)	MMS	TTV (%)	MMS	TTV (%)
Block	9	1515.6	28	65381	24	11177.11	26	54107	22	272.94	40
Treatment	4	2890.58	53**	156392	57**	24520	58**	150069	60**	151.42	22^{ns}
Error	36	1065.24	19	50600	19	6683	16	48086	18	258.10	38
Total	49	5470.98	100	272373	100	42380.1	100	240262	100	682.45	100

^{ns} = non-significant at P ≥ 0.05; ** = significant at P ≤ 0.05.

Table 3.4 Impact of different harvesting times on potassium (K), calcium (Ca) and magnesium (Mg) of *Jatropha zeyheri* leaves (n = 50).

	K		Ca		Mg	
Harvesting months	Variable	RI (%)[z]	Variable	RI (%)	Variable	RI (%)
February	7503.0^{by} ± 777.77	–	8246.0^{bc} ± 919.68	–	3227.0^{b} ± 306.67	–
March	8245.6^{a} ± 806.46	10	11857.9^{a} ± 1622.75	44	4255.0^{a} ± 713.98	32
April	7150.0^{b} ± 220.34	–5	10993.0^{ab} ± 623.93	33	4096.0^{ab} ± 119.31	27
May	6678.9^{b} ± 687.52	–11	9642.8^{c} ± 1230.14	–17	3242.8^{b} ± 497.00	1
June	6008.0^{b} ± 462.29	–20	9129.0^{abc} ± 680.32	11	2950.3^{b} ± 241.47	-9

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[z] Relative impact = [(treatment/control – 1) × 100].

Table 3.5 Impact of different harvesting times on sulphur (S), nickel (Ni) and phosphorus (P) of *Jatropha zeyheri* leaves (n = 50).

	S		Ni		P	
Harvesting months	Variable	RI (%)[z]	Variable	RI (%)	Variable	RI (%)
February	944.8^{by} ± 108.85	–	95.3^{b} ± 11.31	–	647.9^{b} ± 113.31	–
March	1183.5^{a} ± 145.22	25	112.6^{a} ± 12.72	18	896.2^{a} ± 129.50	38
April	1036.0^{b} ± 43.37	10	106.1^{b} ± 5.79	11	812.5^{b} ± 44.49	25
May	928.7^{b} ± 118.23	–2	109.6^{b} ± 12.82	15	715.3^{b} ± 101.08	10
June	846.2^{b} ± 71.22	–10	119.2^{ab} ± 9.57	25	533.9^{b} ± 74.31	–18

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[z] Relative impact = [(treatment/control – 1) × 100].

Table 3.6 Impact of different harvesting times on copper (Cu), iron (Fe) and manganese (Mn) of *Jatropha zeyheri* leaves (n = 50).

	Cu		Fe		Mn	
Harvesting months	Variable	RI (%)[z]	Variable	RI (%)	Variable	RI (%)
February	58.8[by] ± 2.49	–	448.4[c] ± 41.93	–	657.1[b] ± 58.76	–
March	64.2[a] ± 3.12	9	614.8[a] ± 94.21	37	940.7[a] ± 115.59	43
April	62.6[b] ± 1.23	7	615.4[ab] ± 25.08	37	961.2[a] ± 55.63	46
May	62.9[b] ± 3.12	7	549.6[c] ± 68.14	23	853.7.6[b] ± 53.86	30
June	62.8[ab] ± 2.08	7	517.2[bc] ± 41.76	15	770.6[ab] ± 63.43	17

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[z] Relative impact = [(treatment/control – 1) × 100].

Table 3.7 Impact of different harvesting times on aluminium (Al), cobalt (Co), chromium (Cr) and sodium (Na) of *Jatropha zeyheri* leaves (n = 50).

Harvesting months	Al		Co		Cr		Na	
	Variable	RI (%)[z]	Variable	RI (%)	Variable	RI (%)	Variable	RI (%)
February	801.5^{by} ± 51.08	–	100.8^{b} ± 11.12	–	194.6^{c} ± 29.35	–	750.0^{b} ± 32.57	–
March	961.0^{a} ± 95.91	20	119.8^{a} ± 12.46	19	238.9^{a} ± 31.99	23	890.0^{a} ± 89.77	19
April	947.9^{ab} ± 23.28	18	115.9^{ab} ± 5.46	15	221.6^{bc} ± 18.77	14	845.1^{b} ± 40.27	13
May	960.6^{b} ± 81.77	20	117.8^{b} ± 13.59	17	234.9^{bc} ± 30.40	21	842.6^{b} ± 70.20	12
June	995.1^{ab} ± 63.34	24	124.9^{ab} ± 9.19	24	269.0^{ab} ± 35.20	38	942.6^{ab} ± 60.00	26

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[z] Relative impact = [(treatment/control – 1) × 100].

Table 3.8 Optimisation of selected essential mineral elements (Ca, K, Mg, P and S) of *Jatropha zeyheri* indigenous tea.

Variables	Quadratic relationship	R^2	X	P ≤
Ca	$y = –623.99x^2 + 3699.11x + 5740.6$	0.65	2.96	0.05
K	$y = –157.29x^2 + 488.03x + 7713.8$	0.85	1.55	0.05
Mg	$y = –238.27x^2 + 1273.4x + 2356.1$	0.77	2.67	0.05
P	$y = –62.361x^2 + 333.28x + 407.32$	0.90	2.67	0.01
S	$y = –43.026x^2 + 212.94x + 822.32$	0.70	2.47	0.01
	Optimum harvest time		2.46 months	

Calculated optimum harvesting time (x) = $-b_1/2b_2$.

Table 3.9 Comparison of selected essential mineral elements (mg/kg) in different tea leaves.

Tea type	Mg	S	Ca	K	P	Reference
Jatropha zeyheri	4096	1036	10993	7150	607.5	Current study
Camellia sinensis (black tea)	1914	2630	4616	17 250	2906	Olivier *et al.* (2012)
Camellia sinensis (green tea)	1956	2892	5252	13 896	2492	Olivier *et al.* (2012)
Monsonia burkena (special tea)	2700	–	26 800	12 500	2800	Mamphiswana *et al.* (2011)
Aspalathus linearis (rooibos)	1704	874	1792	2762	680	Olivier *et al.* (2012)
Athrixa phylicoides (bush tea)	3830	1720	10 676	14 662	992	Olivier *et al.* (2012)
Cyclopia intermedia (honeybush)	908	752	1886	3658	532	Olivier *et al.* (2012)
Ilex paraguareinsis (mate)	4972	1210	6196	13 448	1190	Olivier *et al.* (2012)
Erythroxylum coca (coca tea)	3478	2330	10 556	10 550	3750	Olivier *et al.* (2012)

