SELECTION OF APPROPRIATE PLANT PARTS WITH SUITABLE CHEMICAL PROPERTIES FOR THE DEVELOPMENT OF *JATROPHA ZEYHERI* INDIGENOUS TEA BEVERAGE

MAMABOLO LERATO KATEDI

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

SUPERVISOR : DR K.G. SHADUNG

CO-SUPERVISOR : DR M.Y. MAILA

2020

TABLE OF CONTENTS

DECLARATION

I, Lerato Katedi Mamabolo, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree 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: Lerato Katedi Mamabolo	Signature	Date

DEDICATION

To my grandmother (Mrs Mogafe Mamabolo), my parents (Mr Thabakgolo and Mrs Mankhubu Mamabolo) and my siblings (Maisha, Letago and Phenyo).

ACKNOWLEDGEMENTS

Firstly, I would like to extend words of gratitude and acknowledgements to the Almighty God who has always been faithful and gave me strength, courage and wisdom during my studies. Indeed, He is the God who keeps his promises, and for that, I will forever give him praises. Secondly, I would like to express my deep appreciation to my supervisors, Dr K.G. Shadung and Dr M.Y. Maila for their patient guidance, enthusiastic encouragement and useful critiques of this research work. Their willingness to give their time so generously has been very much appreciated. Thank you for not giving up on me. Special thanks to my research colleagues (Happy Bango and Annah Sehlapelo), your effort and time on various aspect of my research project are appreciated.

I am grateful to the Department of Plant Production, Soil Science and Agricultural Engineering for sponsoring my conference trips to Faculty Research Day in 2018 and 2019, where I presented two oral papers. I would like to thank Limpopo Agro-Food Technology Station (LATS) for financial assistance on consumables, analysis and sponsoring my conference trip during MSc programme to Combined Congress, University of Free State in 2019, where I presented an oral paper. Also, my appreciations are extended to National Research Foundation (NRF) for financially supporting me from 2018-2020.

My love goes to my family especially my beloved parents Mr T.F. Mamabolo and Mrs M.E. Mamabolo, I say thank you for always praying and encouraging me. Also, my siblings (Tshepo, Letago and Phenyo), thank you for your love and moral support. Lastly, Miss Jennifer Sekwakwa, Mrs Given Mogwanye and Mr Matidi Thaba, thank

you for always supporting, loving and encouraging me throughout my study. May the Lord bless you, keep you and make His face to shine upon you.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Most rural communities harvest different plant parts of *Jatropha zeyheri* for nutritional and medicinal purposes. However, the decision of choosing to harvest the plant part with desired chemical components is essential for quality purposes. Nevertheless, there is a lack of information regarding the distribution of chemical composition in different plant parts of *J. zeyheri* tea. Therefore, the objectives of the study were to: (1) investigate the effect of different plant parts (stems, roots and leaves) of *J. zeyheri* on mineral composition and, (2) determine the effect of different plant parts (stems, roots and leaves) of *J. zeyheri* on phytochemicals and antioxidant activity. A study was conducted with three treatments, namely stems, roots and leaves arranged in a randomised complete block design (RCBD), with 10 replications. Plant materials were collected in the wild from mature plants between June-July 2018. Leaves and stems were manually separated, while roots were cut into smaller pieces, dried at 60°C for 48 hours in an air-forced oven and later finely ground using an electric grinder. Mineral elements were determined using Inductive Coupled Plasma Emission (ICPE-9000 Shimadzu, Japan). Phytochemical tests were performed to detect the presence of flavonoids, tannins, alkaloids and saponins, whereas 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant qualitative test was performed using TLC plates. Antioxidant activity and phytochemicals constituents were quantified using UV/Visible spectrophotometer. Results of this study demonstrated that plant parts had a highly significant effect on essential mineral elements, copper (Cu), iron (Fe), potassium (K), magnesium (Mg), zinc (Zn), manganese (Mn), phosphorus (P) and nickel (Ni) contributing 82, 75, 97, 88, 86, 96, 79 and 82% to total treatment variation (TTV), respectively, whereas, calcium (Ca) had a significant effect contributing 69%, while no significant effect on sulphur (S) was observed. Among the tested essential mineral

elements, Ca, Zn, Cu and Ni were consistently the highest in the leaf, followed by stem whereas, the root had the lowest contents. Manganese and Mg were consistently the highest in the stem, followed by leaf whereas, the root had the lowest content. Iron was highest in the leaf followed by root and was lowest in the stem. Also, the stem had the highest content of K whereas, the leaf and the root had moderately lower content, and P was high in the leaf, whereas, stem and root had lower content. Similarly, plant parts had a highly significant effect on non-essential mineral elements, aluminium (Al), sodium (Na), cobalt (Co) and chromium (Cr) contributing 85, 72, 84 and 81% in TTV, respectively, whereas, not significant on silicon (Si). Among the tested non-essential mineral elements, the leaf had the highest content of Al, Na, Cr, and Co followed by the stem, whereas, the root had the lowest. The screening of phytochemicals showed that flavonoids, tannins, alkaloids and saponins were present within different plant parts of *J. zeyheri*. The results from DPPH qualitative assay of *J. zeyheri* plant parts showed more yellow spots in leaf and root whereas, there was lesser amount in stem indicating that the plant exhibited antioxidant activity. Plant parts had highly significant effect on total flavonoids contributing 72% in TTV, however, had a significant effect on tannins and antioxidant activity contributing 56 and 79% in TTV, respectively. In contrast, plant parts were not significant on total phenols. The leaf had the lowest of total flavonoids content, whereas root and stem reported the higher contents and root had a lower content of tannins and antioxidant activity, whereas stem and leaf reported higher contents. In conclusion, the majority of chemical properties were recorded in leaves followed by stems and lastly in the roots. The result of this study suggested that *J. zeyheri* tea beverage can be brewed from leaves predominantly followed by stems or alternatively they can be combined.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Jatropha zeyheri is an indigenous plant growing naturally in the wild and is from Euphorbiaceae family. It is a perennial densely hairy plant with simple or sparsely branched stems, thick roots and alternate, sessile and shortly petioled leaves (Arnold *et al*., 2002). Genus, *Jatropha* comprises of about 170 species which are widely distributed in South Africa and neighbouring countries such as Botswana, Zimbabwe and Swaziland. In South Africa (SA) it is predominantly found in Limpopo, Gauteng, North West, Mpumalanga and KwaZulu-Natal province (Van Wyk, 2008). The roots of *J. zeyheri* are used by traditional practitioners to treat sexually transmitted diseases, urinary tract infection and irregular menstrual pains (Van Wyk and Gericke, 2000), whereas the leaves of *J. zeyheri* are used to make tea beverage. The plant is also used as a general blood purifier to promote fertility and used by small scale farmers to treat wounds in livestock (Van Wyk and Gericke, 2000).

Tea is one of the oldest beverages which is manufactured from various plant parts, and it is largely consumed worldwide (Mudau *et al.,* 2007). It is the second-largest beverage consumed after water (Ho *et al*., 2009; Zhen, 2002) and its popularity depends on the flavour that is divided into taste and aroma (Hara *et al.,* 1995). According to Food and Agriculture Organization (2015), world tea production (black, green and white) increased significantly by 6% to 5.07 million tonnes in 2013. Tea is produced in more than 50 countries, with major producers being China, India, Kenya, Sri Lanka, Vietman, Turkey, Indonesia and Iran (Food and Agriculture Organization, 2014). China remains one of the largest tea producing country with an output of 1.9 million tonnes (Food and Agriculture Organization, 2015). The production of tea in SA

was minimal constituting less than 0.12% of the world's production in 2008 (Food and Agriculture Organization Statistical Database, 2010).

The chemical composition useful for tea properties is reported to be concentrated in different plant parts, which could be leaves, stems and roots (Cunningham, 1993). In honeybush (*Cyclopia genistoides*), the leaves and stems are used to make tea beverage (Department of Agriculture, Forestry and Fisheries, 2016). Additionally, the dried or fresh leaves of bush tea (*Aspalathus linearis*) are used as a tea beverage (Mabogo, 1990; Swanepoel, 1997), while infusion made of leaves and stems is used to treat hypertension, circulation and heart problems, diabetes, diarrhoea and vomiting (Rampedi and Olivier, 2005). Also, decoction from roots of bush tea (*Aspalathus linearis*) is used as a purgative and a cough medicine, whereas, stems are used to make brooms (Van Wyk and Gericke, 2000). In *J. zeyheri* the stem is used to speed up the healing process of burns and cuts, whereas, the infusion of the roots is used to treat irregular menstrual pains. Foetal development during pregnancy is also improved, and the dried leaves are boiled, and the extract is drunk with sugar as a tea beverage (Van Wyk and Gericke, 2000).

Tea harvesting, processing, leaf maturation, botanical varieties, geographical origin and agricultural practices have a significant effect on the selection of suitable plant part for use in tea production, which in turn could affect its taste and chemical composition (Gonzalex de Mejia *et al*., 2009). Careful handling, using appropriate methods for harvesting different plant parts and transportation are important factors to consider in maintaining the tea quality (Ahmed and Stepp, 2012). Appropriate selection of plant part(s) with suitable tea properties and desired chemical components

is important for tea quality purposes (Ho *et al.,* 2009; Yashin *et al*., 2005). Therefore, the current study focuses on the selection of appropriate plant parts with suitable chemical properties for the development of *J. zeyheri* indigenous tea.

1.2 Problem statement

Worldwide, indigenous teas are playing a vital role through their nutritional, stimulant, cultural, relaxation and medicinal properties to the lives of rural communities. Currently, appropriate plant parts to be harvested in *J. zeyheri* indigenous teas have not been determined. The researcher intends to establish the distribution of phytochemicals, antioxidant activity and mineral elements in stems, roots and leaves of *J. zeyheri* in order to select suitable plant parts that should be used for the development of the tea beverage.

1.3 Rationale of the study

Most of the rural communities harvest different parts of *J. zeyheri* in their dry state after physiological maturity of the plant in order to make tea. This is contrary to what is practised in the tea industry because old plant parts are regarded as agricultural waste and believed to have lost important tea compounds (Yasari *et al.,* 2009). The decision of choosing to harvest the part with desired chemical components is important for quality purposes. However, there is a lack of information regarding the distribution of chemical components in different plant parts of *J. zeyheri* tea plant. The current research focuses on finding appropriate plant part to improve the standard of *J. zeyheri* tea.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study is to evaluate the quality of various plant parts of *J. zeyheri* for the development of an indigenous tea beverage.

1.4.2 Objectives

The objectives of this study are:

(i) To investigate whether plant parts (stems, roots and leaves) of *J. zeyheri* would have similar mineral composition.

(ii) To determine whether plant parts (stems, roots and leaves) of *J. zeyheri* would have similar phytochemicals and antioxidant activity.

1.4.3 Hypotheses

(i) Mineral composition in stems, roots and leaves of *J. zeyheri* do not differ.

(ii) Phytochemicals and antioxidant activity in stems, roots and leaves of *J. zeyheri* do not differ.

1.5 Reliability, validity and objectivity

In this study, the reliability of data 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 results are discussed on the basis of empirical evidence, thereby eliminating all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was reduced by minimising the experimental error by increasing the number of replications and randomising treatments.

1.7 The scientific significance of the study

Findings of this study would expand knowledge and provide the opportunity to harvest appropriate plant parts that would be useful for the development of *J. zeyheri* indigenous tea and thus, improve the tea quality.

1.8 Structure of mini-dissertation

The mini-dissertation was designed using the Senate-approved format of the University of Limpopo. Subsequent to the description and detailed outlining of the research problem (Chapter 1), work done, and the work not done on the research problem were reviewed (Chapter 2). Then, each of the two objectives would constitute a separate Chapter (Chapters 3-4). In the final chapter (Chapter 5), results from all chapters would be summarised and integrated to provide the significance of the results and recommendations with respect to future research and then culminated in an overall conclusion of the study. The Harvard referencing style, as approved by the University Senate, was adopted in this mini-dissertation.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Globally, indigenous teas have gained popularity due to the presence of chemical compounds in different plant parts which are responsible for its medicinal, nutritional, relaxation and cultural properties (Maroyi, 2017). Chemical compounds such as phytochemicals, mineral elements and antioxidant activity have been reported to affect the quality of tea which in turn affect its price (Mudau *et al*., 2007; Owour *et al*., 2000). This literature review will be limited to chemical composition and economic importance of tea.

2.2 Chemical composition of tea

2.2.1 Phytochemicals and antioxidant activity

Phytochemicals are a group of naturally and biologically occurring chemical compounds found in plants (Hasler and Blumberg, 1999). They accumulate in different plant parts, such as in the root, stem, leaf, flower, fruit and seed (Costa *et al*., 1999). These compounds protect plants from diseases and environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Gibson *et al.,* 1998; Mathai, 2000). There are several known categories within phytochemicals such as phenolic compounds, tannins, terpenoids, alkaloids, saponins, flavonoids, carotenoids and etc. (Koche *et al*., 2016).

Phenolic compounds: Phenolic compounds are secondary metabolites that are derivatives from pentose phosphate, shikimate and phenylpropanoid pathways in plants (Randhir *et al*., 2004). These compounds represent the largest group of

phytochemicals and are of considerable physiological and morphological importance in plants (Walton *et al*., 2013). These compounds play an important role in growth and reproduction, providing protection against pathogens and predators (Bravo, 1998). They also contain different physiological properties, such as anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Benavente-Garcia *et al*., 1997; Middleton *et al*., 2000; Puupponen-Pimia *et al*., 2001). Tea contains phenolic compounds such as quercetin, myricetin and kaempferol, which exhibit powerful antioxidant capacities (Keli *et al*., 1996). A study by Mathivha and Mudau, (2017) reported that special tea (*Monsonia burkeana*) has higher contents of total phenols than bush tea (*Athrixia phylicoides*). Moreover, a study by Sheikh *et al*. (2015) reported that Egyptian green tea contains a high content of total phenols than black tea (*Camellia sinensis*). The phenolic content in tea is of great importance because it is associated with health benefits (Yang and Liu, 2012).

Flavonoids: Flavonoids are reported to be one of the most important categories because they contain antioxidants. The major examples of flavonoids in tea include iso-flavones, flavones, flavonols, anthocyanins and proanthocyanidins (Wang *et al*., 2000). The major flavonols in tea are: catechin (C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG) (Du *et al*., 2012). Flavonols have recently received much attention due to their pharmaceutical functions such as antioxidative, antitumor and anticarcinogenic activities (Conney *et al*., 1992; Wang *et al*., 2000). Bush tea (*A. phylicoides*) have been reported to contain major flavonoids such as 5-hydroxy 6, 7, 8, 3, 4, 5 -hexamethoxy-flavon-3-ol (Mashimbye *et al*., 2006), whereas, green tea (*C. sinesis*) contains

catechins, epicatechin, gallocatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Gramza *et al*., 2005).

