INTERACTIVE EFFECT OF HARVESTING SEASONS AND DRYING METHODS ON THE QUALITY OF *JATROPHA ZEYHERI* TEA LEAVES

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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

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DECLARATION

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

Candidate: Makgabiso Constance Ngoetjana	Signature	Date

DEDICATION

To my dearest parents (Mr Masenya Charles Ngoetjana and Mrs Matlou Christine Ngoetjana).

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ABSTRACT

Worldwide, second to water, tea is in the upper class of the most consumed beverages and has greater popularity. The increased consumption and popularity of tea is associated with its health promoting properties and medicinal use. *Jatropha zeyheri* is being used for various purposes due to its nutritional and medicinal properties. In some parts of South Africa, the leaves of *J. zeyheri* are harvested during winter when the leaves are dry to make tea beverage. However, appropriate harvesting seasons and suitable drying methods that will contribute towards optimising the quality of *J. zeyheri* tea leaves is not documented. Therefore, the objectives of this study were two folds, namely, (i) determine whether harvesting seasons and drying methods have an effect on phytochemicals and antioxidants activity of *J. zeyheri* leaves and (ii) investigate whether harvesting seasons and drying methods have an effect on essential and non-essential mineral elements of *J. zeyheri* leaves. Leaves of *J. zeyheri* were collected from Khureng village, Lepelle-Nkumpi Municipality, Limpopo Province, South Africa. To achieve the objectives, a 3 x 4 factorial experiments, with first factor comprising harvesting seasons (autumn, winter and summer), while the second factor constituted the drying methods (shade, sun, oven and freeze drying), were arranged in a randomised complete block design (RCBD), with 9 replications. Approximately, 1 g of ground powdered plant materials were extracted with 10 mL of acetone. After the preparations, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay was used to quantify the antioxidant activity (AA) of the acetone extracts of plants. The total phenol content (TPC) and total tannin content (TTC) in each plant extract were determined using the Folin-Ciocalteu assay method. The total flavonoid content (TFC) was determined using the Aluminium Chloride colorimetric assay. The absorbance for AA and phytochemicals were achieved using UV/visible

spectrophotometer. Mineral elements were determined using Inductively Coupled Plasma Emission Spectrometer-9000. Harvesting seasons had highly significant ($P \leq 0.01$) effects on TPC, TFC, TTC and AA contributing 68, 86, 80 and 65% in TTV, respectively. Drying methods had highly significant effects on TPC, TFC, and AA contributing 18, 10 and 18% in TTV, respectively, whereas drying methods had no significant ($P \leq 0.05$) effect on TTC. Interaction of drying methods and harvesting seasons had highly significant effects on TPC and AA, contributing 10 and 14% in TTV, respectively, whereas total TFC was significantly affected, contributing 2% in TTV. However, TTC was not affected by the interaction between harvesting seasons and drying methods. Summer harvesting season was more efficient in retaining the highest TPC and AA, autumn harvesting season retained the highest TFC while, winter retained TTC. Drying methods demonstrated that oven drying is more efficient in retaining TPC and TFC of *J. zeyheri* tea leaves, as compared to other drying methods. However, freeze drying is more effective in retaining AA of *J. zeyheri* tea leaves. Interactively, the results of this study conclude that summer and oven drying had the highest TPC and TFC, however summer and freeze drying had the highest AA. Harvesting seasons had highly significant effect on essential mineral elements, Ca, Cu, Fe, K, Mn, Ni, and P contributing 14, 50, 25, 42, 53, 74 and 49% in TTV, respectively, whereas Zn was significantly affected, contributing 13% in TTV. However, harvesting seasons had no significant effect on Mg. Drying methods had highly significant effects on Ca, Fe, Mg, Mn and P contributing 57, 37, 57, 25 and 19% in TTV, respectively, whereas, Cu and K were significantly affected, contributing 14 and 13% in TTTV. However, no significant effect was observed on Ni and Zn. Interaction of harvesting seasons and drying methods had highly significant effects on Ca, Cu, Fe, K, Mn, P and Zn contributing 25, 26, 30, 32, 20, 24 and 48% in TTV,