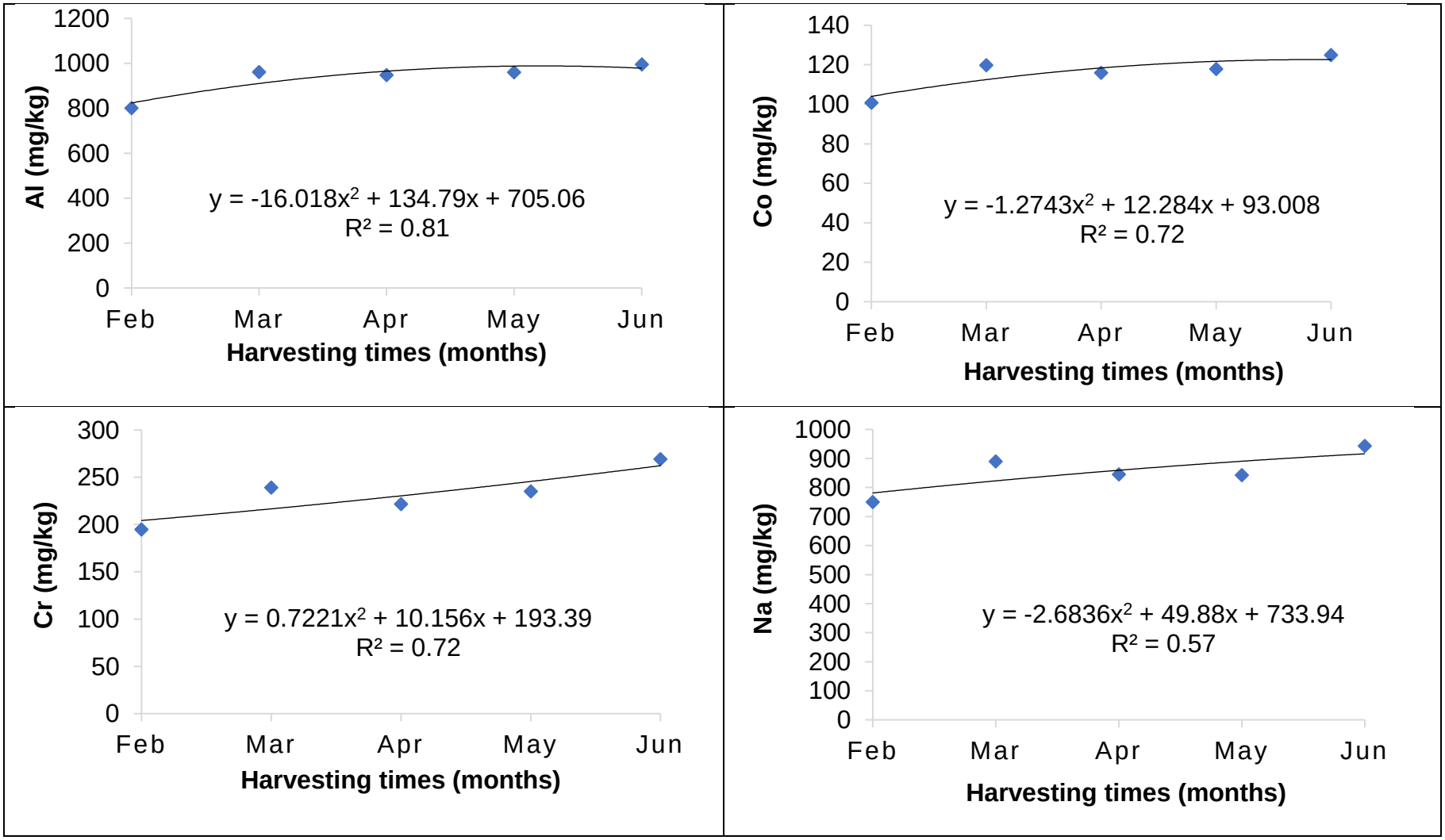

Figure 3.2 The response of Al, Co, Cr and Na non-essential mineral elements to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

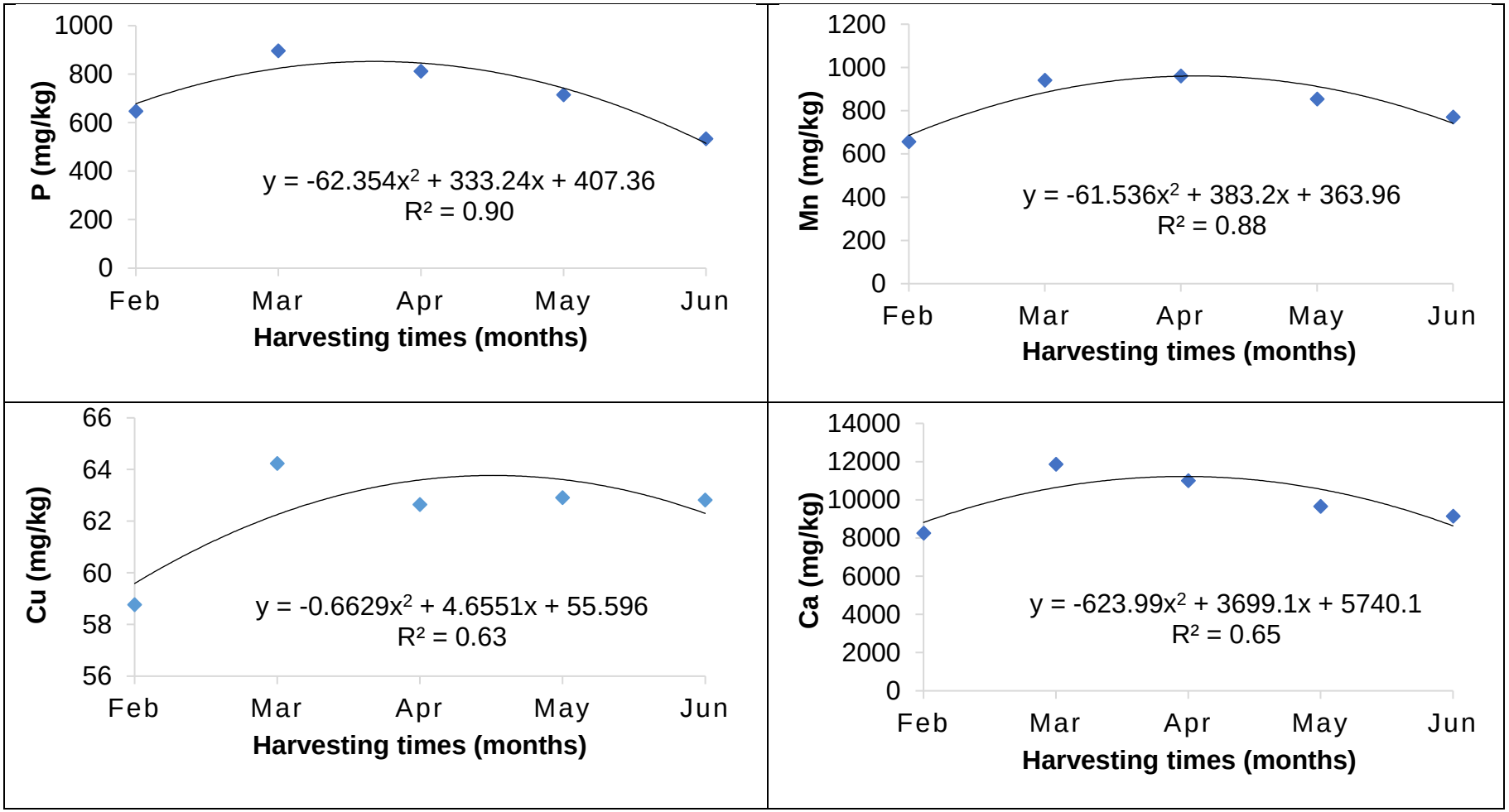

Figure 3.3 The response of P, Mn, Cu and Ca essential mineral elements to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