Tannins: Tannins are water-soluble polyphenols, commonly referred to as tannic acid. They are divided into two groups namely hydrolysable and non-hydrolysable (Akiyama *et al*., 2001). They are found in leaves, stems, fruits and seeds of many plants. The main function of these group of compounds is to provide protection against microbial pathogens, harmful insects and other herbivores (Lattanzio *et al*., 2004; Panjehkeh *et al*., 2009). Many herbivores such as cattle, deer and apes do not eat plants with high tannins content because they cause a sharp sensation in the mouth of many mammalian herbivores due to their ability to bind to salivary proteins (Mazid *et al.,* 2011). Khasnabis *et al*. (2015) reported that tannins have antioxidant properties and may act as antinutritional when present at high concentrations. A study suggests that black tea from *C. sinesis* contains the highest amount of tannin as compared to green tea (*C. sinesis*) which contained the lowest amount (Khasnabis *et al.*, 2015). Tannins have received attention in recent years, since the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent different diseases (Serafini *et al*., 1994).

Antioxidant activity: Antioxidants are substances that inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*.,1998). They contain a compound that can protect the biological system from the harmful effect of reactions that can cause excessive oxidation, involving reaction of oxygen and nitrogen, therefore, prevent damage caused by free radicals to cellular components (Javanmardi *et al*., 2003; Mogotlane *et al*., 2007). Tea is an

important dietary source of antioxidants (Carloni *et al*., 2012; Dufresne and Farnworth, 2001). The presence of antioxidants in tea plants provide protection against diseases, scavenge free radicals and reduce their effect on cell damage (Gulcin, 2012; Konan *et al*., 2014). The main antioxidants in tea are catechins, theaflavins, thearubigins, oxyaromatic acids, flavonols and flavones (Yashin *et al*., 2011). Green tea (*C. sinesis*) is high in a class of antioxidants known as catechins whereas, black tea (*C. sinesis*) contains theaflavins and thearubigins (Balentine *et al*., 1997; Lambert and Elias, 2010).

2.2.2 Mineral elements

Mineral elements are considered essential to plant growth and development if the element is involved in plant metabolic functions, and the plant cannot complete its life cycle without the element (Martens and Westerman, 1991). Non-essential mineral elements are elements that are not required for human nutrition (Gjorgieva *et al*., 2010; Maiga *et al*., 2005). Generally, the vegetative parts of the plant such as leaves, stems and roots vary to a greater extent in their mineral composition than fruits, tubers and seeds (Mengel and Kirkby, 1987). Tea plants have been reported to have 28 mineral elements and high amounts of fluorine (F), manganese (Mn), arsenic (As), nickel (Ni), selenium (Se), iodine (I), aluminium (Al) and potassium (K). Additionally, the tea mineral elements within each plant part differ during processing (Zhen, 2002). Moreover, Sultana *et al*. (2014) reported that the most abundant mineral elements in tea plants are N, K, Ca, P, S, Mg and Zn.

2.2.3 Proximate composition

Moisture content is important in the production of tea as it affects processing, energy consumption, production cost, taste, texture, flavour, shelf life and product safety, legal and labelling requirement, economic, microbial stability and food quality (Isengard, 2001). In order to maintain quality in tea, the moisture content should be between 2.5-6.5% (Venkatesan *et al*., 2006). However, Makanjuola (2016) found that moisture content in tea bags was between 6.6-7.2% and this may be due to fermentation process and packaging material. Ash content is the measure of the total amount of mineral elements or inorganic substance after high-temperature combustion (Afify *et al*., 2017; Golding *et al*., 2010). Determining the ash content is important as it is the first step in preparing food samples for specific elemental analysis by using many spectroscopic procedure and forms part of the proximate analysis for nutritional evaluation (Afify *et al*., 2017). Rehman *et al*. (2002) reported that in order to maintain quality and increase shelf life of tea, ash content should be less than 5.54 %. High ash content in tea may be due to less moisture content and less ash content may be due to adulteration using extracted raw material to produce tea which leads to poor quality of tea (Rehman *et al*., 2002).

Crude fibre is the insoluble residue which remains after acid and alkaline hydrolysis. Determination of crude fibre is the measurement of the quantity of indigestible cellulose (Awadasseid *et al*., 2019). The fibre content in tea is an important quality parameter (Adnan *et al*., 2013). The low fibre content in teas may be due to younger tea leaves while high fibre content in tea may be due to use of impurities such as stems (Adnan *et al*., 2013). Previous researchers reported that in order to maintain a high quality of tea during storage, the fibre content should be less than 16.5%

(Smiechowska and Dmowski, 2006; Venkatesan *et al*., 2006). Crude fibre is the total nitrogen content of a product (Young, 2010). Crude protein is calculated by multiplying nitrogen content by the conversion factor (Young, 2010). Tea protein contains bioactive properties such as antioxidant, anti-mutation and protects biological cells against mutagenesis (Li *et al*., 2001 ; Wang and Hu, 2005). Rehman e*t al*. (2002) reported that in order to maintain tea quality, the protein content shoud be between 1-2%.

Crude lipid is the components that are soluble in organic solvents (such as ether, hexane or chloroform) but are insoluble in water (Luthria *et al*., 2004). It is Important to determine crude lipid in tea because lipid content changes during heating, oxidation, processing and storage. The changes in lipid content result in loss of amino acids, browning and formation of bitter taste which in turn affect the quality of tea (Jimoh and Oladiji, 2005; Lien and Nawar,1974). Carbohydrates are commonly classified based on the carbon atom present. The four classes of carbohydrates present in tea are monosaccharides, disaccharides, oligosaccharides and polysaccharides (Asif *et al*., 2011). Carbohydrates present in tea have been reported to provide energy through oxidation, supply carbon for synthesis of cell components and serve as a form of stored chemical energy (Trouvelot *et al*., 2014).

2.3 Plant parts used for tea

Generally, the production of tea involves the use of different plant parts which can either be roots, stems, fruits and leaves (Zwokunda, 2007). However, according to Zeng *et al.* (2017) the oolong tea (*Camellia sinesis*) made from a combination of leaves and stems is considered to have more aroma than when it is made from leaves only.

Also, bush tea (*A. phylicoides*) harvested for tea brewing purposes, leaves and twigs are selected (Zwokunda, 2007). However, Mabogo (1990) and Swanepoel (1997) argued that bush tea (*A. phylicoides*) is made from roots, berries and leaves. The production of white tea (*C. sinesis*) was reported to involve the use of new shoots growth and tender leaves (Soni *et al*., 2015). Rooibos tea (*Aspalathus linearis*) is produced from the harvested shoots and leaves (Rhoda, 2006). In addition, tender shoots of tea consisting of two or three leaves and a bud are harvested periodically to produce either black or green tea (*C. sinesis*) (De costa *et al*., 2007). Harvesting the appropriate plant parts is an important factor that determines the quality of tea (Ho *et al*., 2009; Yashin *et al.,* 2005).

2.4 Economic importance of tea

Tea is an economically important crop contributing significantly to foreign exchange earnings and rural development (Food and Agriculture Organization, 2015). Around 25% of the world import demand and the revenue from tea, represents almost 50% of the countries' foreign currency earnings (Anon, 1996). Millions of livelihoods around the world depend on tea production. The tea industry has become a significant contributor to the economies of producing countries such as Kenya, Sri Lanka, India and China (Gunathilaka and Tularam, 2016). For example, approximately 273 000 ha are devoted to tea cultivation which creates employment for about 2 million people and generates 65% of export agriculture revenue and contributes approximately 4.2% of island's GDP in Sri Lanka (Munasinghea *et al*., 2017). However, for a country such as South Africa which imports most of its tea, tea trade is also important for the economy (Van der Wal, 2008).

2.5 Work not done on the problem statement

The effect of different plant parts on the quality of *J. zeyheri* indigenous tea beverage has not yet been documented. Therefore, the research intended on evaluating the effect of plant parts on mineral elements, phytochemical constituents and antioxidant activity of *J. zeyheri* indigenous tea.

CHAPTER 3
DISTRIBUTION OF ESSENTIAL AND NON-ESSENTIAL MINERAL ELEMENTS ON DIFFERENT PLANT PARTS OF *JATROPHA ZEYHERI*

3.1 Introduction

The uptake of mineral elements by plant roots and their subsequent distribution within the plant has been the subject of many studies for many decades (Karley and White, 2009; Miller *et al*., 2009; Miwa *et al*., 2009; White and Broadley, 2009). Generally, the mineral elements are acquired from the soil solution by plant roots and are dispersed throughout the other plant parts (Hondrogiannis *et al*., 2012). The quantity of essential and non-essential mineral element nutrient in different plant parts depends on the part's accumulative capabilities and the interactions of the mineral element in that specific plant part (Salisbury and Ross, 1992). Information on the accumulative capability of different plant parts is important since it provides the nutritional value of the plant part and assists in selecting parts that are important instead of harvesting the whole plant. Therefore, the determination of mineral element composition in various plant parts is important to understand their overall distribution (Nookabkaew *et al*., 2006). Consequently, the quality of tea is affected by the plant part, which is harvested prior to tea manufacturing (Ho *et al*., 2009; Yashin *et al*., 2005). Therefore, the objective of the study was to investigate whether plant parts (stems, roots and leaves) of *Jatropha zeyheri* would have an effect on essential and non-essential mineral composition.

3.2 Material and methods

3.2.1 Description of the study area

Jatropha zeyheri plant materials (Figure 3.1) were collected at Khureng village (24°33'53" S, 29°23'4" E), Lepelle-Nkumpi Municipality, in Limpopo Province, South

Africa. Khureng village is characterised by semi-arid climate, with maximum and minimum temperatures that average 30/10°C and an average rainfall of less than 400 mm per annum. Plant parts were harvested randomly during mid-June and early July 2018. The stems, roots and leaves were transported to Limpopo Agro-Food Technology Station (LATS) laboratory in a paper bag for further preparations prior analysis.

Figure 3.1 *Jatropha zeyheri* plant.

3.2.2 Research design, treatments and procedure

Three treatments namely, stems, roots and leaves (Figure 3.2) were collected from 5 x 5 m plots arranged in a randomised complete block design (RCBD), with 10 replications. After harvesting plant parts, leaves and stems were separated, while the

roots were cut into smaller pieces prior to drying at 60°C for 48 hours in an air-forced oven (Kissinger *et al.,* 2005). Dried plant parts were ground using an electric grinder to pass through 1mm pore sieve (MF 10 basic microfile grinder drive, IKA-Werke, United States).

3.2.3 Data collection

A microwave digestion system (PerkinElmer, Titan MPS, USA) was used to prepare the samples. Approximately, 0.5 g of each sample was weighed, transferred into the digestion vessel, and 10.0 mL of HNO_3 was added. The mixture was allowed to cool for 10 minutes prior to closing the vessels and inserted into the microwave digester to run for 48 minutes. The microwave digester vessels were cooled down to room temperature (24°C) for 20 minutes. The solution was transferred into 50 ml centrifuge tubes and diluted with deionized water to top up to 50 ml. The essential mineral elements, *viz*., iron (Fe), phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), calcium (Ca), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) and non-essential mineral elements, *viz.*, aluminium (Al), chromium (Cr), cobalt (Co), sodium (Na) and silicon (Si) were determined using Inductive Coupled Plasma Emission (ICPE-9000 Shimadzu, Japan).

.

Figure 3.2 *Jatropha zeyheri* plant parts; A) Stems, B) Roots and C) Leaves.

3.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using the Statistix 10.0 (SAS Institute Inc, 2008). When the treatments were significant ($P \leq 0.05$) at the probability level of 5%, the degrees of freedom and their associated mean sum of squares were partitioned (Appendix 3.1-3.15) to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was done using Fischer's Least Significant Difference test.

3.3. Results

Plant parts had a highly significant effect on ($P \leq 0.01$) essential mineral elements, Cu, Fe, K, Mg, Zn, Mn, P and Ni contributing 82, 75, 97, 88, 86, 96, 79 and 82% in TTV (Table 3.1), respectively, whereas, Ca had a significant effect ($P \leq 0.05$) contributing 69% in TTV (Table 3.2). However, no significant effect was observed on S in the tested plant parts (Table 3.2). Similarly, plant parts had a highly significant effect on ($P \leq 0.01$) non-essential mineral elements, Al, Na, Co and Cr contributing 85, 72, 84 and 81% in TTV, respectively, whereas, not significant on Si (Table 3.3).

The three plant parts had different distribution of essential mineral elements (Table 3.4 and 3.5). Potassium (K) content was high in the stem (13824 ± 969.35 mg/kg) compared to in leaf (5971 ± 371.70. mg/kg) and root (4063 ± 303.09 mg/kg) (Table 3.4). The stem (3692.0 ± 348.41 mg/kg) and leaf (3544.0 ± 302.44 mg/kg) had highest content of Mg, whereas it was lowest in the root (1640.4 ± 231.69 mg/kg). The Mn content was highest in the stem (933.60 ± 76.67 mg/kg) and leaf (897.80 ± 86.75 mg/kg), whereas lower in the root (122.41 ± 11.02 mg/kg). The Ca content in leaf (9280.0 ± 801.38 mg/kg) and stem (8659.0 ± 818.32 mg/kg) was not statistically

different but different with root (5935.0 ± 853.92 mg/kg). The leaf (54.67 ± 4.05 mg/kg) and stem (53.98. ± 6.03 mg/kg) had the highest content of Zn whereas, root had the lowest (25.52 ± 3.88 mg/kg). Similarly, leaf (66.28 ± 2.08 mg/kg) and stem (64.70 ± 1.91 mg/kg) had highest Cu content, whereas, root had the lowest content (53.64 ± 2.58 mg/kg).

The leaf (527.20 ± 40.54 mg/kg) and root (433.50 ± 82.07 mg/kg) had highest content of Fe and was lowest in stem (267.50 ± 20.00 mg/kg). Leaf had highest content of P (627.80 ± 38.22 mg/kg) whereas, stem (489.50 ± 38.65 mg/kg) and root (372.54 ± 59.85 mg/kg) reported lower content. The leaf (137.10 ± 9.24 mg/kg) and stem (119.06 ± 8.63 mg/kg) had highest content of Ni whereas, root had the lowest (85.79 ± 9.26 mg/kg).