respectively, whereas Mg was significantly affected contributing 14% in TTV. However, no significant effect was observed on Ni. Summer harvesting season accounted for the highest content of essential mineral elements (Fe, Ni, P and Zn), additionally summer harvesting accounted for the highest non-essential mineral elements (Al and Na). Drying methods demonstrated that sun and oven drying accounted for the lowest contents of the selected elements, whereas most of the elements were retained by freeze and shade drying. Interactively summer and shade drying retained most of the mineral elements. In conclusion, the results of this study suggest that harvesting of *J. zeyheri* tea leaves should be done during the summer season and subjected to oven-drying for improved accumulation of phytochemicals. However, for improved accumulation of AA harvested leaves should be freeze-dried, whereas, for improved accumulation of mineral elements, the leaves of *J. zeyheri* should be subjected to shade and freeze-drying methods.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Jatropha zeyheri is a native plant belonging to the Euphorbiaceae family (Arnold *et al.,* 2002). The plant is perennial and densely hairy with thick rhizomes, simple to sparingly branched stems with alternate leaves (Arnold *et al*., 2002). The genus, *Jatropha* comprises about 170 species with 70 species native to Africa and 1 species to Madagascar. In Africa the plant is distributed in Botswana, Zimbabwe, northern parts of South Africa and Swaziland (Semenya and Maroyi, 2018). *Jatropha zeyheri* is mostly used for nutritional and medicinal purpose in southern Africa (Archer and Victor, 2005). Fresh tubers of *J. zeyheri* are used in women health care such as regulation of menstrual cycles, ease of uterine pain and treatment of urinary tract infections (Van Wyk and Gericke, 2007). Also, the rhizomes are used to heal wounds and boils (Van Wyk and Gericke, 2007). Mature dried leaves of *J. zeyheri* are brewed with hot water and consumed as tea beverage in some parts of rural South Africa (Mutshekwa, 2017).

Second to water, tea is in the upper class of the most consumed beverages and has greater popularity worldwide (Khan and Mukhtar, 2013). Globally, tea is part of the huge beverage market. Large populations of Asia, America, Middle East countries and Africa produce tea. About 25% of the world import demand and tea revenue represent 50% of the country's producing tea currency earning (Anon, 1996). These highlight that tea has a huge contribution to the economy of the producing countries. The South African tea industry is one of the oldest industries with huge employment numbers mostly in the rural areas (Chasomeris *et al*., 2015). Tea is an important commodity for a number of developing countries as it contributes significantly to job creation and

export earnings and millions of livelihoods around the world depend on tea production (Chasomeris *et al*., 2015). The increased consumption and popularity of tea are associated with its health promoting properties and medicinal use (Khan and Mukhtar, 2013).

Tea is a rich source of different classes of bioactive compounds such as antioxidants, alkaloids, amino acids, carbohydrates, vitamins, lipids, minerals, proteins and phytochemicals (Parajuli *et al*., 2020). These components attribute to the quality, richness, taste, flavour and health benefits of different types of teas (Adnan *et al*., 2013). Antioxidant activity in tea is associated with anti-cancer health properties. The phytochemicals in tea protect cells from free radicals damage, assist with metabolic rate and the decrease in cholesterol blood levels, which make tea a healthy drink (Chen *et al*., 2008). The quality of tea is affected by pre- and post-harvest factors, which include climatic conditions, variety, soil, harvesting season, manufacturing processing, drying methods and storage (Aidoo, 1993).

Harvesting season is one of the most critical factors that affect quality of tea (Tounekti *et al.,* 2013). Harvesting seasons have variations of climatic conditions such as temperature and precipitation that alter the complex balance of chemicals that give tea its flavour and prospective health benefits (Nowogrodzki, 2019). Tea processing is the transformation of fresh tea leaves into dry leaves for brewing. Tea processing involves various drying methods, which are important in the preservation of natural health promoting properties in tea (Singh *et al*., 2014). Drying methods are one of the processing techniques that have great effect on tea quality. Tea quality determines the market price of the tea and the degree of its health benefits (Hajiboland, 2017).

However, there is limited of knowledge on the suitable harvesting seasons and drying methods of *J. zeyheri* leaves. In most rural areas the leaves are harvested while they are already dry, which is unsuitable for high quality tea production. Therefore, the objective of this study was to determine whether harvesting seasons and drying methods would have an effect on phytochemicals, antioxidants activity and mineral elements of *J. zeyheri* leaves.