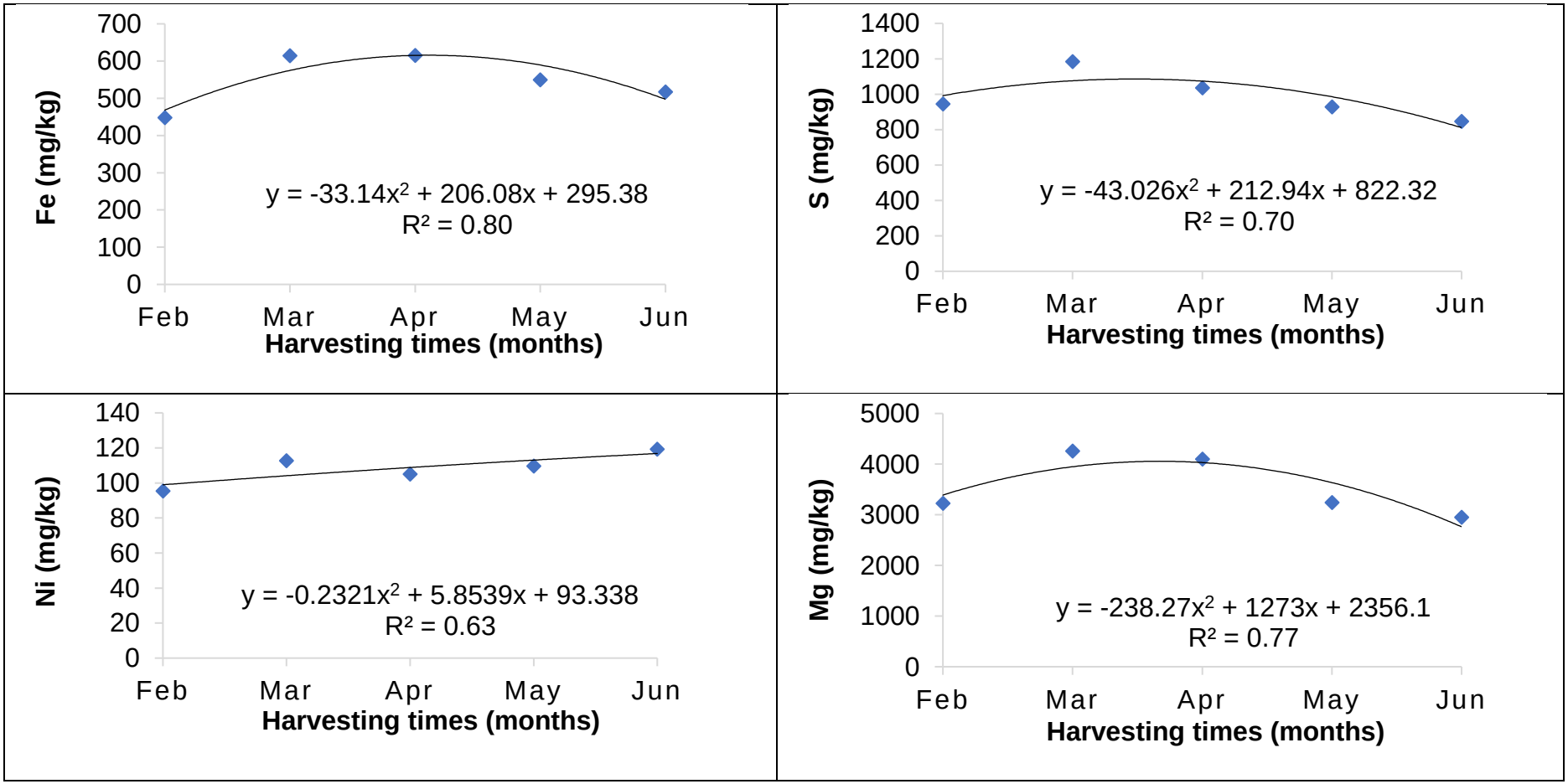

Figure 3.4 The response of Fe, S, Ni and Mg essential mineral elements to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

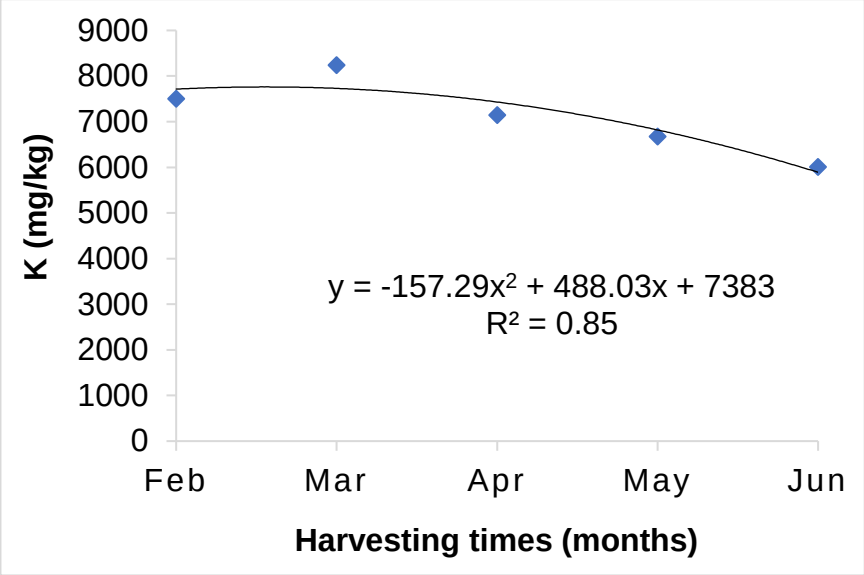

Figure 3.5 The response of K essential mineral element to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

3.4 Discussion

Harvesting times affected majority of essential mineral elements of *J. zeyheri* leaves. Similar findings were observed in green tea leaves (*C. sinensis*) on P, Ca, K, Mg, S and Mn (Ercisli *et al.*, 2008) and on Ca, Cu, Mg, Fe and S (Demir and Bostan, 2018). Harvesting times affected P, K, Ca, Mg, Cu, Fe and Mn on yerba mate tea leaves (*I. paraguariensis*) (Bastos *et al.,* 2018; Rakocevic *et al.,* 2007). The source of variation among mineral elements in plants may be attributed to different environmental factors. For instance, Ercilsi *et al.* (2008) explained that the mineral composition of plants depends on the species or varieties, the growing conditions such as soil and geographical condition. Levickienė *et al.* (2018) also reported similar findings on *Morus alba* (L.) where harvesting times affected Ca, P, K, Mg and S. Similar results were observed on *Olea europaea* (L.) where harvesting times affected P, K, Ca, Mg, Cu, Fe

and Mn (Fernândez-Escobar *et al.,* 1999). Harvesting times affected Fe, Mg, P, K, Ca and Cu in *Ficus carica* (L.) (Brown, 1994).

Contrary findings were observed on *C. sinensis* varieties black tea (Karimzadeh *et al.,* 2013), green tea (Zhao and Zhang, 2011) and oolong tea (Peng *et al.,* 2018) where harvesting times did not affect Cu. Also, harvesting times did not affect the contents of Mn, Cu, Zn and Fe on black tea leaves (*C. sinensis*) (Omwoyo, 2017). Selected mineral elements (P, K, Ca, Mg, S, Zn, Fe, Cu, Mn and Ni) in green tea leaves (*C. sinensis*) were not affected by harvesting times (Ercisli *et al.,* 2009). For instance, in earlier harvesting months, high rainfall and humidity depresses the uptake if some mineral elements such as Zn, Cu and Fe in plant leaves (Sud *et al.,* 1995). In this current study, Zn was not affected by harvesting times. Similar results were reported on black tea (Omwoyo, 2017) and green tea of *C. sinensis* (Ercisli *et al.,* 2009). However, different findings were reported on green tea (*C. sinensis*) (Huang *et al.,* 2019) and *Mentha longifolia* (L.) (Ahmad *et al.,* 2011) where harvesting times affected Zn content.

The response of essential mineral elements to different harvesting times can be attributed to varying climatic and environmental factors which might lead to different mineral elements uptake capabilities (Marschner, 1995; Zhao and Zhao, 2019). Adequate rainfall during the growing season may lead to adequate soil moisture for increased mineral elements uptake. In the current study, it was observed that Mg and Fe contents were higher during high temperature months. This might be due to increased transportation and uptake of mineral elements within the plants by high temperature and atmospheric humidity. Sud *et al.* (1995) explained that the presence

of Mg and Fe in most tea plants may be due to high temperature conditions. For instance, during colder harvesting months when the temperatures are low, the plant experiences the reduction of mineral elements uptake (Yadav, 2010). Alternatively, rainfall changes may also lead to moisture stress which will result in reduced uptake of mineral elements by the tea plants (Wright, 2005). The high contents of Mg and Fe are reported to suppress the uptake of Zn in lea leaves (Sud *et al.,* 1995; Barpujari and Dey, 1982).

In this current study, harvesting times affected non-essential mineral elements of *J. zeyheri* leaves. Similar findings were reported in green tea leaves (*C. sinensis*) on Al and Na (Zhao *et al.,* 2017; Huang *et al.,* 2019) and Na, Cr, Co and Al (Zhao and Zhao, 2019). Harvesting times affected Al in green tea (*C. sinensis*) (Peng *et al.,* 2018) and Na, Cr, and Al in black currant herbal tea (*Ribes nigrum* L.) (Nour *et al.,* 2014). Also, harvesting times affected Na on yerba mate tea (*I. paraguariensis*) (Bastos *et al.,* 2018), *M. longifolia* (Ahmad *et al.,* 2011) and on *Diospyros kaki* (L.f.) (Hossain *et al.,* 2018). In contrast, harvesting times did not affect Al in green tea (*C. sinensis*) (Ercisli *et al.,* 2009) and in *Rubus fruticosus* (Strik, 2015). In this current study, harvesting times did not affect the contents of Si. Similar findings were reported on *Vellozia gigantea* where harvesting times did not affect Si contents (Morales *et al.,* 2015).

Under certain conditions, plants may accumulate high levels of non-essential mineral elements such as Al, Cr and Co (Soliman, 2016). These non-essential mineral elements are regarded as heavy metals which may be accumulated through fertilizers and pesticides use (Ebadi *et al.,* 2005). Heavy metals are regarded as natural soil components which may be beneficial for plant growth and human body at low levels

(Lasat, 2000). However, at excess levels, the intake of heavy metals may have toxic effects on human health and plant quality (Korfali *et al.,* 2013). For instance, when Al is at high concentrations, it may be toxic and have detrimental effects on human nervous system which will lead to memory loss (Krewski *et al*., 2007). Excess levels of Cr in plants beyond the permissible limit may negatively affect the biological factors of the plant and lead to chlorosis and necrosis (Jaishankar *et al.,* 2014; Ghani, 2011). High concentrations of Co may pose negative effects on human health such as lung and heart diseases (Basu *et al.,* 2010; Devi *et al.,* 2014).