The three plant parts had different distribution of non-essential mineral elements (Table 3.6). Leaf (995.10 ± 62.59 mg/kg) and stem (839.20 ± 55.96 mg/kg) had highest content of Al whereas, root had the lowest (603.50 ± 53.72 mg/kg). Similarly leaf (1090.9 ± 68.36 mg/kg) and stem (1019.7 ± 69.15 mg/kg) had highest content of Na whereas, the root had the lowest (767.9 ± 81.61 mg/kg). Leaf (140.83 ± 8.19 mg/kg), and stem (122.34 ± 8.26 mg/kg) had highest content of Co whereas, root had lowest content (86.36 ± 9.05 mg/kg). The leaf (308.10 ± 20.03 mg/kg) and stem (253.40 ± 19.56 mg/kg) had highest content of Cr whereas, root had the lowest (185.15 ± 24.10 mg/kg).

Table 3.1 Partitioning mean sum of squares for essential mineral elements (Cu, Fe, K, Mg, Zn, Mn, P and Ni) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

		Copper (Cu) (mg/kg)		Iron (Fe) (mg/kg)		Potassium (K) (mg/kg)		Magnesium (Mg) (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	60.362	10	28835	12	4407887	2	947062	6
Treatment	2	474.316	82***	172966	75***	2.676E+08	97***	1.309E+07	88***
Error	18	42.892	8	29483	13	3644397	1	859183	6
Total	29	577.52	100	231284	100	275652284	100	14896245	100
		Zinc (Zn) (mg/kg)		Manganese (Mn) (mg/kg)		Phosphorus (P) (mg/kg)		Nickel (Ni) (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	226.73	7	55074	2	22268	11	748.11	9
Treatment	2	2766.95	86***	2100901	96***	163274	79***	6775.08	82***
Error	18	225.75	7	40092	2	21552	10	769.83	9
Total	29	3219.43	100	2196067	100	207094	100	8293.02	100

***Treatments effects were highly significant at P ≤ 0.01, DF = Degree of Freedom, MSS = Mean Sum of Squares, TTV (%) = Percentage of Total Treatment Variation.

Table 3.2 Partitioning mean sum of squares for essential mineral elements (Ca and S) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

		Calcium (Ca) (mg/kg)		Sulphur (S) (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)
Block	9	8134283	18	81075.4	52
Treatment	2	3.166E+07	69**	20767.0	13^{ns}
Error	18	6138077	13	55567.7	35
Total	29	45932360	100	157410.1	100

**Treatments effect were significant at $P \leq 0.05$, ^{ns} non-significant at $P \geq 0.05$, DF = Degree of Freedom, MSS = Mean Sum of Squares, TTV (%) = Percentage of Total Treatment Variation.

Table 3.3 Partitioning mean sum of squares for non-essential mineral elements (Al, Na, Co, Si and Cr) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

		Aluminium (Al) (mg/kg)		Sodium (Na) (mg/kg)		Cobalt (Co) (mg/kg)		Silicon (Si) (mg/kg)		Chromium (Cr) (mg/kg)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	39272	8	63060	16	775.87	8	352.972	43	3860.9	8
Treatment	2	388683	85***	288003	72***	7672.37	84***	224.045	28^{ns}	37944.8	81***
Error	18	30038	7	49047	12	697.54	8	237.158	29	4891.8	11
Total	29	457993	100	400110	100	9145.78	100	814.185	100	46697.5	100

***Treatments effects were highly significant at P ≤ 0.01, ^{ns} non-significant at P ≥ 0.05, DF = Degree of Freedom, MSS = Mean Sum of Squares, TTV (%) = Percentage of Total Treatment Variation.

Table 3.4 Responses of essential mineral elements (K, Mg, Mn, Ca, Zn, Cu, Fe and P) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

	Potassium (K) (mg/kg)	Magnesium (Mg) (mg/kg)	Manganese (Mn) (mg/kg)	Calcium (Ca) (mg/kg)
Treatment	Variable	Variable	Variable	Variable
Stem	13824^{ay} ± 969.35	3692.0^{a} ± 348.41	933.60^{a} ± 76.67	8659.0^{a} ± 818.32
Root	4063^{c} ± 303.09	1640.4^{b} ± 231.69	122.41^{b} ± 11.02	5935.0^{b} ± 853.92
Leaf	5971^{b}± 371.70	3544.0^{a} ± 302.44	897.80^{a}± 86.75	9280.0^{a} ± 801.38
	Zinc (Zn) (mg/kg)	Copper (Cu) (mg/kg)	Iron (Fe) (mg/kg)	Phosphorus (P) (mg/kg)
Treatment	Variable	Variable	Variable	Variable
Stem	53.98^{a} ± 6.03	64.70^{a} ± 1.91	267.50^{b} ± 20.00	489.50^{b} ± 38.65
Root	25.52^{b} ± 3.88	53.64^{b} ± 2.58	433.50^{a} ± 82.07	372.54^{b} ± 59.85
Leaf	54.67^{a} ± 4.05	66.28^{a} ± 2.08	527.20^{a} ± 40.54	627.80^{a} ± 38.22

^{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.

Table 3.5 Responses of essential mineral element (Ni) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

	Nickel (Ni) (mg/kg)
Treatment	Variable
Stem	119.06[ay] ± 8.63
Root	85.79[b] ± 9.26
Leaf	137.10[a] ± 9.24

[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.

Table 3.6 Responses of non-essential mineral elements (Al, Na, Co and Cr) to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

	Aluminium (Al) (mg/kg)	Sodium (Na) (mg/kg)	Cobalt (Co) (mg/kg)	Chromim (Cr) (mg/kg)
Treatment	Variable	Variable	Variable	Variable
Stem	839.20^{ay} ± 55.96	1019.7^{a} ± 69.15	122.34^{a} ± 8.26	253.40^{a} ± 19.56
Root	603.50^{b} ± 53.72	767.9^{b} ± 81.61	86.36^{b} ± 9.05	185.15^{b} ± 24.10
Leaf	995.10^{a} ± 62.59	1090.9^{a} ± 68.36	140.83^{a} ± 8.19	308.10^{a} ± 20.03

^{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.

3.4 Discussion

Different plant parts of *J. zeyheri* affected majority of essential mineral elements. Similar findings were reported on special tea (*Monsonia burkeana)* (Mamphiswana *et al.,* 2011)*, Lippia multiflora* (Christine *et al*., 2017), *Agaratum conyzoides*, *Trimelia grandifolia* and *Rhamnus prinoides* (Agbafor *et al*., 2015), where selected minerals were affected by different plant parts. In contrast, the findings on black tea (*Camellia sinesis*) differed where Fe, Zn, Mn, Ca, and Mg were not affected by selected plant parts (Salahinejad and Aflaki 2010). Also, plant parts did not affect Zn, Cu and Mg of special tea (*M. burkeana)* (Mamphiswana *et al*., 2011). Copper from *A. conyzoides and Dovyalis abyssinica, Todalia asiastica and Clutia abyssinica* were also not affected by plant parts (Agbafor *et al*., 2015; Nathan *et al*., 2014), whereas*,* Fe from *T. grandifolia, R. prinoides, D. abyssinica, T. asiastica, Calyusea abyssinica* was not affected by plant parts (Nathan *et al*., 2014)*.*

Generally, factors such as age of a plant, climatic conditions and mineral composition of the soil plays an important role in the accumulation of different essential and non-essential mineral elements in different plant parts (Serfor-Armah *et al*., 2001). The mineral elements are absorbed by the roots, translocated to stem and distributed to different plant parts and tissues (Gupta *et al*., 2016). Mineral ions are translocated in a plant through series of tissues, beginning with root hair which absorb minerals through xylem and phloem. Xylem is the main tissue responsible for transporting mineral ions within different plant parts (Hopkins and Norman, 2008). Phloem translocate soluble organic compounds known as photosynthates to the parts of the plant where they are required (Lalonde *et al*., 2004).

The essential mineral elements contained in tea play an important role in the human body. For instance, copper is important to the human body since it develops a component in many enzyme systems, such as cytochrome oxidase, lysyl oxidase and an iron-oxidizing enzyme in the blood (Amin *et al.*, 2003; Osuocha *et al.*, 2016). Copper helps produce red and white blood cells and activates the release of iron to form haemoglobin which is the substance that carries oxygen around the body (Idris *et al*., 2011). Manganese is important for the development of normal bone structure, reproduction, metabolism of amino acids, lipids, carbohydrates and operating of the central nervous system (Hussain *et al.*, 2009). Iron is essential for electron and oxygen transfer in human body and it is also important for the synthesis of haemoglobin (Kaya and Incekara, 2003; Wani *et al.,* 2010). Phosphorus is important for the formation of bones and teeth. It plays an important role in the body's utilization of carbohydrates and fats and in the synthesis of protein for the growth, maintenance and repair of cells and tissue (Idris *et al*., 2011).

Magnesium is an activator of many enzymes involved in carbohydrate metabolism and synthesis of nucleic acids (DNA and RNA). It also acts as a binding agent of ribosomal particles where protein synthesis takes place (Indrayan *et al*., 2005). Nickel is essential for maintenance of membrane structure, control of prolactin, nucleic acid metabolism or as a cofactor in enzyme (Soetan *et al*., 2010). Potassium is important for regulating many systems in the body (Ringer and Barlett, 2007). Calcium is essential for normal functioning of cardiac muscles, regulation of cell permeability, blood coagulation, important in the formation of bones and teeth and prevent osteoporosis (Pravina *et al*., 2013). Zinc plays an important role in blood coagulation, neuromuscular transmission, tissue repair and wound healing (Srinivasan e*t al*., 2012). Similarly, different plant parts

of *J. zeyheri* affected majority of non-essential mineral elements. Similar findings were reported on *D. abyssinica, T. Asiatic, C. abyssinica, T. grandifolia, R. prinoides, C. abyssinica* where selected minerals were affected by different plant parts (Nathan *et al*., 2014). Also, similar findings were observed on mate tea (*Llex paraguariensis*), rooibos tea (*Aspalathus linearis*), honey bush tea (*Cyclopia genistoides*), coca tea (*Erythoxylum coca*) (Olivier *et al*., 2012) where selected minerals were affected by different plant parts. Contrary results were observed in *Blighla Sapida* where Cr was not affected by different plant parts *(*Abolaji *et al.,* 2007).

The non-essential mineral elements in tea play an important role in the human body. For instance, chromium is important for synthesis of fatty acids and cholesterol, increases the insulin action, plays a role in glucose metabolism and regulates carbohydrate, nucleic acid and lipoprotein metabolism (Katz and Salem, 1992). Sodium is important for maintaining water balance within cells and is involved in proper functioning of both nerve impulses and muscles within the body (Soetan *et al.,* 2010). Cobalt is required as a component of vitamin B12 and its metabolism is the same as for vitamin B12. It is a co-factor involved in DNA biosynthesis and amino acid metabolism (Arinola, 2008).

World Health Organization (1998) outlined the threshold limits for Mn, Zn, Cu, Fe, Ni, Co and Cr heavy metals as 200, 50, 10, 450, 10, 1.5 and 1.3 mg/kg, respectively in tea plants. However, in this study majority of heavy metals were above the threshold except for Fe which had acceptable levels in the stems and roots. Also, the roots of *J. zeyheri* had acceptable levels of Mn and Zn in the roots. Determination of mineral elements in tea is important to evaluate their nutritional value and prevents possible ill

effects (Karak and Bhagat, 2010). Consequently, the content of heavy metals in different plant parts is of great importance for consumer safety (Nathan *et al*., 2014). Heavy metals have been reported to cause several types of diseases in the human body and cause damage to different body parts, such as kidney, liver and bones (Singh *et al.,* 2011). Heavy metals have the tendency to change different systems in humans, including respiratory, endocrine, nervous systems, skin, blood, etc (Izah *et al*., 2017). However, some heavy metals are important nutrients, yet becomes toxic at high concentrations such as Cu, Fe, Mg, Mn, Ni, Al, Co, Cr and Zn. High supply of these mineral elements leads to variety of deficiency diseases or syndromes. For example, Zn deficiency leads to retarded growth, low blood pressure, retarded bones, loss of appetite loss of sense of smell and taste, weight loss, pale skin, diarrhoea, hair loss, fatigue and white spots under finger nails (Bhowmik *et al.,* 2010). Sources of heavy metals in plants includes, soil water, fertilisers, sewage sludge, organic manures, smelting and different industrial activities (Nathan *et al*., 2014). In this study, heavy metals such as Co, Cr, Al, Fe, Ni and etc were present in different plant parts of *J. zeyheri* indigenous tea.

Among the tested essential mineral elements, Ca, Zn, Cu and Ni were consistently the highest in the leaf, followed by stem whereas, root had the lowest content. Similar results were observed in *D. abssinica*, *T. grandifolia* and *C. abyssinica* where leaf and stem had high content of Cu and root had the lowest (Nathan *et al*., 2014). Also, Ca of *Grewia mollis* was high in leaf and stem and low in root (Adamu *et al*., 2016). Contrary results were observed in *T. asiastica and R. prinoides*, where stem and root had high content of Cu and leaf had the lowest. Mamphiswana *et al.* (2011) reported that special tea (*M. burkeana*) had high content of Zn in root and leaf, whereas, low in

stem. Also, Ni of *G. mollis* was high in leaf and root, whereas stem had the lowest (Adamu *et al*., 2016), however, in *A. conyzoides* root had high content of Zn and leaf had low content (Agbafor *et al*., 2015). Additionally, Zn of *G. mollis* was high in stem and root and low in leaf (Adamu *et al.,* 2016), whereas, Ni of *Datura straminium* was high in root and leaf stem (Olowoyo *et al*., 2012). Calcium is absorbed by root in free ionic from the soil and transported towards the aerial part. The distribution of Ca in soil affect the physiology of plants (Demarty *et al*., 1984); for instance, plants grown in soil containing low content of Ca cause poor growth and yield (Singh *et al*., 2014). Calcium does not only decrease the toxic effects of various cations; however, it also increases the absorption and translocation of certain essential mineral elements such as phosphorus (Patel *et al*., 2011; Shah *et al*., 2006). Plants absorb Ni through the root by passive diffusion and active transport mechanisms, thereafter due to exposure to soil containing high levels of Ni, the Ni content are usually highest in the root with much less amount in leaf and stem (Sharma and Dhiman, 2013). The high content of of Ca, Cu, Ni and Zn in leaf and stem might be due to the ability of the roots to absorb mineral elements from the soil and translocate to other plant parts of *J. zeyheri* indigenous tea.