1.2 Problem statement

Harvesting seasons and post-harvest treatment highly influence the quality and value of tea. Generally, tea is harvested in different seasons namely spring, summer, winter and autumn. The spring tea is harvested before late May and it has high consumer preference because of its bitter taste and increased flavour complexity. Summer tea and autumn tea are accredited as more astringent and bitter than spring tea, which ranks them lower in economic value (Pan *et al*., 2015). In winter, leaves dry out and old dry leaves are considered a waste in the tea industry as they would have lost important phytochemicals (Mutshekwa, 2017). Drying methods may lead to loss, maintenance and/or improvement of certain chemical composition. Therefore, the study proposed to investigate the interactive effect of harvesting seasons and drying methods on the quality of *J. zeyher*i tea.

1.3 Rationale

Jatropha zeyheri tea is an indigenous plant of nutritional and medicinal properties; therefore, understanding chemical composition is vital for increasing its quality (Mutshekwa, 2017). There is lack of knowledge on the effect of harvesting seasons of *J. zeyheri* tea with various suitable drying methods that are favourable for production

of high-quality tea. Quality of tea changes seasonally due to changes in the climate. According to Ahmed (2011), spring harvested tea contributed to high quality of green tea leaves in terms of taste, aroma and health promoting properties as compared to the one cultivated during rainy season. Black tea leaves harvested in summer season are considered more bitter contrary to tea leaves of other seasons (Tounekti *et al*., 2013). This is due to high temperature and prolonged sunlight exposure that lead to high oxidation of the leaves (Tounekti *et al*., 2013). Tea leaves harvested in autumn are considered richer in nutrients than tea leaves harvested in summer, the cooler season exposes the leaves to low temperature and sunlight, which influence the concentration of catechin that also contribute to overall effect of tea quality (Tounekti *et al*., 2013). Tea harvested in summer and autumn months are considered as more bitter than those harvested in spring months (Pan *et al*., 2015). Therefore, the determination of harvesting seasons and favourable drying methods have an opportunity to contribute towards increasing the quality of *J. zeyheri* indigenous tea and thereby increasing its health, nutritional, economic benefits as well as its market value in the future.

1.4 Purpose of the study

1.4.1 Aim

The aim of this study was to assess the interactive effect of harvesting seasons and drying methods on quality of *J. zeyheri* tea.

1.4.2 Objectives

The objectives of this study were to:

(i) Determine whether harvesting seasons and drying methods have an effect on phytochemicals and antioxidants activity of *J. zeyheri* leaves.

(ii) Investigate whether harvesting seasons and drying methods have an effect on essential and non-essential mineral elements of *J. zeyheri* leaves.

1.4.3 Hypotheses

(i) Harvesting seasons and drying methods have an effect on phytochemicals and antioxidants activity of *J. zeyheri* leaves.

(ii) Harvesting seasons and drying methods have an effect on essential and non-essential mineral elements of *J. zeyheri* leaves.

1.5 Reliability, validity and objectivity

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

1.6 Bias

Bias was minimised through reduction of experimental error by increasing the number of replications. Also, treatments were assigned randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Scientific significance of the study

Findings of this study will impart knowledge to villagers and farmers on suitable harvesting seasons and drying methods for *J. zeyheri* leaves, which will improve its

health benefits and quality by optimising its chemical constituents. Furthermore, the findings will be useful for commercialisation of *J. zeyheri* tea.

1.8 Structure of the mini dissertation

The research problem was outlined and described in detail (Chapter 1), the work done and the work not done on problem statement were reviewed (Chapter 2). Then, each of the two objectives was discussed in distinct chapters (Chapters 3 and 4). In the final chapter (chapter 5), results from all chapters were summarised and integrated to provide the significance of the results and recommendations with respect to future research and culminated an overall conclusion of the study. The citation in text and references used in the study were as in the Harvard style as prescribed by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Tea is a standout drink amongst other popular drinks because of its ability to induce cognition, relaxation and convergent thinking (Einöther and Martens, 2013). Moreover, it has the ability to burn fat as fuel, which leads to improved muscle endurance (Einöther and Martens, 2013). Tea protects against diseases such as cardiovascular, degenerative diseases, inflammatory bowel diseases and metabolic diseases (Khan and Mukhtar, 2013). However, the health benefits of tea depend on its quality attributes. Tea quality is affected by pre- and post-harvest factors, which include harvesting seasons, climatic conditions, and agronomic practices, plucking, withering and drying (Yao *et al*., 2005). These factors have an effect on the chemical composition of tea such as antioxidant activity, phytochemicals and minerals. The chemical compositions determine the outcome of quality characteristics of tea such as aroma, taste and colour (Thea *et al*., 2012).