The results of the present study suggested that majority of the essential mineral elements were increased with an increase in harvesting times, except for K which was gradually decreased. Similar trend was observed in green tea (*C. sinensis*) (Ertuk *et al.,* 2010) and yerba mate tea (*I. paraguariensis*) (Bastos *et al.,* 2018), where P, Ca, Mg, Fe, Cu and Mn were increased with increasing harvesting times. A gradual decrease of K in this study agreed with those previously reported on green tea (*C. sinensis*) (Zhao and Zhao, 2019; Takayanagi *et al.,* 1985), yerba mate tea (*I. paraguariensis*) (Bastos *et al.,* 2018) and *M. alba* (Levickienė *et al.,* 2018). Ryugo (1988) suggested that the K leaf concentration in most plants is reduced as the growing season progresses. In contrast, the behaviour of K, Ca, Mg, S and Mn exhibited density-dependent growth (DDG) patterns when exposed to different harvesting times of green tea (*C. sinensis*) (Ercisli *et al.,* 2008). Different harvesting times decreased the contents of Fe and Mg in green tea (*C. sinensis*) (Demir and Bostan, 2018) and Mg, Fe and Cu in *M. longifolia* (Ahmad *et al.,* 2011). Harvesting times decreased the contents of P and Mg in green tea leaves (*C. sinensis*) (Takayanagi *et al.,* 1985).

Generally, climate conditions cause fluctuations in the uptake and transportation of mineral elements due to the rise in temperature, extreme weather conditions and unpredictable patterns which affect the accumulation of mineral elements. For instance, high temperature increases the metabolism and transpiration processes in plants, which would bring more mineral elements to the plant (Criddle *et al.,* 1994). Brouder and Volenec (2008) also suggested that the rate of mineral element absorption in plants increases with an increase in temperature. Rainfall changes may lead to changes in the solubility of mineral elements in the soil making some more and some less available for plant uptake (Fung and Wong, 2001; Zhang *et al.,* 2006; Meier and Leuschner, 2014).

Non-essential mineral elements of the present study were increased with later harvesting months. Similar trends were reported in green tea (*C. sinensis*) on Na and Al (Zhao *et al.,* 2017), Na and Co (Zhao and Zhao, 2019), Al (Demir and Bostan, 2018) and on Na (Ertuk *et al.,* 2010; Ercisli *et al.,* 2008). Li *et al.* (2015) suggested that the increasing content of Al in most teas may be due the fact that tea plants are one of the few Al accumulating plants. Harvesting times also increased the contents of Na on *M. longifolia* (Ahmad *et al.,* 2011). Contrary findings were observed on yerba mate tea (*I. paraguariensis*) (Bastos *et al.,* 2018), black currant herbal tea (*R. nigrum*) (Nour *et al.,* 2014) and *D. kaki* (Hossain *et al.,* 2018) where harvesting times decreased Na contents. The behaviour of Al on oolong tea leaves (*C. sinensis*) (Peng *et al.,* 2018) and of Na on green tea leaves (*C. sinensis*) (Sud *et al.,* 1990) exhibited DDG pattern when exposed to different harvesting times.

Generally, plants accumulate the mineral elements from the soil. Therefore, the contents of mineral elements in plant leaves are closely related to the soil environment (Özyazici *et al.,* 2011). For instance, acidic soils promote the retention of mineral elements in the soil but limit the uptake of mineral elements by the plants (Nath, 2013). Lynch and St. Clair (2004) postulated that acidic soils may lead to Al deficiencies, while alkaline soils may lead to poor P availability in plants. Saline soils reduce the availability of mineral elements by disrupting the mineral elements uptake and translocation (Niste *et al.,* 2014). Clay and moist soil conditions are favourable for the supply of mineral elements to the roots through mass flow and diffusion (Kreuzwieser and Geßler, 2010). Harvesting in winter months effectively limits soil compaction when soils are frozen to a depth that is adequate to resist the pressure applied by harvesting equipment (Kolka *et al.,* 2012). For instance, Stone and Elioff (1998) reported that harvesting *Populus grandidentata* (Michx.) in winter months on frozen soils had a little effect on soil physical properties.

In the current study, *J. zeyheri* leaves had high contents of Ca than in *C. sinensis* (black and green tea), *A. linearis, A. phylicoides, C. intermedia, I. paraguareinsis* and *E. cocoa*. Magnesium contents in *J. zeyheri* were higher than those of that *C. sinensis* (black and green tea), *A. linearis, A. phylicoides, M. burkena, I. paraguareinsis* and *E. cocoa*. However, contents of S and K in *J. zeyheri* leaves were only higher than those of *A. linearis* and *C. intermedia*, whereas P was higher than that of *C. intermedia*. The high contents of Ca and Mg in *J. zeyheri* agreed with previous studies which indicated the abundance of these mineral elements within tea leaves and medicinal plants (Ajasa *et al.,* 2004; Basgel and Erdemoglu, 2006; Lesniewicz *et al.,* 2006). Nookabkaew *et al.* (2006) tested mineral elements in different teas and found that

green tea (*C. sinensis*) had lower contents of Ca and Mg than *M. alba* and *Gynostemma pentaphyllum* Thunb. High contents of Mg, Ca and K were found in herbal tea (*Lippia multiflora* L.) than in green tea (*L. multiflora*) (Christine *et al.,* 2017). Gallaher *et al.* (2006) also compared mineral elements in different teas and found that P and K were higher in *Taraxacum officinale* (L.) than other compared teas. However, Ca was found to be higher in *Trifolium pratense* (L.) whereas Mg was higher in *Vaccinium myrtillus* (L.) when compared to the other teas.

Findings from this current study indicated that *J. zeyheri* is a good source of Ca and Mg. Generally, the presence of high Ca and Mg contents in plants is important for human health as insufficient intake of either mineral may pose threat to human health (Nile and Khobragade, 2009). These two mineral elements work together in transmitting nerve impulse in the brain and relieving patients with depression (Powell *et al.,* 1998). In plants, Ca is important for various metabolic process of the plants. Inadequate supply of Ca to the plants will result in deficiencies such as necrosis and stunted growth of the plant. Magnesium is required for the physiological and biochemical processes of the plant. Insufficient levels of Mg may result in the impairments in growth of the plant (Cakmak and Yazici, 2010).

Harvesting time and mineral elements exhibited positive quadratic relationships. The observed positive quadratic models also provided optimum harvest time at which the selected mineral elements of *J. zeyheri* plant would be at the optimum contents. Using $X = -b_1/2b_2$ relations (Gomez and Gomez, 1984), the optimisation suggested the harvesting of *J. zeyheri* to be approximately between April-May. The results of this study agree with the general practise in most tea industries. Honeybush tea (*C.*

intermedia) (DAFF, 2016b) and black tea (*C. sinensis*) (DAFF, 2016a) are harvested in late summer (November-December) and early autumn (February-April). In contrast, North *et al.* (2017) reported the optimum harvest time of Honeybush tea (*C. intermedia*) in September. Consequently, Joubert *et al.* (2011) reported the harvest time of *C. subternata* between April and June. This might be attributed by different tea varieties which have different adaptations to climatic conditions. The harvest seasons of tea may differ due to the variability in climate (Ahmed, 2011). Generally, the change in weather conditions during different seasons is responsible for the fluctuations in mineral elements in tea plants (Hasselo, 1965). Determination of optimum harvest time is of importance as the quality of the plant is dependent on an optimum harvesting time.

3.5 Conclusion

The study revealed that *J. zeyheri* indigenous plant is a beneficial source of mineral elements. Late harvesting times increased majority of essential and non-essential mineral elements, whereas K was significantly decreased by different harvesting times. The presence of heavy metals such as Al, Cr and Co were found in *J. zeyheri* leaves, which may be toxic to plant growth and human health at high concentrations. The results of this study suggested that harvesting of *J. zeyheri* leaves be done between April-May when the plant is between the fruiting stage and maturity, for increased mineral elements within the tea leaves.