Among the tested essential mineral elements, Mn and Mg were high in stem and leaf, whereas, root had the lowest content. Similar results were observed in Mg of *Pentaclethra macrophylla* where stem and leaf had high content and root lowest content (Ogbonna *et al*., 2018). Contrary results were observed in Mg of special tea (*M. burkeana*) where leaf, stem and root had similar content and Mn was low in the stem (Mamphiswana *et al.,* 2011). Also, Mn and Mg of *A. conyzoides* were high in roots and low in leaf *(*Agbafor *et al*., 2015*).*

Iron was highest in leaf and root, whereas, lowest in stem. Similar results were observed in special tea (*M. burkeana*) where leaf and root had high content of Fe, whereas, stem had lowest content (Mamphiswana *et al*., 2011). Also, *G. mollis* root had high content of Fe and stem had low content (Adamu *et al*., 2016). Contary results were observed in *T. asiastica* where stem and leaf had high content of Fe, whereas, root had lowest content *(*Nathan *et al*., 2014*)*. In addition, stem *of Cassia nigricans* had high content of Fe (Gbekele-Oluwa Ayo, 2013). The high content of Fe in roots may be attributed to the ability of the root to absorb and store Fe in the root of *J. zeyheri*. In order to resist the damaging effect of absorbing more than the allowable limit of trace metal in soil, plants adopt different strategies which include reducing the transfer of trace metal to the shoot and adsorbed it to the cell walls of the roots (Ogbonna *et al*., 2018).

Phosphorus had highest content in leaf whereas, stem and root reported lower content. Contrary results were observed on *P. macrophylla* with stem containing high content of P and leaf containing lower content (Ogbonna *et al*., 2018). Also, stem of special tea (*M. burkeana)* had high content of P whereas, leaf reported lower content (Mamphiswana *et al.,* 2011). In addition, *A. conyzoides* had high content of P in root and low content in leaf (Agbafor *et al*., 2015). The high content of P in the leaf may be attributed to the availability of these element in the soil and the ability of the plant to take it up and translocate to the leaf.

Potassium content was high in the stem and low in leaf and root. Similar results were reported in *G. mollis* where stem had high content of K (Adamu *et al.,* 2016). Also, stem of special tea (*M. burkeana*) had high content of K (Mamphiswana *et al.,* 2011).

Contrary results were reported in *A. conyzoides* with root having high content of K (Agbafor *et al*., 2015). Potassium is usually obtained from the soil through plant roots, but many factors affect the efficiency of K acquisition. First, the chemistry and composition of certain soils can make it harder for plants to absorb nutrient. The nutrients may not be available in certain soils or may be present in forms that the plants cannot use. Soil properties like water content, pH and compaction may aggravate these problems (Morgan and Connolly, 2013). The transfer of trace metal such as K is reduced by the plant to the aerial part of the plant (e.g. leaves) by compartmentation of the metal in the vacuole through the production of organic acids and formation of metal binding polypeptides known as phytochelatins (Hall, 2002). In this context, it is important to point out that stems are not simply structural components but also key plant parts for the uptake, transport, accumulation, and storage of nutrients for plant biosynthesis (Chapin *et al*., 1990).

Among the tested non-essential mineral elements leaf and stem had highest content of Al, Na, Co and Cr, whereas, root had the lowest. Similarly, Nathan *et al*. (2014) reported that leaf and stem of *T. asiastica* had high content of Cr, whereas leaf had the lowest. Also, Co of *C. abyssinica* had high content in leaf and stem, whereas, root had lower content (Nathan *et al*., 2014). Moreover, leaf of *P. macrophylla* reported high content of Na in leaf (Ogbonna *et al*., 2018). In contrast, Co and Cr of *G. mollis* were high in root and leaf, whereas, stem had lower content (Adamu *et al.,* 2016). Also, Cr of *Amaranthus spinosus* was high in root and stem had lower content (Olowoyo *et al*., 2012). The phytotoxic effects of Cr are primarily dependent on the speciation of the metal, which determines its uptake, translocation and accumulation (Shanker *et al.,* 2005). The distribution and absorption mechanism of Cr in the different

plant parts are still not fully understood (Hayat *et al*., 2012). It has been reported that Cr is transported and accumulated in plants via carrier ions such as sulfate or iron and is not directly absorbed by plants (Gajalakshmi *et al.,* 2012; Singh *et al.,* 2013). Chromium is absorbed as both Cr^{3+} and Cr^{6+}, but there is no detailed mechanism for Cr absorption that has yet been proposed (Oliveira, 2012; Singh *et al.,* 2013). Chromium causes harmful effects on physiological processes such as photosynthesis, water relations, mineral nutrition and can also generate morphological changes (Daud *et al*., 2014; Rodriguez *et al*., 2012; Singh *et al*., 2013).

3.5 Conclusion

Most of essential and non-essential mineral elements were maintained in leaves and stems than in roots. However, heavy metals were also present in slightly higher concentration which can have negative adverse to human health. More studies are necessary to establish the source of heavy metals. In conclusion, the results suggested that leaves and stems could be used in the brewing of *J. zeyheri* tea beverage.

CHAPTER 4
EFFECT OF DIFFERENT PLANT PARTS ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *JATROPHA ZEYHERI*

4.1 Introduction

Tea has gained popularity over the years as it contains phytochemicals and antioxidant activity which are responsible for its health benefits (Yashin *et al.,* 2005). Moreover, tea possess antioxidative, antimicrobial immune-stimulatory, anti-inflammatory and bone mineralization enhancement activities (Hamilton-Miller, 1995; Sajilata *et al.,* 2008; Sano *et al*., 2004; Shen *et al*., 2011). Flavonoids and polyphenols present in tea have shown a wide range of biological and pharmaceutical benefits, including prevention of cancer, obesity, type-2 diabetes, depressive symptoms and cardiovascular diseases (Deka and Vita, 2011; Zhang *et al*., 2013). These beneficial effects may be attributed to antioxidant activity possessed by the polyphenolic compounds in tea (Cabrera *et al*., 2006). Generally, tea contains phytochemicals and antioxidant activity, therefore the use of inappropriate plant parts could have an effect on the quality of tea developed from *Jatropha zeyheri* plant parts. Wild bush tea (*Athrixia phylicoides* DC.) contains phytochemicals such as tannins which are main indicators of medicinal potential due its antioxidant activity (Hirasawa *et al*., 2002). Moreover, Samadi and Fard (2020) reported that green tea (*Camellia sinensis*) had higher levels of total phenols, flavonoids tannins and antioxidant activity. Also, white tea contains tannins, flavonoids, glycosides and saponins (Ekayanti *et al*., 2017). Therefore, the objective of the study was to investigate whether plant parts (stems, roots and leaves) of *J. zeyheri* would have an effect on phytochemicals and antioxidant activity.

4.2 Materials and methods

4.2.1 Description of the study area

Jatropha zeyheri plant materials were collected at Khureng village (24°33’53” S, 29°23’4” E), Lepelle-Nkumpi Municipality, in Limpopo Province, South Africa. Khureng village is characterised by semi-arid climate, with maximum and minimum temperatures that average 30/10°C and an average rainfall of less than 400 mm per annum. Plant parts were harvested randomly during mid-June and early July 2018. The stems, roots and leaves were transported to Limpopo Agro-Food Technology Station (LATS) laboratory in a paper bag for further preparations prior analysis.

4.2.2 Research design, treatments and procedure

Three treatments namely, stems, roots and leaves were collected from 5 x 5 m plots arranged in a randomised complete block design (RCBD), with 10 replications. The plant materials were collected and prepared as described previously (Chapter 3).

4.2.3 Extraction process

One gram of each ground plant materials from the *J. zeyheri* plant was extracted with 10 mL of acetone in different 50 mL polyester centrifuge tubes. The tubes were shaken for 10 minutes in a series 25 shaking incubator (New Brunswick Scientific Co., Inc) at 200 rpm. Post-shaking, the mixtures were filtered using a filter and cotton wool, the supernatants were decanted into glass vials. The solvents were evaporated under a stream of cold air at room temperature (24°C), the mass obtained were determined and the extracts were reconstituted to a final concentration of 10 mg/mL in acetone.

4.2.4 Data collection

4.2.4.1 Qualitative analysis of phytochemical constituents

Tannins: Ground plant materials were separately dissolved in 5 mL of distilled water, gently boiled and there after cooled. The solution (1 mL) of each sample was put in a test tube and 3 drops of ferric chloride solution was added to each solution. The samples were observed for a blue-black, green or blue-green color to draw inference as described by Trease and Evans (1989).

Flavonoids: Diluted ammonia (5 mL) solution was added to a portion of the aqueous filtrate (0.3 g + 10 mL of distilled water) of each sample extract, followed by addition of concentrated sulphuric acid. The samples were observed for color changes to draw inference as described by Borokini and Omotayo (2012).

Alkaloids: Ground plant materials (0.2 g) were extracted with 95% ethanol in a Soxhlet extractor for six hours and the ethanolic extracts were evaporated to dryness using vacuum evaporator at 45°C. The residue was redissolved in 5 mL of 1% HCL and 5 drops of Drangendoff's reagent was added. Color change was observed to draw inference as described Harborne (1973).

Saponins: One gram of each powdered sample was suspended in 30 mL of tap water. The mixture was vigorously shaken and heated. The sample was observed for formation of froth to draw inference as described by Odebiyi and Sofowora (1978).

4.2.4.2 Qualitative DPPH assay

Qualitative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed to screen plant parts for antioxidants which are compounds with the capability to scavenge or reduce free radicals. The chromatograms were dried in a fume-hood and then later sprayed with 0.2% (w/v) of DPPH (Sigma®) in methanol as an indicator. The antioxidant compounds present were detected by the yellow spots against a purple background on the TLC (Thin-layer chromatography) plates (Deby and Margotteaux, 1970).

4.2.4.3 Antioxidant activity assay

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 plants. In test tubes, the plant extracts were serially diluted with distilled water to make a volume of 1 mL at different concentrations (0.0625 mg/mL to 1 mg/mL) and then mixed with 1 mL of 0.2% DPPH solution in methanol. The method was further modified by diluting the samples 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 minutes in the dark and the absorbance was 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.

4.2.4.4 Phytochemical constituents quantification

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.,* 2011). After incubation for 5 minutes, 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 minutes at room temperature (24°C) in the dark. 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 gallic acid equivalents (GAE) per g of the extract (Hlahla *et al.,* 2010).

Total tannin content: The Folin-Ciocalteu assay was also 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. One millilitre 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 the dark at room temperature (24°C) 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). An amount of 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 minutes, and 0.3 mL of 10% aluminium chloride was added thereafter, 2 mL of 1M sodium hydroxide (NaOH) was added after 5 minutes. 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 minutes 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 quercetin equivalents (QE) per gram of plant extract.

4.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using the Statistix 10.0 (SAS Institute Inc, 2008). When the treatments were significant ($P \leq 0.05$) at the probability level of 5%, the degrees of freedom and their associated mean sum of squares were partitioned (Appendix 4.1-4.5) to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fischer's Least Significant Difference test.

4.3. Results

4.3.1 Phytochemical constituents screening

The results showed that all phytochemicals (tannins, flavonoids, alkaloids and saponins) screened in this study were present in all the plant parts of *J. zeyheri* indigenous tea (Table 4.1).

Table 4.1 Phytochemical constituents screening of plant parts from *Jatropha zeyheri* indigenous tea.

Compound	Stems	Roots	Leaves
Tannins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+

Key words: + = present; - = absent

4.3.2 Qualitative DPPH assay

The antioxidant activity of plant parts was screened using the qualitative 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay on TLC plates. The presence of antioxidant compounds was indicated by the yellow spots against the purple background. The results from DPPH qualitative assay of the plant parts showed more yellow spots in roots and leaves whereas, there was lesser amount in stems (Figure 4.1).

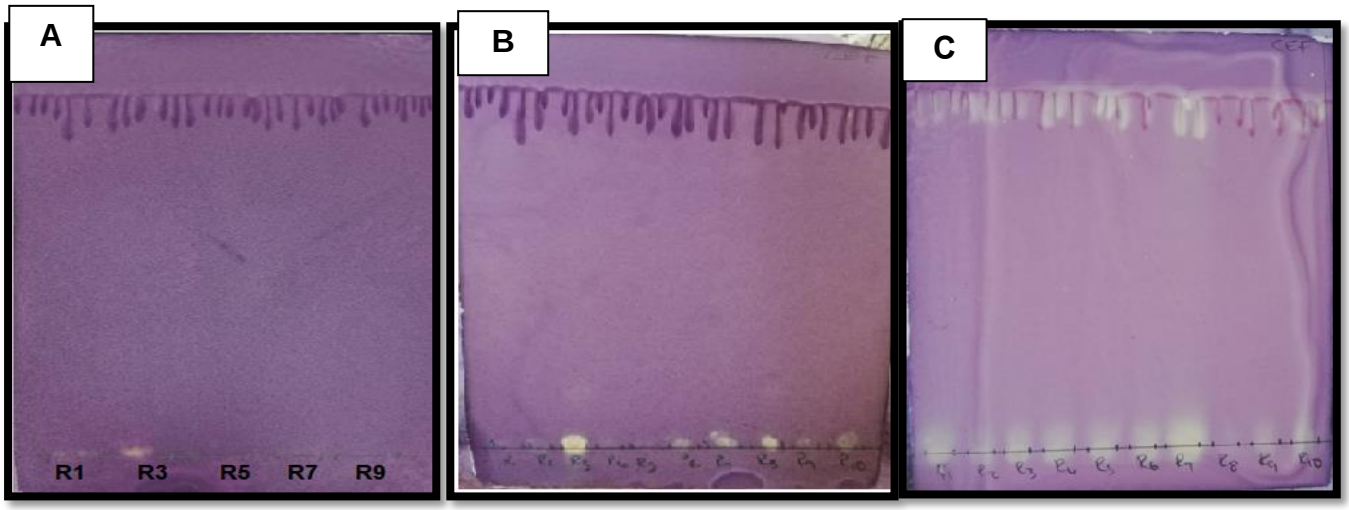

Figure 4.1: TLC plates after DPPH qualitative assay; A) Stems, B) Roots and C) Leaves of *Jatropha zeyheri*.