CHAPTER 4
EFFECT OF HARVESTING TIMES ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *JATROPHA ZEYHERI* LEAVES

4.1 INTRODUCTION

Phytochemicals in tea are receiving a lot of attention due to their potential health benefits when consumed as part of a varied diet on a regular basis and at effective levels (Karori *et al.,* 2007). These include total phenol and flavonoid contents which contribute to the astringency and bitterness of green tea, which is regarded as a desirable attribute (Botwright, 1997; Wang *et al.*, 2012). Tannins found in teas are also reported to prevent cancers, heart problems and reduction of the tendency of blood platelets to stick together (Stensveld *et al*., 1992). These phytochemicals contain biological properties such as antioxidant activity and antimicrobial effects and are the potential indicators of tea quality (Hara *et al.,* 1995; Mudau *et al.,* 2007b). The high antioxidant activity found in tea leaves is important for human health through the dietary intake (Duh *et al.,* 1999; Hara *et al.,* 1995).

Phytochemicals and antioxidant activity of tea are mostly influenced by harvest time, geographic location, environmental and husbandry factors (Kaur *et al*., 2014). *Jatropha zeyheri* leaves are currently harvested when they are already dry, without the consideration of the influence of time of harvest on the phytochemical constituents and antioxidant activity. Therefore, the objective of the study was to determine the effect of harvesting times on phytochemicals and antioxidant activity of *J. zeyheri* indigenous tea leaves.

4.2 Materials and methods

4.2.1 Description of the study area

Jatropha zeyheri plant material was collected at Khureng village, Lepelle-Nkumpi Municipality (24°33’53” S, 29°23’4” E) in Limpopo Province, South Africa between February and June 2018. Khureng village is characterised by semi-arid climate with maximum/minimum temperature that averages 30/10°C and an average rainfall of less than 400 mm per annum (Shadung *et al.,* 2012). The soil is predominately clay with bushveld vegetation. The harvesting of leaves was done as previously described in Chapter 3.

4.2.2 Research design, treatments and procedures

Five harvesting times namely, February, March, April, May and June were used as treatments arranged in a randomised complete block design with 10 replications. A 5×5 (25 m^2) was demarcated and the plant leaves were harvested randomly within the demarcated area. Leaves were harvested on a monthly basis, cleaned by dusting and brushing between the leaves for soil particles and dirt. Harvested leaves were dried at 60°C in an air-forced oven for 24 hrs (Kissinger *et al.,* 2005). The dried leaves were ground using an electric grinder to pass through 1 mm sieve (MF 10 basic, IKA WERKE, United States) prior to analysis.

4.2.3 Data collection

One gram of the ground powdered plant materials was extracted with 10 mL of acetone. The filtrates were filtered into pre-weighed vials and the solvents were evaporated at room temperature. The mass extracted was determined and samples

were reconstituted in acetone to a final concentration of 10 mg/mL for subsequent assays.

Antioxidant activity assay: The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was used to quantify the antioxidant activity of the acetone extracts of plant materials. The plant extracts were serially diluted with distilled water in test tubes to make a volume of 1 mL at different concentrations (1 mg/mL to 0.0625 mg/mL) and then mixed with 1 mL of 0.2% DPPH solution in methanol. The samples were diluted with 10 mL of methanol for optimal colour development. Methanol was used as blank and DPPH solution a standard control. The mixtures were then incubated for 20 min in dark environment and the absorbance were measured at 517 nm using a UV/visible spectrophotometer (Beckman Coulter-DU730, USA) and ascorbic acid was used as reference control. The EC50 value of ascorbic acid was compared with that of the extracts (Brand-Williams *et al.,* 1995). The radical scavenging activity was calculated from the linear regression formula. The antioxidant activity was expressed as mg of GAE/g of the extract.

Total phenol content: The total amount of phenols in each plant extract was determined using the Folin-Ciocalteu method. Extracts infusion of 0.1 mL was diluted with 0.9 mL of distilled water then mixed with 1 mL of Folin-Ciocalteu reagent and shaken well (Wang *et al.,* 2011b). After incubation for 5 min, 1 mL of Sodium Carbonate (7%) was added to the mixtures and the mixtures were made up to 25 mL with distilled water. The standard was prepared using a serial dilution of quercetin (1 to 0.0625 mg/mL) in place of the extract. The mixtures were then incubated for 90 min at room temperature in dark environment. The absorbance for test and standard

solutions were determined against blank reagent using a UV/visible spectrophotometer (Beckman Coulter-DU730, USA) at 765 nm. The total phenol content was expressed as mg of GAE/g of the extract (Hlahla *et al.,* 2010).

Total tannin content: The Folin-Ciocalteu assay was used to determine the total tannin content of the plant extracts. In a volumetric flask (10 mL) a volume of 0.1 mL of the plant extract was mixed with 7.5 mL of distilled water, into which 0.5 mL of the Folin-Ciocalteu phenol reagent was added. Approximately, 1 mL of 35% solution of Sodium Carbonate was added and the mixture was diluted with 10 mL of distilled water. The mixture was then shaken well and incubated in dark environment at room temperature for 30 minutes. Gallic acid was used as reference standard in varying concentrations (1 to 0.0625 mg/mL) prepared using the same procedure as test samples. The absorbance for the standard and the test samples was determined against the blank reagent at 725 nm using UV/visible spectrophotometer (Beckman Coulter-DU730, USA). The tannin content was expressed as mg of GAE/g of extract.

Total flavonoid content: The total flavonoid content was determined using the Aluminium Chloride colorimetric assay as described by Zhishen *et al.* (1999). Approximately, 1 mL of plant extract was diluted with 4 mL of distilled water in a volumetric flask and 0.3 mL of 5% sodium nitrite. The mixture was incubated for 5 min and 0.3 mL of 10% aluminium chloride was added. An amount of 2 mL of 1M Sodium Hydroxide (NaOH) was added after 5 min. The standard was prepared using a serial dilution of quercetin (1 to 0.0625 mg/mL) in place of the extract. The mixture was then diluted to 10 mL with distilled water and left to stand for 30 min after which the absorbance was recorded at 510 nm using UV/visible spectrophotometer (Beckman

Coulter-DU730, USA). The total flavonoid content was expressed as mg of QE/g of plant extract.

4.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using the Statistix 10.0. When the treatments were significant at the probability level of 5%, the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fisher's Least Significant Difference Test ($P \leq 0.05$). The variable with significant ($P \leq 0.05$) treatment means were further subjected to lines of the best fit using phytochemicals and antioxidant activity responses to different harvesting times. Unless otherwise stated, only treatment means significant at the probability level of 5% were discussed.

4.3 Results

Different harvesting times had highly significant effects ($P \leq 0.01$) on antioxidant activity, total flavonoid and phenol contents contributing 62, 88 and 60% in TTV, respectively (Table 4.1). However, harvesting times did not affect the total tannin contents (Table 4.1). Relative to harvesting in February, harvesting times increased total flavonoid contents by 2-137% (Table 4.2). However, harvesting times decreased the antioxidant activity and total phenol contents by 10-28 and 29-52%, respectively (Table 4.2). Antioxidant activity, total flavonoid and phenol contents over different harvesting times of *J. zeyheri* leaves exhibited negative quadratic relations with the models explained by 50, 95 and 87% respectively (Figure 4.1-4.3). Different harvesting times increased the antioxidant activity and total phenol contents, but displayed the

existence of negative quadratic relationships (Figure 4.1-4.2). The total flavonoid contents over different harvesting times were increasing displayed the existence of negative quadratic relationships (Figure 4.3). However, optimum harvest time (x) using $-b_1/2b_2$ could not be achieved since the phytochemicals and antioxidant activity displayed the existence of negative quadratic relationships.

Table 4.1 Partitioning mean sum of squares of antioxidant activity (AA), total flavonoids (TFC), phenol (TPC) and tannins contents (TTC) to different harvesting time on quality of *Jatropha zeyheri* leaves (n=50).

		AA (mg GAE/g)		TFC (mg QE/g)		TPC (mg GAE/g)		TTC (mg GAE/g)	
Source	DF	MSS	TTV (%)[z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	550.78	26	2195.3	4	376.93	24	114663	29
Treatment	4	1350.33	62***	48390.1	88***	941.23	60***	189736	49[ns]
Error	36	261.79	12	4150.2	8	242.14	16	84245	22
Total	49	2162.89	100	54735.6	100	1560.30	100	388644	100

[ns] = non-significant at P ≥ 0.05; *** = highly significant at P ≤ 0.01.

Table 4.2 Impact of different harvesting times on antioxidant activity (AA), total flavonoid (TFC) and total phenol (TPC) contents of *Jatropha zeyheri* leaves (n = 50).