4.3.3 Phytochemical constituents and antioxidant activity quantification

Plant parts had highly significant effect on ($P \leq 0.01$) total flavonoids content contributing 72% to TTV, whereas plant parts had significant effect ($P \leq 0.05$) on tannins and antioxidant activity contributing 58 and 79%, respectively. In contrast, plant parts had no significant effect on total phenol content (Table 4.2).

The three plant parts had different distribution of total flavonoid, total tannin content and antioxidant activity (Table 4.3). The root (206.12 ± 48.70 mg QE/g) and stem

(181.14 ± 23.70 mg QE/g) contained the highest total flavonoid content, whereas leaf had the lowest content (60.83 ± 15.58 mg QE/g). The total tannin content varied in different plant parts and ranged from (161.89 ± 46.54 to 436.57 ± 109.32 mg GAE/g). The highest content of total tannin was detected in stem (436.57 ± 109.32 mg GAE/g) and leaf (324.72 ± 58.17 mg GAE/g) whereas, root had the lowest content (161.98 ± 46.54 mg GAE/g). Similarly, the stem (57.65 ± 9.35 mg GAE/g) and leaf (36.36 ± 5.44 mg GAE/g) had highest content of antioxidant activity whereas, root had the lowest content (25.13 ± 4.22 mg GAE/g).

Table 4.2 Partitioning mean sum of squares of total flavonoid, total tannin, total phenol content and antioxidant activity to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

		Total flavonoid (mg QE/g)		Total tannin (mg GAE/g)		Total phenol (mg GAE/g)		Antioxidant activity (mg GAE/g)	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Block	9	7705.5	18	52901.4	32	11094.0	12	100.10	3
Treatment	2	30172.2	72***	95327.2	58**	54996.7	62^{ns}	2810.77	79**
Error	18	4088.0	10	17301.0	10	23480.5	26	623.36	18
Total	28	41965.7	100	165529.6	100	89571.20	100	3534.23	100

*** Treatments effects were highly significant at P ≤ 0.01, ** Significant at P ≤ 0.05, ^{ns} non-significant at P ≥ 0.05, DF = Degree of Freedom, MSS = Mean Sum of Squares, TTV (%) = Percentage of Total Treatment Variation.

Table 4.3. Responses of total flavonoid, total tannin content and antioxidant activity to different plant parts of *Jatropha zeyheri* indigenous tea (n = 30).

	Total flavonoid (mg QE/g)	Total tannin (mg GAE/g)	Antioxidant activity (mg GAE/g)
Treatment	Variable	Variable	Variable
Stem	181.14^{ay} ± 23.70	436.57^{a} ± 109.32	57.65^{a} ± 9.35
Root	206.12^{a} ± 48.70	161.98^{b} ± 46.54	25.13^{b} ± 4.22
Leaf	60.83^{b} ± 15.58	324.72^{ab} ± 58.17	36.36^{ab} ± 5.44

[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.

4.4 Discussion

The results obtained from phytochemical screening showed that flavonoids, tannins, alkaloids and saponins were present within the tested plant parts of *J. zeyheri* indigenous tea plant. The presence of phytochemicals in plants play a significant role as defence mechanisms against environmental stress, attacks by insects and pests. Plant protects themselves by producing odours and repelling substances that resist pests that might cause damage on the plant (Ogbonna *et al.,* 2018; Salvet *et al.,* 2001). In addition, saponins helps with absorption of nutrients and hormonal activity (Chukwuebuka and Chinenye, 2015).

The observed findings of this current study agree with those by Singh *et al*. (2012) on the presence of saponins, flavonoids, alkaloids and tannins in green tea (*C. sinensis*) leaf. Similarly, Ogbonna *et al.* (2018) observed the presence of saponins, flavonoids, alkaloids and tannins in the leaf, root and stem of *Pentaclethra macrophylla.* Reports by Elgailani (2015) suggested the presence of tannin, saponin and alkaloid, with flavonoids being absent in black tea (*Camellia sinensis*) leaf. Additionally, Reid *et al.* (2001) reported the presence of saponins and tannins in the leaf and stem of *Dombeya rotundifolia.* In contrast, tannins and saponins were absent in root of *Acorus calamis,* whereas, tannins and flavonoids were absent in leaf and stem of *Moringa olifera* (Shrestha *et al*., 2015).

An antioxidant is a substance that inhibit oxidation by reducing the number of free radicals (Halliwell and Gutteridge, 1995; Mattson and Cheng, 2006). When using qualitative DPPH TLC plate analysis, a compound with antioxidant activity is seen by a yellow colour change on the purple background and DPPH signify the ability of the

compounds to donate electrons to scavenge free radicals (Naik *et al*., 2003). The results from DPPH qualitative assay of *J. zeyheri* plant parts showed more yellow spots in leaf and root, whereas, there was lesser amount in stem indicating that the plant exhibited antioxidant activity. The plant parts were able to reduce the stable free radical of DPPH to the yellow coloured diphenyl picrylhydrazine. This proofs that the *J. zeyheri* indigenous plant parts contain some active constituents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity.

Different plant parts of *J. zeyheri* affected total flavonoid, total tannin contents and antioxidant activity. Similar results were reported on *P. macrophylla (*Ogbonna *et al.,* 2018*), Monsonia burkeana* (special tea) *(*Mamphiswan*a et al.,* 2010*), Stevia rebaudiana* (Kumari *et al*., 2016), *Clinacanthus nutans* (Raya *et al*., 2015), *Chromolaena odorata* (Ugwoke *et al*., 2017) where antioxidant activity and selected phytochemicals were affected by different plant parts. For instance, the presence of total tannin in different plant parts act as a defence mechanism by protecting plant against predation as pesticides and in plant growth regulation (Ndukwe and Ikpeama, 2013). Tannins are used as treatment of intestinal disorders such as diarrhea and dysentery and healing of wounds (Akindahunsi and Salawu, 2005; Ndukwe and Ikpeama, 2013). Flavonoids in tea reduce oxidation of low-density lipoprotein strengthen blood capillaries, reduces cramps of smooth muscles, lower the blood level of cholesterol and triglycerides and improves circulation in the coronary arteries (Ogbonn*a et al.,* 2018). Furthermore, flavonoids act as anti-inflammatory and anti-allergic effect for inhibition of tumor production whereas, antioxidant activity delay and

reduce the free radicals in the body, thus prevent cellular damage caused by oxidative stress (Mattson and Cheng, 2006).

In this study, leaf had the lowest of total flavonoid content, whereas stem and root reported the higher content. Similarly, Ogbonna *et al*. (2018) reported high content of total flavonoid in root and stem, whereas, leaf contained low content in *P. macrophylla*. Contrary results were observed on *Teucrium chamaedrys* and *Cleome cilate* with leaf containing high content of total flavonoid (Milan *et al*., 2010; Okeke and Chinelo, 2018). Also, Karimi *et al.* (2011) also reported high content of total flavonoid in leaf and root of *Labisia pumila* and low content in stem. In addition, Ugwoke *et al*. (2017) reported high content in stem and low content in root. High content of total flavonoid in roots may be attributed to the ability of *J. zeyheri* roots to absorb and store flavonoids in roots over time (Ogbonna *et al*., 2018). The high content of total flavonoid in root and stem is also an indicative of its strong antioxidant effect, suggesting that the plant may be useful as an antibacterial, anti-inflammatory, antiallergic, antiviral, antithrombotic, antimultagic, and vasodilatory compound (Panche *et al*., 2016).

The root had lower content of total tannin, whereas, stem and leaf reported higher content. Similar results were reported on *Memecylon umbellatu* with high content of total tannin in leaf (Killedar and More, 2010). Contrary results were observed in *Cissus pipulnes* where root reported higher content and stem lower contents (Soladaye and Chukwuma, 2012), *Acacia confuse* whereby root reported high content of total tannin and leaf lower content (Wei *et al*., 2010). The presence of high total tannin content in leaf and stem have been reported to have anti-flammatory effects which help control

all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders (Hayashi *et al*., 1993).

The root had lower content of antioxidant activity, whereas stem and leaf reported higher content. Results of this study agree with those in *Centella asiatica* (Sneha *et al.,* 2017); *M. burkeana* (Mamphiswana *et al.,* 2010), creosote bush (*Larrea tridentata*), cup plant (*Silphium perfoliatum*), *Hypericum maculatum*, sweet amber (*Hypericum androsaemum*) (Hyder *et al*., 2002; Radusiene *et al*., 2004 ; Valentao *et al*., 2003) reporting high content of antioxidant activity in leaf and stem and root with lower content . In addition, it contradicts with study reported by Brighente *et al.* (2007) on *Trichilia catigua*, *Waltheria indica* (Olajuyigbe *et al*., 2011). The higher content of antioxidant activity in the leaf might be due to presence of high content of flavonoids and variety of other pigments in the leaf (Sharanabasappa *et al*., 2007).

Generally, the differences in the results obtained for stems, roots and leaves of tea may be due to ecological factors and extraction solvents that have led to differences in the total tannin, total flavonoid, total phenol content and antioxidant activity (Bhakuni *et al*., 1969; Thaker and Anjaria, 1986). Furthermore, factors such as growth stage, storage duration, light, excessive rainfall, drought, insect and pathogenic damage and temperature can affect the quantity and quality of total tannin, total flavonoid, total phenol content and antioxidant activity in different plant parts of indigenous tea (Raya e*t al*., 2015; Vlachos *et al*., 1997).

4.5 Conclusion

Phytochemical constituents screening showed that the plant parts possess flavonoids, tannins, alkaloids and saponins. The DPPH qualitative assay showed that different plant parts of *J. zeyheri* contain antioxidant activity. The higher antioxidant activity and total tannin content were recorded in stems and leaves, whereas, high content of total flavonoid was recorded in stems and roots. The outcome of this study suggests that the leaves and stems of *J. zeyheri* have good medicinal properties and have the ability to improve human health.

CHAPTER 5
SUMMARY, SIGNIFANCE OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

The study investigated the distribution of essential and non-essential mineral elements, phytochemicals and antioxidant activity on different plant parts of *Jatropha zeyheri* indigenous tea. The quantities of essential and non-essential mineral elements in stem, root and leaf differed. The leaves and stems had highest quantities of most determined essential mineral elements. However, Fe was high in leaves and roots, whereas P was high in leaves only and K was high in stems only. Plant parts had no significant effect on S. The leaves and stems had highest quantities of most determined non-essential mineral elements whereas and plant parts had no significant effect on Si.

Plant parts had effect on total flavonoid, total tannin content and antioxidant activity, however it had no effect on total phenol content. The screening of phytochemicals showed that different plant parts exhibited flavonoids, tannins, alkaloids and saponins. The DPPH qualitative assay showed that plant parts contain antioxidant activity. Total tannin and antioxidant activity were high in both stems and leaves, whereas total flavonoid content was high in stems and roots.

5.2 Significance of findings

The results obtained in this study demonstrated that leaves and stems of *J. zeyheri* contained most of determined essential and non-essential mineral elements. Moreover, the results showed that the specified plant parts are rich in biologically

important elements and have medicinal properties that can boost human immune system. The plant parts of *J. zeyheri* were screened for the presence of different phytochemical constituents. This study showed that phytochemicals such as flavonoids, tannins, alkaloids and saponins were present in different plant parts. These phytochemical constituents have therapeutic value and may possess one or more biological activity hence the importance of qualitative analysis. The results from DPPH qualitative assay showed that plant parts of *J. zeyheri* contain antioxidant activity. Plants that contain this compound have the ability to protect humans from many diseases as such *J. zeyheri* plant have the potential to serve as a medicinal plant. The results of this study demonstrated that total flavonoid content was high in stems and roots whereas, total tannin content and antioxidant activity were high in leaves and stems. Knowledge of the chemical constituents of different plant parts is desirable since they are known to have beneficial effects that improve quality of human health. The results of this study demonstrated that various plant parts of *J. zeyheri* have presence of heavy metals such as Al, Mn, Ni, Fe, Cr and Co. Heavy metals are important in tea because they are associated to human health but can have a negative effect on human health if the concentrations are higher. The findings of the study provided new knowledge on the parts (stems and leaves) with suitable chemical properties that should be harvested for the development of *J. zeyheri* indigenous tea. Knowledge of appropriate parts containing suitable chemical properties would help improve the quality of tea and its taste.

5.3 Recommendations

Quality is one of the important factors in the tea industry. In particular, heavy metals in tea are important indicators in the process of tea quality evaluation. Therefore,

further study should be conducted to determine source of heavy metals. Additionally, tea is consumed as a beverage therefore it is important to monitor the concentration of these metals especially in view of their permissible level for human growth and good health.

5.4 Conclusion

Jathropa zeyheri leaves and stems possessed highest concentrations of both essential and non-essential mineral elements suggesting that leaves and stems are suitable plant parts for use in the development of *J. zeyheri* indigenous tea beverage. The presence of total tannin content and antioxidant activity in stems and leaves confirms that the tested plant parts can be used to improve quality of *J. zeyheri* indigenous tea with proper medicinal properties. The findings of this study would benefit rural communities to know which plant parts to harvest to avoid generalisation in using leaves only for making tea beverage.

REFERENCES

Abolaji, O.A., Adebayo, A.H. and O.S. Odesanm. 2007. Nutritional qualities of three medicinal plant parts (*Xylopia aethiopica*, *Blighia sapida* and *Parinari polyandra*) commonly used by pregnant women in the Western part of Nigeria. *Pakistan Journal of Nutrition* 6: 665-668.

Adamu, H.M., Mshelia, E.H., Maigari, A.U., Umar, H.Y. and O.L. Balogun. 2016. Determination of some trace elements and macro minerals in *Grewia mollis* plant parts. *International Journal of Pure and Applied Science Research* 11: 1-16.

Adnan, M., Ahmad, A., Khalid, N., Hayat, I. and I. Ahmed. 2013. Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pakistan Journal of Botany* 45: 901-909.

Afify, A.S., Abdalla, A.A., Elsayed, A., Gamuhay, B., Abu-Khadra, A.S., Hassan, M., Ataalla, M. and A. Mohamed. 2017. Survey on the moisture and ash contents in agricultural commodities in al-rass governorate, Saudi Arabia in 2017. *Journal of Agricultural Science* 48: 55-62.

Agbafor, K.N., Engwa, A.G. and I.K. Obiud. 2015. Analysis of chemical composition of leaves and roots of *Ageratum conyzoides*. *International Journal of Current Research and Academic Review* 3: 60-65.

Ahmed, S. and S.R. Stepp. 2012. Green Tea: Plants, Processing, Manufacturing and Production. In: Preedy, S. (eds.). Tea in Health and Disease Prevention. Elsevier Science and Technology: USA.

Akindahunsi, A.A. and S.O. Salawu. 2005. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. *African Journal of Biotechnology* 4: 497-501.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. and K. Iwatsuki. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* 48: 487-491.

Amin, N.S., Kaneco, T. and K. Ohta. 2003. Slurry sampling electrochemical atomic absorption spectrometric determination of copper in herbal medicines samples with a molybdenum tube atomizer. *Microchemical Journal* 74: 181-186.

Anon. 1996. Tea Production from Sri Lanka. Sri Lankan Tea Boards: Colombo.

Arinola, O.G. 2008. Essential trace elements and metal binding proteins in Nigerian consumers of alcoholic beverages. *Pakistan Journal of Nutrition* 7: 763-765.

Arnold, T.H., Prentice, C., Hawker, L., Snyman, E., Tomalin, M., Crouch, N. and C. Pottas-Bircher. 2002. Medicinal and Magical Plants of Southern Africa: An Annotated Checklist. Strelitzia 13. National Botanical Institute: Pretoria, South Africa.

Asif, H.M., Akram, M., Saeed, T., Khan, M.I., Akhtar, N., Rehman, R., Ali-Shah, I.M., Ahmed, K. and S. Ghazala. 2011. Carbohydrates. *International Research Journal of Biochemistry and Bioinformatics* 1: 1-5.

Awadasseid, A., Adam, I.M., Jamal, M., Khameis, F. and F. Teia. 2019. Physicochemical properties of kakamut gum (*Acacia polyacantha*) and hashab gum (*Acacia senegal). International Journal of Applied Agricultural Sciences* 5: 138-143.

Balentine, D.A., Wiseman, S.A. and L.C. Bouwens. 1997. The chemistry of tea flavonoids. *Critical Reviews in Food Science and Nutrition* 37: 693-704.

Benavente-Garcia, O., Castillo, J., Marin, F.R., Ortuno, A. and J.A. Del-Rio. 1997. Uses and properties of citrus flavonoids. *Journal of Agricultural and Food Chemistry* 45: 4505-4515.

Bhakuni, D.S., Dhar, M.L., Dhar, M.M., Dhawan, B.N. and B.N. Mehrotra. 1969. Screening of Indian plants for biological activity. *Indian Journal of Experimental Biology* 7: 250-262.

Bhowmik, D., Chiranjib, K.P. and S. Kuma. 2010. A potential medicinal importance of zinc in human health and chronic disease. *International Journal of Pharmaceutical Biomedical* 1: 5-11.

Borokini, T.I. and T.O. Omotayo. 2012. Phytochemical and ethnobotanical study of some selected medicinal plants from Nigeria. *Journal of Medicinal Plants Research* 6: 1106-1118.

Brand-Williams, W., Cuvelier, M.E. and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology* 28: 25-30.

Bravo, L. 1998. Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews* 56: 317-333.

Brighente, I.M.C., Dias, M., Verdi, L.G. and M.G. Pizzolatti. 2007. Antioxidant activity and total phenolic content of some Brazilian species. *Pharmaceutical Biology* 45: 156-161.

Cabrera, C., Artacho, R. and G. Rafael. 2006. Beneficial effects of green tea. *Journal of the American College of Nutrition* 25: 79-99.

Carloni, P., Tiano, L., Padella, L., Bacchetti, T., Customu, C., Kay, A. and E. Damiani. 2012. Antioxidant activity of white, green and black tea obtained from the same tea cultivar. *Food Research International* 53: 900-908.

Chapin, F.S., Schulze, E.D. and H.A. Mooney. 1990. The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics* 21: 423-447.

Christine, E.A., Albert, Y.K. and K.C. Seraphin. 2017. Determination of the minerals of the herbal tea and tea green from *Lippia multiflora*. *American Journal of Plant Sciences* 8: 2608-2621.

Chukwuebuka, E. and I.J. Chinenye. 2015. Biological functions and anti-nutritional effects of phytochemicals in living system. *Journal of Pharmacy and Biological Sciences* 10: 10-19.

Conney, A.H., Wang, Z.Y., Ho, C., Yang, C.S. and M. Huang. 1992. Inhibitory Effect of Green Tea on Tumorigenesis and Tumor Growth in Mouse Skin. In: Huang, M., Ho, C. and C.Y. Lee (eds.). Phenolic Compounds in Food and Their Effects on Health II. American Chemical Society Symposium Series: Washington, DC.

Costa, M.A., Zia, Z.Q., Davin, L.B. and N.G. Lewis. 1999. Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops. In: Romeo, J.T. (eds.). Recent Advances in Phytochemistry, Phytochemicals in Human Health Protection, Nutrition and Plant Defense. Kluwer Academic: New York.

Cunningham, A.B. 1993. African Medicinal Plants: Setting Priorities at the Interface Between Conservation and Primary Health Care. People and Plants Working Paper 1. UNESCO: Paris.

Daud, M.K., Mei, L., Variath, M.T., Ali, S., Li, C., Rafiq, M.T. and S.J. Zhu. 2014. Chromium (VI) uptake and tolerance potential in cotton cultivars: Effect on their root physiology, ultramorphology and oxidative metabolism. *BioMed Research International* 8: 11-12.

De Costa, W.A.J.M., Weerakoon, W.M.W., Chinthaka, K.G.R., Herath, H.M.L.K. and R.M.I. Abeywardena. 2007. Genotypic variation in the response of rice (*Oryza*

sativa L.) to increased atmospheric carbon dioxide and its physiological basis. *Journal of Agronomy and Crop Science* 193: 117-130.

Deby, C. and G. Margotteaux. 1970. Relationship between essential fatty acids and tissue antioxidant levels in mice. *Comptes Rendus des Seances de la Societe de Biologie et de ses Filiales* 165: 2675-2681.

Deka, A. and J.A. Vita. 2011. Tea and cardiovascular disease. *Pharmacology Research* 64: 136-145.

Demarty, M., Morvan, C. and M. Thellier. 1984. Calcium and the cell wall. *Plant Cell Environment* 7: 441-448.

Department of Agriculture, Forestry and Fisheries (DAFF). 2016. Honeybush Tea. Briza Publications: Pretoria, South Africa.

Du, G.J., Zhang, Z., Wen, X.D., Yu, C., Calway, T., Yuan, C.S. and C.Z. Wang . 2012. Epigallocatechin Gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. *Nutrients* 4: 1679-1691.

Dufresne, C.J. and E.R. Farnworth. 2001. A review of latest research findings on the health promotion properties of tea. *Journal of Nutritional Biochemistry* 12: 404-421.

Ekayanti, M., Ardiana, L., Najib, S.Z., Sauriasari, R. and B. Elya. 2017. Pharmacognostic and phytochemical standardization of white tea leaf (*Camellia sinensis* L. Kuntze) ethanolic extracts. *Pharmacognosy Journal* 9: 221-226.

Elgailani, I.E.H. 2015. Spectrophotometric and phytochemical analysis of black tea (*Camellia sinensis*) leaves. *Journal of Applied and Industrial Sciences* 3: 167-171.

Food and Agriculture Organization (FAO). 2014. World Tea Production and Trade Current and Future Development. Food and Agriculture Organization of the United Nations: Rome.

Food and Agriculture Organization (FAO). 2015. World Tea Production and Trade: Current and Future Development. Food and Agriculture Organization of the United Nations: Rome.

Food and Agriculture Organization Statistical Database (FAOSTAT). 2010. Statistical Database of the Food and Agriculture Organization of the United Nations. FAO: Rome.

Gajalakshmi, S., Iswarya, V., Ashwini, R., Divya, G., Mythili, S. and A. Sathiavel. 2012. Evaluation of heavy metals in medicinal plants growing in Vellore district. *European Journal of Experimental Biology* 2: 1457-1461.

Gbekele-Oluwa Ayo, R. 2013. Proximate and mineral compositions of the leaves and stem bark of *Cassia nigricans*. *International Journal of Medicinal Plants Research* 2: 242-246.

Gibson, E., Wardel, J. and C.J. Watts. 1998. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite* 31: 205-228.

Gjorgieva, D., Kadifkova-Panovska, T., Bačeva, K. and T. Stafilov. 2010. Some toxic and essentials metals in medicinal plants growing in Repubic of. Macedonia. *American Eurasian Journal of Toxicology and Science* 2: 57-61.

Golding, S.D., Uysal, I.T., Boreham, C.J., Kirste, D., Baublys, K.A. and J.S. Esterle 2010. Adsorption and mineral trapping dominate CO_2 storage in coal systems in proceedings of the 10th international conference on greenhouse gas. *Control Technologies* 4: 3131-3138.

Gonzalex de Mejia, E., Ramirez-Marez, M.V. and S. Puangpraphant. 2009. Bioactive compounds of tea: Cancer, inflammation and behavior. *Brain, Behavior and Immunity* 23: 721-731.

Gramza, A., Korczak, J. and A. Ryszard. 2005. Tea polyphenols, antioxidant properties and biological activity. *Polish Journal of Food and Nutrition Sciences* 3: 219-235.

Gulcin, I. 2012. Antioxidant activity of food constituents: An overview. *Toxicology* 86: 345-391.

Gunathilaka, R.P.D. and G.A. Tularum. 2016. The tea industry and a review of its price modelling in major tea producing countries. *Journal of Management and Strategy* 7: 21-36.

Gupta, N., Ram, H. and B. Kumar. 2016. Mechanism of zinc absorption in plants: Uptake, transport, translocation and accumulation. *Reviews in Environmental Science and Biotechnology* 15: 89-109.

Hall, J.L. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* 53: 1-11.

Halliwell, B. and J.C. Gutteridge. 1995. The definition and measurement of antioxidants in biological systems. *Free Radical Biology and Medicine* 18: 125-126.

Hamilton-Miller, J.M. 1995. Antimicrobial properties of tea (*Camellia sinensis* L.). *Antimicrobial Agents and Chemotherapy* 39: 2375-2377.

Hara, Y., Lou, S.J., Wickremasinghe, R.L. and T. Yamanishe. 1995. Special issue on tea. *Food Reviews International* 11: 371-545.

Harborne, J.B. 1973. Phytochemical Methods. Chapman and Hall: London.

Hasler, C.M. and J.B. Blumberg. 1999. Phytochemicals: Biochemistry and physiology. *Journal of Nutrition* 129: 756-757.

Hayashi, M., Oychev, E.V., Okamura, K.A., Sugeta, H.C., Okuyama, K. and S. Ebisu. 1993. Heat treatment strengthens human dentin. *Journal of Dental Research* 3: 309-314.

Hayat, M., Abbas, M. and F. Munir. 2012. Potential of plant flavonoids in pharmaceutics and nutracetics. *Journal of Biomology and Biochemistry* 1: 12-17.

Hirasawa, M., Takada K, Makimura, M. and S. Otake. 2002. Improvement of periodontal status by green tea catechin using local delivery system. *Alternative Medicine Review* 37: 1387-1390.

Hlahla, L.N., Mudau, F.N. and I.K. Mariga. 2010. Effect of fermentation temperature and time on the chemical composition of bush tea (*Athrixia phylicoides* DC.) *Journal of Medicinal Plants Research* 4: 824-829.

Ho, C.T., Lin, J.K. and F. Shahidi. 2009. Tea and Tea Products: Chemistry and Health-Promoting Properties. CRC Press: Boca Ranton, USA.

Hondrogiannis, E., Peterson, K., Zapf, C.M., Roy, W., Blackney, B. and K. Daily. 2012. The use of wavelength dispersive X-ray fluorescence and discriminant analysis in the identification of the elemental composition of cumin samples and the determination of the country origin. *Food Chemistry* 135: 2825-2831.

Hopkins, W.G and P.A.H. Norman. 2008. Introduction to Plant Physiology. John Wiley and Sons: London.

Hussain, J., Khan, A.L., Rehman, N., Khan, F. and S.T. Hussain. 2009. Proximate and nutrient investigations of selected medicinal plant species of Pakistan. *Pakistan Journal of Natural Products* 8: 620-624.

Hyder, P.W., Fredrickson, E.L., Estell, R.E., Tallez, M. and R.P. Gibbens. 2002. Distribution and concentration of total phenolics, condensed tannins, and nordihydroguaiaretic acid (NDGA) in creosotebush (*Larrea tridentata*). *Biochemial Systematics and Ecolology* 30: 905-912.

Idris, S., Iyaka, Y.A., Ndamitso, M.M. and Y.B. Paiko. 2011. Nutritional Composition of the Leaves and Stems of *Ocimum gratissimum*. Department of Chemistry, Federal University of Technology. Minna Niger State: Nigeria.

Indrayan, A.K., Rajput, M.A., Alam, R., Khan, R.A. and T. Assad. 2005. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Science* 89: 1252-1255.

Isengard, H.D. 2001. Water content, one of the most important properties of food. *Food Control* 12: 395-400.

Izah, S.C., Inyang, I.R., Angaye, T.C.N. and I.P. Okowa. 2017. A review of heavy metal concentration and potential health implications of beverages consumed in Nigeria. *Toxics* 5: 1-15.

Javanmardi, J., Stushnoff, C., Locke, E. and J.M. Vivanco. 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chemistry* 83: 547-550.

Jimoh, F.O. and A.T. Oladiji. 2005. Preliminary studies on piliostigma thonningii seeds: Proximate analysis, mineral composition and phytochemical screening. *African Journal of Biotechnology* 4: 1439-1442.

Karak, T. and R.M. Bhagat. 2010. Trace elements in tea leaves, made tea and tea infusion: A review. *Food Research International* 43: 2234-2252.

Karimi, E., Jaafar, H.Z.E. and S. Ahmad. 2011. Phenolics and flavonoids profiling and antioxidant activity of three varieties of Malaysian indigenous medicinal herb *Labisia pumila* Benth. *Journal of Medicinal Plants Research* 5: 1200-1206.

Karley, A.J. and P.J. White. 2009. Moving cationic minerals to edible tissues: Potassium, magnesium and calcium. *Current Opinion in Plant Biology* 12: 291-298.

Katz, S.A. and H. Salem. 1992. The toxicology of chromium with respect to its chemical speciation: A review. *Journal of Applied Toxicology* 13: 217-224.

Kaya, I. and N. Incekara. 2003. Contents of some wild plant's species consumed as food in Aegean region. *Turkish Journal of Weed Science* 8: 56-64.

Keli, S.O., Hertog, M.G.L., Feskens, E.J.M. and D. Kromhout. 1996. Dietary flavonoids, antioxidant vitamins, and incidence of stroke. *Archives of Internal Medicine* 156: 637-642.