	AA (mg GAE/g)		TFC (mg QE/g)		TPC (mg GAE/g)	
Harvesting months	Value	RI (%)[z]	Value	RI (%)	Value	RI (%)
February	39.1^{aby} ± 0.19	–	106.9^{bc} ± 0.15	–	30.8^{a} ± 0.16	–
March	32.3^{bc} ± 0.16	–17	108.8^{b} ± 0.19	2	21.9^{c} ± 0.03	–29
April	28.1^{c} ± 0.11	–28	103.4^{c} ± 0.13	–3	14.8^{c} ± 0.05	–52
May	35.4^{a} ± 0.14	–10	137.7^{b} ± 0.10	29	19.4^{ab} ± 0.04	–37
June	30.7^{bc} ± 0.10	–21	253.5^{a} ± 0.13	137	16.9^{bc} ± 0.02	–45

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Fisher's Least Significant Difference test.

[z] Relative impact = [(treatment/control – 1) × 100].

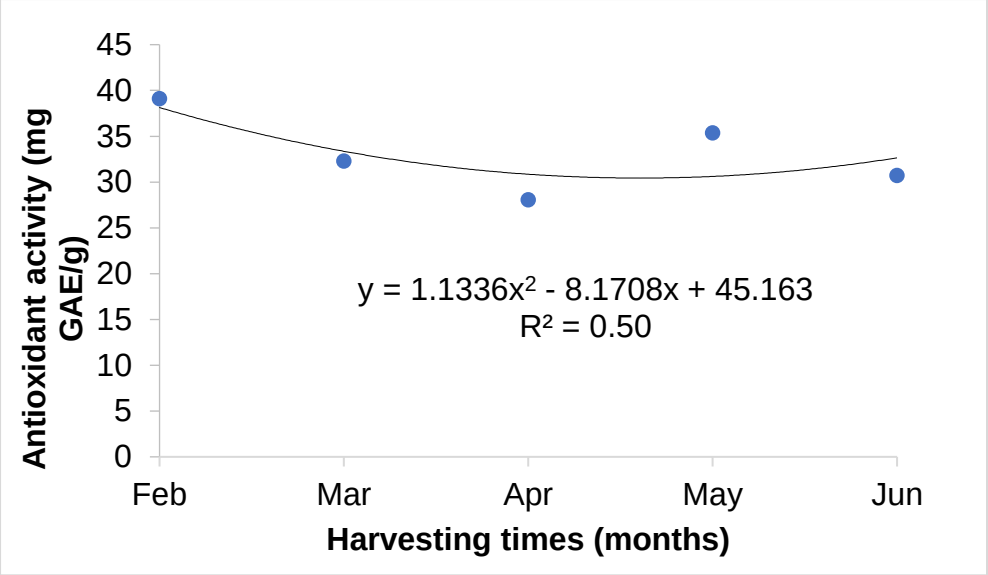

Figure 4.1 The response of antioxidant activity to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

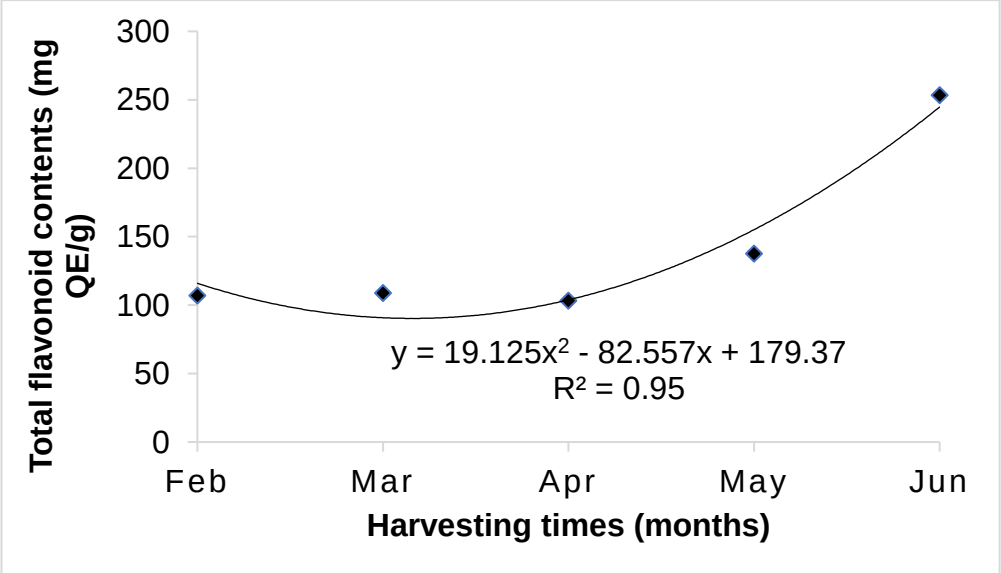

Figure 4.2 The response of total flavonoid contents to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

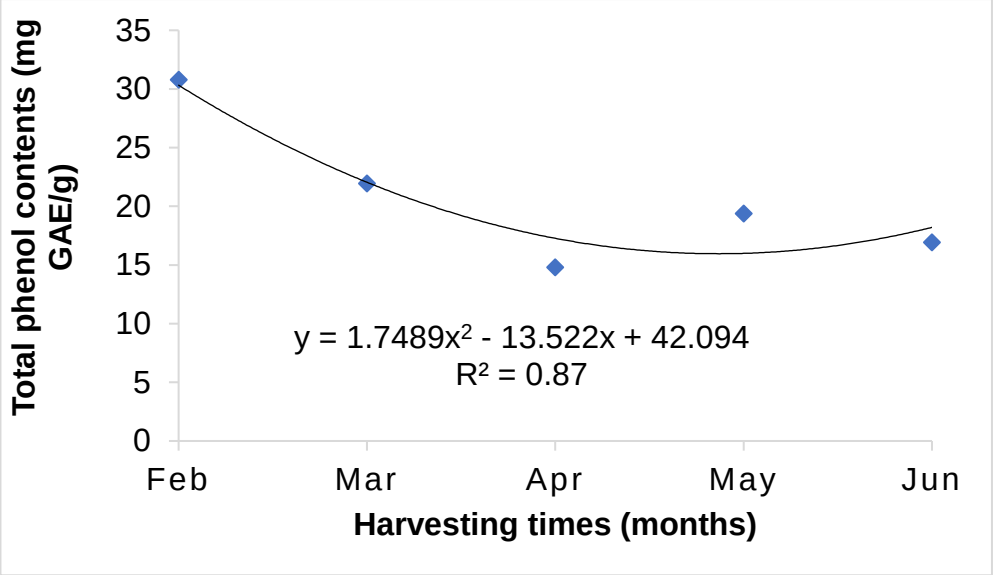

Figure 4.3 The response of total phenol contents to different harvesting times of *Jatropha zeyheri* leaves (n = 50).

4.4 Discussion

In the current study, harvesting times had an effect on antioxidant activity of *J. zeyheri* leaves. Similar results were reported on antioxidant activity of green tea leaves (*Camellia sinensis* L.) (Ercisli *et al.,* 2008; Lee *et al.,* 2014; Ertuk *et al.,* 2010), wild bush tea (*Athrixia phylicoides* L.) (Mudau *et al.,* 2008), black currant herbal tea (*Ribes nigrum* L.) (Nour *et al.,* 2014) and *Talinum triangulare* (L.) (Brasileiro *et al.*, 2015). In contrast, harvesting times did not affect the antioxidant activity of black tea (*C. sinensis*) (Kaur *et al.,* 2014). In this current study, the total tannin contents were not affected by different harvesting times. Similar results were reported on bush tea (*A. phylicoides*) where the total tannin contents were not affected by harvesting times (Mudau and Makunga, 2018). Contrary findings were observed on bush tea (*A. phylicoides*) (Mudau *et al.,* 2007a) and *Acer truncatum* where harvesting times affected the total tannin contents (Yang *et al.,* 2017).

Phytochemical constituents serve as defence mechanism against unfavourable conditions such as a variety of herbivores or aggression by pathogens and therefore contribute to plant quality (Brunetti *et al.,* 2015). The antioxidant activity in plants is associated with beneficial compounds such as phenols, tannins and flavonoids and is regarded as a great indicator of medicinal potential of herbal tea (Hirasawa *et al.,* 2002). Generally, phytochemicals and antioxidant activity of the plants are affected by temperature fluctuations, rainfall patterns and UV radiation (Gobbo-Neto and Lopes, 2007).