Khasnabis, J., Rai, C. and A. Roy. 2015. Determination of tannin content by titrimetric method from different types of tea. *Journal of Chemical and Pharmaceutical Research* 7: 238-241.

Killedar, S.G. and H.N. More. 2010. Estimation of tannins in different parts of *Memecylon umbellatum* Burm. *Journal of Pharmacy Research* 3: 554-556.

Kissinger, M., Tuvia-alkalai, S., Shalom, Y. and E. Falik. 2005. Characterization of physiological and biological factors associated with post-harvest losses in ripe pepper fruit during storage. *Journal of Horticultural Sciences* 130: 735-741.

Koche, D., Shirsat, R., Syed, I. and D.G. Bhadange. 2016. Phytochemical screening of eight ethnomedicinal plants from Akola district. *International Journal of Pharma and Biosciences* 1: 256-259.

Konan, Y., Witabouna, K.M., Bassirou, B. and K. Kagoriye. 2014. Antioxidant activity and total phenolic content of nine plants from West Africa. *Journal of Applied Pharmaceutical Science* 4: 36-41.

Kumari, N., Rana, R.C., Sharma, Y.P. and S. Kumar. 2016. Dynamics of steviol glycosides (stevioside and rebaudioside-A) with growth and development of *Stevia rebaudiana* Bertoni. *Journal of Applied and Natural Science* 8: 1953-1958.

Lalonde, S., Wipf, D. and W.B. Frommer. 2004. Transport mechanisms for organic forms of carbon and nitrogen between source and sink. *Annual Review of Plant Biology* 55: 341-372.

Lambert, J.D. and R.J. Elias. 2010. The Antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Archives of Biochemistry and Biophysics* 501: 65-72.

Lattanzio, V., Terzano, R., Cicco, N., Cardinali, A., Di Venere, D. and V. Linsalata. 2004. Seed coat tannins and bruchid resistance in stored cowpea seeds. *Journal of the Science of Food and Agriculture* 85: 839-846.

Leedy, P.D. and J.E. Ormrod. 2005. Practical Research: Planning and Design. Pearson Education: New Jersey.

Li, Y., Cai, D.L., Xia, X.J., Chen, X.L., Hu, T.J., Yuan, J.J. and Z.P. Xiao. 2001. Effects of tea protein against mutagenesis caused by irradiation. *Carcinog Teratog Mutagen* 13: 32-36.

Lien, Y.C. and W.W. Nawar. 1974. Thermal interaction of amino acids, triglycerides, valine and tricaproin. *Journal of Food Sciences* 39: 917:919.

Luthria, D.L., Noel, K. and D. Vinjamoori. 2004. Impact of sample preparation on the determination of crude fat content in corn. *Journal of the American Oil Chemists Society* 81: 999-1004.

Mabogo, D.E.N. 1990. The ethnobotany of the Vhavenda. Masters Disseratation submitted to University of Pretoria, Pretoria, South Africa.

Maiga, A., Diallo, D., Bye, R. and B.S. Paulsen. 2005. Determination of some toxic and essential metal ions in medicinal and edible plants from Mali. *Journal of Agricultural and Food Chemistry* 53: 2316-2321.

Makanjuola, O.M. 2016. Routine chemical analysis of tea commercially available in Ogun State, South West, Nigeria. *International Journal of Research Studies in Bioscience Volume* 4: 15-18.

Mamphiswana, N.D., Mashela P.W. and L. K. Mdee. 2010. Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. *African Journal of Agricultural Research* 5: 2570-2575.

Mamphiswana, N.D., Mashela P.W. and L. K. Mdee. 2011. Accumulative capabilities of essential nutrient elements in organs of *Monsonia burkeana*. *African Journal of Biotechnology* 10: 16849-16853.

Maroyi, L. 2017. *Lippia javanica* (Burm.f.) Spreng. Traditional and commercial uses and phytochemical and pharmacological significance in the African and Indian subcontinent. Evidence-Based Complementary and Alternative Medicine 2017: 1-34.

Martens, D.C. and D.T. Westerman. 1991. Fertilizer Applications for Correcting Micronutrient Deficiencies. In: Mortvedt, J.J., Cox, F.R., Shuman, L.M. and R.M. Welch (eds.). Micronutrients in Agriculture. Soil Science Society of America: Madison.

Mashimbye, M.J., Mudau, F.N., Van Ree, T. and P. Soundy. 2006. A new flavonol from bush tea *(Athrixia phylicoides*). *South African Journal of Chemistry* 59: 1-2.

Mathai, K. 2000. Nutrition in the adult years in krause's food. *Nutrition and Diet Therapy* 271: 274-275.

Mathivha, L.M. and F.N. Mudau. 2017. Response of total phenolic content and antioxidant activities of bush tea and special tea using different selected extraction solvents. *Journal of Consumer Sciences* 45: 27-33.

Mattson, M.P. and A. Cheng. 2006. Neurohormetic phytochemicals: Low-dose toxins that induce adaptive neuronal stress responses. *Trends Neuroscience* 29: 632-639.

Mazid, M., Khan, T.A. and F. Mohammad. 2011. Role of secondary metabolites in defence mechanisms of plants. *Biology and Medicine* 3: 232-249.

Mengel, K. and E.A. Kirkby. 1987. Principles of Plant Nutrition. International Potash Institute, Worblaufen-Bern: Switzerland.

Middleton, E., Kandaswami, C. and T.C. Theoharides. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. *Pharmacological Reviews* 52: 673-751.

Milan, S., Topuzovic, M., Solujic, S. and V. Mihailovic. 2010. Total phenolic content, flavonoid concentrations and antioxidant activity of the whole plant and plant parts extracts from *Teucrium montanum* and *Supinum reichenb*. *Journal of Medicinal Plants Research* 4: 1-9.

Miller, A.J., Shen, Q. and Q. Xu. 2009. Freeways in the plant: Transporters for nickel, phosphorus and sulphur and their regulation. *Current Opinion in Plant Biology* 12: 284-290.

Miwa, K., Kamiya, T. and T. Fujiwara. 2009. Homeostasis of the structurally important micronutrients, boron and silicon. *Current Opinion in Plant Biology* 12: 307-311.

Mogotlane, I.D., Mudau, F.N., Mashela, P.W. and P. Soundy. 2007. Seasonal responses of total antioxidant contents in cultivated bush tea (*Athrixia phylicoides* L.) leaves to fertilizer rates. *Medicinal and Aromatic Plants Science and Biotechnology* 1: 77-79.

Morgan, J.B. and E.L Connolly. 2013. Plant-soil interactions: Nutrient uptake. *Nature Education Knowledge* 4: 2-6

Mudau, F.N., Soundy, P. and E.S. Du-Toit. 2007. Nitrogen, phosphorus and potassium nutrition on total polyphenols content of bush tea in a shaded nursery environment. *Horticultural Science*s 42: 334-338.

Munasinghea, M., Deraniyagalaa, Y., Dassanayakeb, N. and H. Karunarathn. 2017. Economic, social and environmental impacts and overall sustainability of the tea sector in Sri Lanka. *Sustainable Production and Consumption* 12: 155-169.

Naik, G.H., Priyadarsini, K.I., Satav, J.G., Banavalikar, M.M., Sohoni, D.P., Biyani, M.K. and H. Mohan. 2003. Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. *Phytochemistry* 63: 97-104.

Nathan, O., Bethpurity, M., Mosima, A.O. and W.N. Omwoy. 2014. Determination of the levels of selected leavy metals in medicinal plants from Narok country, Kenya and variations in their levels due to hot water infusion. *International Research Journal of Environment Sciences* 3: 5-10.

Ndukwe, O.K. and A. Ikpeama. 2013. Comparative evaluation of the phytochemical and proximate constituents of oha (*Pterocarpus soyansii*) and nturukpa (*Pterocarpus santalinoides*) leaves. *International Journal of Academic Research* 2: 22-31.

Nookabkaew, S., Rangkadilok, N. and J. Satayavidad. 2006. Determination of trace elements in herbal tea products and their infusions consumed in Thailand. *Journal of Agricultural and Food Chemistry* 54: 6939-6944.

Odebiyi, O.O. and E.A. Sofowora. 1978. Phytochemical screening of Nigerian medicinal plants II. *Lloydia* 41: 234-246.

Ogbonna, P.C., Pricewill-Ogbonna, I.L., Ngegbule, E.C., Mpamah, I.C. and N.G. Nwachuchu. 2018. Levels of biologically active compounds and some essential metal in parts of African oil bean tree (*Pentaclethra macrophylla*). *Journal of Applied Sciences and Environmental Management* 22: 1315-1320.

Okeke, C. and A. Chinelo. 2018. Ezeabara phytochemical screening and in vitro antimicrobial activity of various parts of *Cleome ciliata*. *Bioscience Horizons* 11: 1-7.

Olajuyigbe, O.O., Babalola, A.E. and A.J. Afolayan. 2011. Antibacterial and phytochemical screening of crude ethanolic extracts of *Waltheria indica*. *African Journal of Microbiology Res*earch 5: 3760-3764.

Oliveira, H. 2012. Chromium as an environmental pollutant: Insights on induced plant toxicity. *Journal of Botany* 2012: 1-8.

Olivier, J., Symington, E.A., Jonker, C.Z., Rampedi, I.T. and T.S. Van Eeden. 2012. Comparison of the mineral composition of leaves and infusions of traditional and herbal teas. *South African Journal of Science* 108: 1-7.

Olowoyo, J.O., Okedeyi, O.O., Mkolo, N.M., Lion, G.N. and S.T.R. Mdakane. 2012. Uptake and translocation of heavy metals by medicinal plants growing around a waste dump site in Pretoria, South Africa. *South African Journal of Botany* 78: 116-121.

Osuocha, K.U., Akubugwo, E.I., Chinyere, G.C. and A.E. Ugbogu. 2016. Seasonal impact on phyto-accumulation potentials of selected edible vegetables grown in Ishiagu quarry mining effluent discharge soils. *African Journal of Environmental Science and Technology* 10: 34-43.

Owour, P.O., Ng'etich, K.W. and M. Obanda. 2000. Quality response of clonal black tea to nitrogen fertilizer, plucking interval and plucking standard. *Journal of the Science of Food and Agriculture* 70: 47-52.

Panche, A.N, Diwan, A.D. and S.R. Chandra. 2016. Flavonoids: An overview. *Journal of Nutritional Science* 5: 1-15.

Panjehkeh, N., Backhouse, D. and A. Taji. 2009. Role of proanthocyanidins in resistance of the legume *Swainsona formosa* to *Phytophthora cinnamomi*. *Journal of Phytopathology* 158: 365-371.

Patel, N.T., Panchal, N.S., Pandey, I.B. and A.N. Pandey. 2011. Implications of calcium nutrition on the response of mimosaceae (*Acacia senegal*) to soil salinity. *Anales de Biología* 33: 23-34.

Pravina, P., Sayaji, D. and A. Mokashi. 2013. Calcium and its role in human body. *International Journal of Research in Pharmaceutical and Biomedical Science* 4: 659-668.

Puupponen-Pimia, R., Nohynek, L., Meier, C., Kahkonen, M., Heinonen, M.A. and A. Hopia. 2001. Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology* 90: 494-507.

Radusiene, J., Bagdonaite, E. and S. Kazlauskas. 2004. Morphological and chemical evaluation on *Hypericum perforatum* and *Hypericum maculatum* in Lithuania. *Acta Horticulturae* 629: 55-62.

Rampedi, I. and J. Olivier. 2005. The use and potential commercial development of *Athrixia phylicoides*. *Acta Academia* 37: 165-183.

Randhir, R., Lin, Y.T. and K. Shetty. 2004. Phenolics, antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asia Pacific Journal of Clinical Nutrition* 13: 295-307.

Raya, K.B., Ahmad, S.H., Farhana, S.F., Mohammad, M., Tajidin, N.E. and A. Parvez. 2015. Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration. *Bragantia*

Rehman, S., Almas, K., Shahzadi, N. Bhatti, N. and A. Saleem. 2002. Effect of time and temperature on infusion of tannins from commercial brands of tea. *International Journal of Agriculture and Biology* 4: 285-287.

Reid, K.A., Jager, A.K. and J. Van Staden. 2001. Pharmacological and phytochemical properties of *Dombeya rotundifolia*. *South African Journal of Botany* 67: 349-353.

Rhoda, L. 2006. Sustainability harvesting of wild rooibos (*Aspalathus linearis*) in the Suid Bokkeveld, Northern Cape. Masters Dissertation submitted to the University of Cape Town, Cape Town, South Africa.

Ringer, J. and Y. Barlett. 2007. The significance of potassium. *The Pharmaceutical Journal* 278: 497-500.

Rodriguez, E., Santos, C., Azevedo, R., Moutinho-Pereira, J., Correia, C. and M.C. Dias. 2012. Chromium (VI) induces toxicity at different photosynthetic levels in pea. *Plant Physiology and Biochemistry* 53: 94-100.

Sajilata, M.G., Bajaj, P.R. and R.S. Singhal. 2008. Tea polyphenols as nutraceuticals. *Comprehensive Reviews in Food Science and Safety* 7: 229-254.

Salahinejad, M. and F. Aflaki. 2010. Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. *Biological Trace Element Research* 134: 109-117.

Salisbury, F.B. and C.W. Ross. 1992. Plant physiology. Wasworth: California.

Salvet, A., Antonnacci, L., Fortunado, R.H., Suarez, E.Y. and H.M. Godoy. 2001. Screening of some plants from Northern Argentina for their antimicrobial activity. *Journal of Ethnopharmacology* 32: 293-297.

Samadi, S. and F.R. Fard. 2020. Phytochemical properties, antioxidant activity and mineral content (Fe, Zn and Cu) in Iranian produced black tea, green tea and roselle calyces. *Biocatalysis and Agricultural Biotechnology* 23: 1-6.

Sano, J., Inami, S., Seimiya, K., Ohba, T., Sakai, S. and T. Takano. 2004. Effects of green tea intake on the development of coronary artery disease. *Circulation Journal* 68: 665-670.

SAS Institute Inc. 2008. SAS/STAT® 9.2 Qualification Tool User's Guide. SAS Inc: Carry, USA.

Serafini, T., Kennedy, T.E., Galko, M.J., Mirzayan, C., Jessell, T.M. and M. Tessier-Lavigne. 1994. The netrins define a family of axon outgrowth-promoting proteins homologous to *C. elegans* UNC-6*. Cell* 78: 409-420.