Harvesting times also significantly affected the total phenol contents in *J. zeyheri* leaves. Similar findings were noted on green tea leaves (*C. sinensis*) (Ercisli *et al.,* 2008; Ertuk *et al.,* 2010), black tea leaves (*C. sinensis*) (Kaur *et al.,* 2014; Jayasekera *et al.,* 2014), black currant herbal tea (*R. nigrum*) (Nour *et al.,* 2014) and on *T. triangulare* (Brasileiro *et al.,* 2015). Contrary findings were reported on green tea (*C. sinensis*) (Ahmed *et al.,* 2014), *Salvia officinalis* (Farmani *et al.,* 2012) and wild carqueja (Silva *et al.,* 2007) where harvesting times did not affect the total phenol contents.

In this current study, harvesting times affected total flavonoid contents in *J. zeyheri* leaves. Similar results were reported on total flavonoid contents on oolong tea (*C. sinensis* L.) (Wang *et al.,* 2006; Chen *et al.,* 2010), green tea (*C. sinensis*) (Hilton and Palmer-Jones, 1973) and on hawk tea (*Litsea coreana* L.) (Chen *et al.,* 2019). Total flavonoid contents were affected by harvesting times on *Lessertia frutescens* (Campbell, 2012), *Pseudobombax marginatum* (Chaves *et al.,* 2013), *Morus alba* (L.)

(Levickienė *et al.,* 2018), *A. truncatum* (Yang *et al.,* 2017), *Diospyros kaki* (L.f.) (Hossain *et al.,* 2018) and on *Armoracia rustican* (Tomsone and Kruma, 2017).

In this current study, different harvesting times decreased the antioxidant activity of *J. zeyheri* leaves. Similar trends were reported on green tea (*C. sinensis*) (Yazici and Goksu, 2017; Ahmed *et al.,* 2014), black tea (*C. sinensis*) (Jayasekera *et al.,* 2011), *Melilotus indicus* (L.) (Ahmed *et al.,* 2012), *A. rustican* (Tomsone and Kruma, 2017) and on *T. triangulare* (Brasileiro *et al.*, 2015). Contrary to the current findings, harvesting times increased antioxidant activity of green tea leaves (*C. sinensis*) (Ertuk *et al.,* 2010; Lee *et al.,* 2014). The behaviour of antioxidant activity exhibited density-dependent growth (DDG) pattern when exposed to different harvesting times on *Chelodonium majus* (L.) (Jakovljević *et al.,* 2013) and *Moringa oleifera* (L.) (Arena and Radice, 2016).

Different harvesting times decreased the total phenol contents in *J. zeyheri* leaves. Similar trends were observed on black tea (*C. sinensis*) (Kaur *et al.,* 2014; Jayasekera *et al.,* 2011), black currant herbal tea (*R. nigrum*) (Nour *et al.,* 2014) and on *M. indicus* (Ahmed *et al.,* 2012). In contrast, harvesting times increased total phenol contents on green tea (*C. sinensis*) (Ertuk *et al.,* 2010; Ölmez and Yilmaz, 2010), *A. truncatum* (Yang *et al.,* 2017) and on *Mentha longifolia* (L.) (Ahmad *et al.,* 2011). The behaviour of total phenol contents exhibited DDG pattern when exposed to different harvesting times on hawk tea (*L. coreana*) (Chen *et al.,* 2019) and *M. oleifera* (Arena and Radice, 2016).

Temperature plays an important role on the synthesis of total phenol contents which in turn affects the antioxidant activity (Aldrich *et al.,* 2011). For instance, the changes in temperature may affect the uptake and accumulation of phenol contents of the leaves and therefore affect antioxidant activity (Digehsara *et al.,* 2012). Generally, the strength of antioxidant activity depends largely on the total phenol contents. Li *et al.* (2009) reported that the antioxidant activity of plants has strong relationship with the total phenol contents. For instance, generally in plants, the higher the total phenol contents the higher the antioxidant activity and vice versa. Kim *et al.* (2016) also suggested that antioxidant activity is affected by the phenolic contents in tea leaves. This may be argued on the postulation by Duda *et al.* (2015) who reported that the phenol contents in plants may differ among organs, tissues and different seasons and may have effect on the antioxidant activity.

Generally, variations in climatic conditions may affect the synthesis and the accumulation of total phenols in tea leaves (Yao *et al.,* 2005; Chen *et al.,* 2010). Harbowy and Balentine (1997) also suggested that the biosynthesis of total phenol contents is induced effectively by daylength and stronger sunlight. The development of leaves may also affect the total phenol contents in plants. Ahmed *et al.* (2012) postulated that more matured leaves have less total phenol contents than young developing leaves, due to their low biosynthetic activity. Heldt (2005) also suggested that the immature plant tissues produce more phenol contents when compared to fully matured tissues. The content of total phenols in tea leaves are important as they are indicators of sensory properties such as astringency, bitterness, flavour and colour of the plant leaves therefore, high quality of final product (Boyer and Liu, 2004).

In the current study, harvesting times had an increasing trend on total flavonoid contents in *J. zeyheri* leaves. Similar trends were reported on total flavonoid contents on oolong tea (*C. sinensis*) (Chen *et al.,* 2010), *Grewia flava* (Gololo *et al.,* 2016), *M. indicus* (Ahmed *et al.,* 2012), blueberry (Cezarotto *et al.,* 2017) and *M. longifolia* (Ahmad *et al.,* 2011). Contrary findings were reported on hawk tea (*L. coreana*) (Chen *et al.,* 2019), *M. alba* (Levickienė *et al.,* 2018), *A. truncatum* (Yang *et al.,* 2017) and *C. majus* (Jakovljević *et al.,* 2013) where total flavonoid contents exhibited DDG patterns in different harvesting times. Harvesting times decreased total flavonoid contents on *A. rustican* (Tomsone and Kruma, 2017), *D. kaki* (Hossain *et al.,* 2018) and on *P. marginatum* (Chave *et al.,* 2013).

The total flavonoid contents in leaves may be affected by the plant physiological status, environmental and climatic conditions (Digehsara *et al.,* 2012). For instance, the biosynthesis of flavonoids is increased during periods of drought and cold temperatures (Winkel-Shirley, 2002; Lillo *et al.,* 2008). Low temperature conditions and low light irradiance which occur during cold harvesting months, are also the main climatic conditions which may enhance the synthesis of flavonoid contents in plant leaves (Buchanan *et al.,* 2000; Harborne and Williams, 2000; Garmash, 2005). The total flavonoid contents in plant leaves are affected by the maturity of the leaves, for instance, more mature leaves have more total flavonoid contents as compared to young leaves (Buchanan *et al.,* 2000; Harborne and Williams, 2000; Garmash, 2005). Also, during different harvesting months, when plant maturity is increasing, most plants first concentrate the total flavonoid contents in other plant parts, then later concentrate the total flavonoid contents in plant leaves (Venskutonis *et al.,* 2016). Total flavonoid contents in plant leaves are important as their consumption is associated with reduced

risk of cardiovascular disease based on wide epidemiological evidence (Hooper *et al.,* 2008).

In this study, optimisation of harvest time for *J. zeyheri* leaves was not determined which means it would still be necessary to establish the appropriate harvesting time by harvesting from early summer to winter. For instance, since harvesting in February resulted in high contents of total phenols, it could imply that harvesting in January would result in even higher total phenol contents. Alternatively, since harvesting in June resulted in high total flavonoid contents, it could imply that harvesting in July would result in even higher total flavonoid contents. Mudau *et al.* (2006) reported that the ideal harvesting time of bush tea is in winter (June-August) and summer (December-February) months for maximum polyphenolic contents. This contradicts to the findings reported by Le Gall *et al.* (2004) that the highest quality green teas with higher phenolic compounds (epigallocatechin gallate, epicatechin gallate, gallic acid), were harvested during the first flush in late April and early May and quality decreases in later harvests. Concurrently, teas harvested in summer and autumn months had less phenol contents and therefore, are considered as more astringent than those harvested in spring months (Pan *et al.,* 2015).