Serfor-Armah, Y., Nyarko, B.J.B., Akaho, E.H.K., Kyere, A.W.K., Osae, S., Oppong-Boachie, K. and E.K.J. Osae. 2001. Activation analysis of some essential elements in five medicinal plants used in Ghana. *Journal of Radioanalitical and Nuclear Chemistry* 250: 173-176.

Shah, A., Ryushi, K., Fumihito, A., Shigeru, K. and K. Shigenao. 2006. Alleviation of manganese phytotoxicity in barley with calcium source. *Journal of Plant Nutrition* 29: 59-74.

Shanker, A.K., Cervantes, C., Laza-Tavera, H. and A. Subramanian. 2005. Chromium toxicity in plants. *Environmental International* 31: 739-753.

Sharanabasappa, G.K., Mallikharjuna, P.B., Rajanna, L.N. and Y.N. Seetharam. 2007. Antibacterial and phytochemical evaluation of three medicinal plants. *Journal of Natural Products* 5: 327-334.

Sharma, A. and A. Dhiman. 2013. Nickel and cadmium toxicity in plants. *Journal of Pharmaceutical and Scientific Innovation* 2: 20-24.

Sheikh, R., Amin, A.S., Atwa, M.A., Gouda, A.A. and A.A. Abdullah. 2015. Determination of phenolic components and antioxidant activity of some Egyptian tea samples*. International Journal of Pharmacy and Pharmaceutical Sciences* 7: 198-202.

Shen, C.L., Yeh, J.K., Cao, J.J., Chyu. M.C. and J.S. Wang. 2011. Green tea and bone health: Evidence from laboratory studies. *Pharmacological Research* 64: 155-161.

Shrestha, P., Sulis, M., Simmer, C. and S. Kollet. 2015. Impacts of grid resolution on surface energy fluxes simulated with an integrated surface-groundwater flow model hydrol. *Journal of Earth System Science* 19: 4317-4326.

Singh, H.P., Mahajan, P., Kaur, S., Batish, R.D. and R.K. Kohli. 2013. Chromium toxicity and tolerance in plants. *Environmental Chemistry Letters* 11: 229-254.

Singh, R., Gautam, N., Mishra, A. and R. Gupta. 2011. Heavy metals and living systems: An overview. *Indian Journal of Pharmacology* 43 :246-253.

Singh, U.M., Pandey, D. and A. Kumar. 2014. Determination of calcium responsiveness towards exogenous application in two genotypes of *Eleusine coracana* L. differing in their grain calcium content. *Acta Physiology Plantarum* 36: 2521-2529.

Singh, V.K., Singh, A., Singh, S.P., Ellur, R.K., Choudhary, V., Sarkhel, S., Singh, D., Nagarajan, M., Vinod, K.K., Singh, U.D., Rathore, R., Prasanthi, S.K., Agrawal, P.K., Bhatt, J.C., Mohapatra, T., Prabhu, K.V. and A.K. Singh. 2012. Incorporation of blast resistance into an elite Basmati rice restorer line, through marker assisted backcross breeding. *Field Crop Research* 128: 8-16.

Smiechowska, M. and P. Dmowski. 2006. Crude fibre as a parameter in the quality evaluation of tea. *Food Chemistry* 94: 366-368.

Sneha, V., Pooja, A., Amrita, D., Divya, K. and V.C. Kolluru. 2017. Antimicrobial and antioxidant activities in the root, stem and leaf extracts of *Centella asiatica. Advances in Biotechnology and Microbiology* 3: 1-6.

Soetan, K.O., Olaiya, C.O. and O.E. Oyewole. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Science* 4: 200-222.

Soladaye, M.O. and E.C. Chukwuma. 2012. Phytochemical analysis of the stem and root of *Cissus populnea* (Vitaceae) – An important medicinal plant in Central Nigeria. *Phytologia Balcanica* 18: 149-153.

Soni, R.P., Katoch, M., Kmar, A., Ladohiya, R. and V. Parmod. 2015. Tea: Production, consumption and its potential and antimicrobial agent. *International Journal of Food and Fermentation Technology* 5: 95-106.

Srinivasan, M.G., Ndeezi, G. and C.K. Mboijana. 2012. Zinc adjunct therapy reduces case fatality in severe childhood pneumonia: A randomized double-blind placebo-controlled trial. *BMC Medicine* 10 :14-18.

Sultana, J., Siddique, M.N.A., Kamaruzzaman, M. and M.A. Halim. 2014. Conventional to ecological: Tea plantation soil management in Panchagarh district of

Bangladesh. *Journal of Science, Technology and Environmental Informatics* 1: 27-35.

Swanepoel, D.P. 1997. The medicinal value of the Southern African Asteraceae. Masters Dissertation submitted to the University of Pretoria, Pretoria, South Africa.

Thaker, A.M. and J.V. Anjaria. 1986. Antimicrobial and infected wound healing response of some traditional drugs. *Indian Journal of Pharmacology* 18: 171-174.

Trease, G.E. and W.C. Evans. 1989. Textbook of Pharmacognosy. W.B. Sanders: London, United Kingdom.

Trouvelot, S., Héloir, M., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., Combier, M., Trdá, L., Daire, X. and M. Adrian. 2014. Carbohydrates in plant immunity and plant protection: Roles and potential application as foliar sprays. *Frontiers in Plant Science* 5: 592-598.

Ugwoke, C.E.C., Norji, J., Anze, S.P.G. and C.V. Ilodibia. 2017. Quantitative phytochemical analysis and antimicrobial potential of the ethanol and aqueous extracts of the leaf, stem and root of *Chromolaena odorata*. *International Journal of Pharmacognosy and Phytochemical Research* 9: 207-214.

Valentao, P., Dias, A., Ferreira, M., Silva, B., Aandre, P.B., Bastos, M.L. and R.M. Seabra. 2003. Variability in phenolic composition of *Hypericum androsaemum*. *Natural Product Research* 17: 135-140.

Van der Wal, S. 2008. Sustainability Issues in the Tea Sector: A Comparative Analysis of Six Leading Producing Countries. Stichting Onderzoek Multinationale Ondernemingen Centre for Research on Multinational Corporations: Amsterdam, The Netherlands.

Van Wyk, B.E. 2008. A broad review of commercially important Southern African medicinal plants. *Journal of Ethnopharmacology* 119: 342-355.

Van Wyk, B.E. and N. Gericke. 2000. People's Plants: A Guide to Useful Plants of Southern Africa. Briza Publications: Pretoria, South Africa.

Velioglu, Y.S., Mazza, G., Gao, L. and B.D. Oomah. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *Journal of Agricultural and Food Chemistry* 46: 4113-4117.

Venkatesan, S., Senthurpandian, V.K., Murugesan, S., Maibuam, W. and M.N.K. Ganapathy. 2006. Quality standards of black teas as influenced by sources of potassium fertiliser. *Journal of the Science of Food and Agriculture* 86: 799-803.

Vlachos, V., Critchley, A.T. and A. Von Holy. 1997. Antimicrobial activity of extracts from selected Southern African marine macroalgae. *South African Journal of Science* 93: 328-332.

Walton, N.J., Mayer, M.J. and A. Narbad. 2013. Molecules of interest: Vanillin. *Phytochemistry* 63: 505-515.

Wang, H.X. and C.Y. Hu. 2005. Study on modification and functional properties of tea drugs protein. *Food Sciences* 26: 135-140.

Wang, L., Kim, D. and C.Y. Lee. 2000. Effects of heat processing and storage on flavanols and sensory qualities of green tea beverage. *Journal of Agricultural and Food Chemistry* 48: 4227-4232.

Wang, S., Meckling, K.A., Marcone, M.F., Kakuda, Y. and R. Tsao. 2011. Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of Agricultural and Food Chemistry* 59: 960-968.

Wani, B.A., Bodha, R.H. and A.H. Wani. 2010. Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plant Research* 4: 2598-2604.

Wei, X., Chen, M., Xiao, J., Liu, Y., Yu, L., Zhang, H. and Y. Wang. 2010. Composition and bioactivity of tea flower polysaccharides obtained by different methods. *Carbohydrates Polymer* 79: 418-422.

White, P.J. and M.R. Broadley. 2009. Biofortification of crops with seven mineral elements often lacking in human diets - Iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182: 49-84.

World Health Organization. 1998. Quality control methods for medicinal plant materials. World Health Organization Geneva: Switzerland.

Yang, J. and R.H. Liu. 2012. The phenolic profiles and antioxidant activity in different types of tea. *International Journal of Food Science and Technology* 20: 1-9.

Yasari, E., Mozafari, S., Shafiee, E. and A. Foroutan. 2009. Evaluation of sink-source relationship of soybean cultivars at different dates of sowing. *Research Journal of Agriculture and Biological Sciences* 5: 786-793.

Yashin, A., Yashin, Y. and B. Nemzer. 2011. Determination of antioxidant activity in tea extracts and their total antioxidant content. *American Journal of Biomedical Sciences* 3: 322-335.

Yashin, I., Yashin, A.Y. and N.I. Chernousova. 2005. Chromatographic determination of tea chemical composition. *Beer and Beverages* 2: 44-49.

Young, E. 2010. Proximate and mineral composition of selected whole invertebrates and nutritional effect of different diets on the field of cricket, *Gryllus bimaculatus*. Honours Dissertation submitted to the University of Natingham Trent, Natingham, Southwell.

Zeng, L.T., Zhou, Y., Fu, X.M., Mei, X., Cheng, S.H., Gui, J.D. and Z.Y. Yang. 2017. Does oolong tea (*Camellia sinensis*) made from a combination of leaf and stem smell more aromatic than leaf only tea? Contribution of the stem to oolong tea aroma. *Food Chemistry* 237: 488-498.

Zhang, Q., Yang, H., Wang, J., Li, A., Zhang, W. and X. Cui. 2013. Effect of green tea on reward learning in healthy individuals: A randomized, double-blind and placebo-controlled pilot study. *Nutrition Journal* 28: 12-84.

Zhen, Y.S. 2002. Tea: Bioactivity and Therapeutic Potential. Medicinal and Aromatic Plants - Industrial Profiles. Taylor and Francis: London, United Kingdom.

Zhishen, J., Mengcheng, T. and W. Jianming. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64: 555-559.

Zwokunda, J.R. 2007. Indigenous knowledge of bush tea (*Athrixia phylicoides*) and effect of fertigation frequency and growing medium of plant growth. Masters Dissertation submitted to the University of Pretoria, Pretoria, South Africa

APPENDICES

Appendix 3.1 Analysis of variance for manganese (Mn) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	495664	55074		
Plant part	2	4201802	2100901	52.40	0.00
Error	18	721655	40092		
Total	29	5419121			

Appendix 3.2 Analysis of variance for sodium (Na) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	567536	63060		
Plant part	2	576006	288003	5.87	0.01
Error	18	882854	49047		
Total	29	2026396			

Appendix 3.3 Analysis of variance for nickel (Ni) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	6733.0	748.11		
Plant part	2	13550.2	6775.08	8.80	0.00
Error	18	13856.9	769.83		
Total	29	34140.0			

Appendix 3.4 Analysis of variance for phosphorus (P) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	200413	22268		
Plant part	2	326547	163274	7.58	0.00
Error	18	387939	21552		
Total	29	914899			

Appendix 3.5 Analysis of variance for sulphur (S) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	729678	81075.4		
Plant part	2	41534	20767.0	0.37	0.69
Error	18	1000219	55567.7		
Total	29	1771431			

Appendix 3.6 Analysis of variance for silicon (Si) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	3176.75	352.972		
Plant parts	2	448.09	224.045	0.94	0.41
Error	18	4268.85	237.158		
Total	29	7893.69			

Appendix 3.7 Analysis of variance for zinc (Zn) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	2040.6	226.73		
Plant part	2	5533.9	2766.95	12.26	0.00
Error	18	4063.4	225.75		
Total	29	11637.9			

Appendix 3.8 Analysis of variance for aluminium (Al) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	353444	39272		
Plant part	2	777366	388683	12.94	0.00
Error	18	540677	30038		
Total	29	1671487			

Appendix 3.9 Analysis of variance for calcium (Ca) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	7.321E+07	8134283		
Plant part	2	6.332E+07	3.166E+07	5.16	0.02
Error	18	1.105E+08	6138077		
Total	29	2.470E+08			

Appendix 3.10 Analysis of variance for cobalt (Co) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	6982.8	775.87		
Plant part	2	15344.7	7672.37	11.00	0.00
Error	18	12555.8	697.54		
Total	29	34883.4			

Appendix 3.11 Analysis of variance for chromium (Cr) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	34748	3860.9		
Plant part	2	75890	37944.8	7.76	0.00
Error	18	88053	4891.8		
Total	29	198691			

Appendix 3.12 Analysis of variance for copper (Cu) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	543.26	60.362		
Plant part	2	948.63	474.316	11.06	0.00
Error	18	772.06	42.892		
Total	29	2263.95			

Appendix 3.13 Analysis of variance for iron (Fe) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	259515	28835		
Plant parts	2	345933	172966	5.87	0.01
Error	18	530698	29483		
Total	24	1136145			

Appendix 3.14 Analysis of variance for potassium (K) content in plant parts of *Jatropha zeyheri.*

Source of variation	DF	SS	MS	F	P
Replication	9	3.967E+07	4407887		
Plant parts	2	5.353E+08	2.676E+08	73.44	0.00
Error	18	6.560E+07	36444397		
Total	29	6.406E+08			

Appendix 3.15 Analysis of variance for magnesium (Mg) content in plant parts of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Replication	9	8523562	947062		
Plant parts	2	2.618E+07	1.309E+07	15.24	0.00
Error	18	1.547E+07	859183		
Total	29	5.017E+07			

Appendix 4.1 Analysis of variance for antioxidant activity in plant parts of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Replication	9	900.9	100.10		
Plant parts	2	5621.5	2810.77	4.51	0.03
Error	18	11220.4	623.36		
Total	29	17742.8			

Appendix 4.2 Analysis of variance for total phenol content in plant parts of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Replication	9	44376	11094.0		
Plant parts	2	109993	54996.7	2.34	0.16
Error	18	187844	23480.5		
Total	29	342214			

Appendix 4.3 Analysis of variance for total tannin content in plant parts of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Replication	9	211606	52901.4		
Plant parts	2	190654	95327.2	5.51	0.03
Error	18	138408	17301.0		
Total	29	540667			

Appendix 4.4 Analysis of variance for total flavonoid content in plant parts of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Replication	9	30822	7705.5		
Plant parts	2	60344	30172.2	7.38	0.02
Error	18	32704	4088.0		
Total	29	123871			