4.5 Conclusion

The results from this study demonstrated that antioxidant activity, total phenol and flavonoid contents were affected by harvesting times. Different harvesting times increased total flavonoid contents but decreased the antioxidant activity and total phenol contents. Antioxidant activity, total phenol and flavonoid contents exhibited negative quadratic relations. The findings further suggested that a study be conducted

where harvesting of *J. zeyheri* leaves could start from early summer until winter to find the optimum harvesting time for phytochemicals and antioxidant activity.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

The study investigated the influence of harvesting times on mineral elements, phytochemicals and antioxidant activity of *Jatropha zeyheri* leaves. The accumulation of essential mineral elements in *J. zeyheri* leaves increased at earlier harvesting times but were decreased at later harvesting times. However, non-essential mineral elements were lower at earlier harvesting times but were higher at later harvesting times. Although majority of essential mineral elements increased with an increase in harvesting times, K was gradually reduced with increasing harvesting times. Considerable levels of heavy metals were found in *J. zeyheri* leaves. When compared to other teas, *J. zeyheri* leaves showed higher contents of Mg and Ca than the majority of selected tea types. Contrarily, contents of K, P and S were lower in *J. zeyheri* leaves as compared to most selected tea types. Furthermore, the optimisation value for selected essential mineral elements was found to be 2.46 months, which was translated to harvesting in between April-May.

Harvesting times had effects on phytochemicals and antioxidant activity of *J. zeyheri* leaves. Total phenol contents and antioxidant activity were higher at earlier harvesting times but were lower at later harvesting times. Contrarily, total flavonoid contents were lower at earlier harvesting times but were higher at later harvesting times. Total phenol contents and antioxidant activity were both lower with increasing harvesting times. In contrast, under increasing harvesting times of *J. zeyheri* leaves, total flavonoids displayed an increasing trend.

5.2 Significance of findings

The results of this study indicated that harvesting times increased the accumulation of essential and non-essential mineral elements in *J. zeyheri* leaves. Findings of this study also indicated that harvesting in April-May could improve the accumulation of most essential mineral elements in *J. zeyheri* leaves. The results showed that non-essential mineral elements were increased with later harvesting months. Additionally, the results demonstrated that *J. zeyheri* leaves are a rich source of several essential mineral elements. The study also indicated that *J. zeyheri* leaves contained different levels of phytochemicals and antioxidant activity. The presence of both essential and non-essential mineral elements, phytochemicals and antioxidant activity in *J. zeyheri* leaves is valuable for improving the quality of tea and human health. The results further demonstrated that the total flavonoid contents were increased with later harvesting times. However, total phenol contents and antioxidant activity were decreased with an increase in harvesting times. The study suggested that harvesting of *J. zeyheri* leaves be done at 2.46 months, which translates to approximately harvesting between April-May. These findings could be used by rural people and small-holder farmers for maintaining quality of *J. zeyheri* indigenous tea.

5.3 Recommendations

In this study, increasing harvesting times have been shown to increase the majority of essential mineral and some non-essential elements. Even though the current findings were not intended to find the factors that contribute to high levels of heavy metals in tea. The latter could be important in understanding the factors, for instance soil types, cultivation methods and site which may contribute to high levels of heavy metals in *J. zeyheri* leaves. Consequently, investigate the acceptable levels of heavy metals which

could be attained since heavy metals are required at low levels by the human body for proper functioning and healthy developments (Watrak *et al.,* 2016). Also, the total phenol contents and antioxidant activity were found to be lower with an increase in harvesting time, it could be necessary to establish a further study where harvesting could commence earlier during first leaf flushes. Since the findings of this study suggested that *J. zeyheri* leaves be harvested at 2.46 months for improving the accumulation of most mineral elements, it would be necessary to extend the harvesting time from months to weeks. This would help in tracing chemical composition changes for leaf flushes until the leaves are mature.

5.4 Conclusions

The results of this study demonstrated that both essential and non-essential mineral elements were increased with an increase in harvesting times of *J. zeyheri* leaves, except for K which was gradually decreased. Also, as shown by the optimisation equation, harvesting of *J. zeyheri* leaves was optimised at 2.46 months, which was translated to harvesting between April-May. The results of the study indicated that certain levels of heavy metals were found in *J. zeyheri* leaves, which suggested further studies to investigate the acceptable levels of heavy metals. The total phenol contents and antioxidant activity were decreased with an increase in harvesting times, whereas the total flavonoid contents were increased with an increase in harvesting times of *J. zeyheri* leaves. The results from phytochemicals and antioxidant activity showed a density dependant growth patterns where increase in harvesting times resulted in either increase or decrease of the parameters. Therefore, the study recommended further studies, to achieve optimisation. The current study indicated that *J. zeyheri* leaves contain high levels of most mineral elements, which is an indicator of good tea

quality. Therefore, the study concluded that harvesting times improved the development and quality of *J. zeyheri* indigenous tea.

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APPENDICES

Appendix 3.1 Analysis of variance for calcium to different harvesting times of J*atropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	9.434E+07	1.048E+07		
Treatment	4	1.481E+08	3.703E+07	3.09	0.03
Error	36	4.317E+08	1.199E+07		
Total	49	6.741E+08			

Appendix 3.2 Analysis of variance for copper to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	765.35	85.039		
Treatment	4	793.23	198.307	3.45	0.02
Error	36	2072.29	57.564		
Total	49	3630.88			

Appendix 3.3 Analysis of variance for iron to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	603532	67059		
Treatment	4	694161	173540	6.34	0.00
Error	36	985011	27361		
Total	49	2282704			

Appendix 3.4 Analysis of variance for potassium to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	4.015E+07	4461136		
Treatment	4	8.998E+07	2.250E+07	5.78	0.00
Error	36	1.402E+08	3894981		
Total	49	2.704E+08			

Appendix 3.5 Analysis of variance for magnesium to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	2.016E+07	2239560		
Treatment	4	2.621E+07	6552792	3.75	0.01
Error	36	6.295E+07	1748525		
Total	49	1.093E+08			

Appendix 3.6 Analysis of variance for manganese to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	370233	41137		
Treatment	4	777351	194338	3.31	0.02
Error	36	2116547	58793		
Total	49	3264131			

Appendix 3.7 Analysis of variance for nickel to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	17365.8	1929.54		
Treatment	4	14015.4	3503.86	3.63	0.01
Error	36	34770.2	965.84		
Total	49	66151.4			

Appendix 3.8 Analysis of variance for phosphorus to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	1322560	146951		
Treatment	4	1889605	472401	5.78	0.00
Error	36	2944782	81799		
Total	49	6156947			

Appendix 3.9 Analysis of variance for sulphur to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	1382961	153662		
Treatment	4	2179715	544929	5.66	0.00
Error	36	3465244	96257		
Total	49	7027920			

Appendix 3.10 Analysis of variance for zinc to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	6616.6	735.183		
Treatment	4	3188.5	797.117	1.89	0.13
Error	36	15177.2	421.588		
Total	49	24982.3			

Appendix 3.11 Analysis of variance for aluminum to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	486965	54107		
Treatment	4	600277	150069	3.26	0.02
Error	36	1659107	46086		
Total	49	2746349			

Appendix 3.12 Analysis of variance for cobalt to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	13636.4	1515.16		
Treatment	4	11562.3	2890.58	2.71	0.04
Error	36	38348.7	1065.24		
Total	49	63547.5			

Appendix 3.13 Analysis of variance for chromium to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	100594	11177.1		
Treatment	4	98080	24520.0	3.67	0.01
Error	36	240587	6683.0		
Total	49	439261			

Appendix 3.14 Analysis of variance for sodium to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	588428	65381		
Treatment	4	625569	156392	3.09	0.03
Error	36	1821614	50600		
Total	49	3035610			

Appendix 3.15 Analysis of variance for silicon to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	2456.4	272.938		
Treatment	4	605.7	151.415	0.59	0.67
Error	36	9291.6	258.101		
Total	49	12353.7			

Appendix 4.1 Analysis of variance for antioxidant activity to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	4957.0	550.78		
Treatment	4	5401.3	1350.33	5.16	0.0022
Error	36	9424.5	261.79		
Total	49	19782.9			

Appendix 4.2 Analysis of variance for total flavonoids to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	19757	2195.3		
Treatment	4	193561	48390.1	11.66	0.00
Error	36	149409	4150.2		
Total	49	362726			

Appendix 4.3 Analysis of variance for total phenolic contents to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	9	3392.4	376.932		
Treatment	4	3764.9	941.227	3.89	0.01
Error	36	8717.2	242.144		
Total	49	15874.5			

Appendix 4.4 Analysis of variance for total tannin contents to different harvesting times of *Jatropha zeyheri* leaves.

Source	Df	SS	MS	F	P
Replication	4	458651	114663		
Treatment	4	758942	189736	2.25	0.11
Error	16	1347920	84245		
Total	24	2565514			