2.2 Work done on the research problem

2.2.1 Pre-harvest factors affecting tea quality

Harvesting seasons: Harvesting seasons have an influence on the physiological and chemical parameters that determine the overall yield and quality of tea (Ahmed *et al*., 2019). The growth and production of tea plant depend on the climatic conditions including temperature, rainfall, humidity and solar radiation (Ahmed *et al*., 2019). Seasonal climate change factors determine the concentration of nutrients, minerals and secondary metabolites. According to Ahmed *et al*. (2019) climate change factors have resulted in a decrease of multiple secondary metabolites in a range of food and

beverages crops including tea. Tea is harvested in different seasons throughout the year depending on the cultivar and the area of production.

Many teas are known to grow through the warmer months of the year. Rakuambo (2011) reported that the growth rate of bush tea (*Athrixia phylicoides*) between the autumn season and winter season was higher as compared to winter and spring season. Erturk *et al.* (2010) reported that summer harvest had the highest total phenolic content of green tea shoots as compared to other seasons, which increase quality of the tea. Harvesting of medicinal herbs, leather leaf barleria (*Barleria dinteri)* and brandy bush (*Grewia flava)* during autumn and winter seasons resulted in high concentration of tannin leading to astringent taste (Gololo *et al*., 2016). Green tea leaves harvested in the rainy season are reported to have the lowest quality in terms of taste, aroma and health promoting properties as compared to those harvested during the spring season (Ahmed, 2011).

Climatic conditions: Variations in climatic conditions alter the complex balance of chemicals that give tea its flavour and potential health benefits (Chen *et al*., 2008). Sunlight is known to affect plant growth and development. In addition, light regulates the biosynthesis of both primary and secondary metabolites (Ghasemzadeh *et al*., 2010). Adequate amounts of temperature are essential for photosynthesis, chloroplast regulation for proper growth of tea plant (Chen *et al*., 2008). Low temperature induced low concentration of amino acids in albino tea (Chen *et al*., 2008). Sud *et al.* (1995) suggested that high temperature induces the uptake of calcium contents in tea leaves.

Ahmed *et al.* (2019) suggested that rainfall reduced green tea quality as it led to low amounts of antioxidant accumulation, which reduced its flavour, medicinal and nutritional properties. High amount of rainfall may cause erosion of essential mineral elements and waterlogging of soil, which reduces nutrient absorption and leads to reduction in tea quality. Higher water availability increased total methyl xanthine concentration, decreased epigallocatechin gallate (ECG) levels and decreased total phenolic contents of tea leaves (Ahmed *et al*., 2019). Shrubs with shallow roots, such as clonal tea shrubs, are particularly susceptible to drought effects and show severe water stress during the dry season (Ahmed *et al*., 2019). Furthermore, Gulati and Ravindranath (1996) suggested that low rainfall leads to reduced accumulation abilities of phytochemicals by tea leaves.

Agronomic practices: Plucking and pruning are amongst major agronomic practices that are essential for optimizing yield and quality of tea (Madamombe, 2016). Plucking is the harvesting of fresh tea shoots that emerge from the axillary buds near the top of the canopy. The shoots can be plucked using various methods and intensities (Madamombe, 2016). Quality of tea is also affected by the growth rate of the pluckable shoots and it improves as growth rate decrease (Madamombe, 2016). Intensity of harvesting tea can be defined in terms of number of leaves or axillary buds left behind after a shoot is harvested. The plucking intervals may be either short or long, with short ones being superior to long intervals with regard to the aflavins, caffeine, brightness and flavour index (Mudau *et al*., 2006). Owuor *et al*. (2011) reported that short plucking intervals ensure production of high-quality tea.

Plucking standards are essential for determining black tea quality and are categorised as fine, medium or coarse (Wright, 2005). Harvesting of the first two leaves and bud picking is fine plucking, with medium or coarse plucking three or four leaves and the bud are harvested (Wright, 2005). According to Wright (2005), fine plucking optimizes the yield and quality of tea. The quality of black tea depreciated with regard to coarse plucking standards in terms of plain and aroma quality parameters (Mahanta and Baruah, 2006). Hand plucking and mechanical plucking are the two methods used for harvesting of tea shoots. Hand plucking increased tea the aflavins, caffeine, brightness and flavour index (Owuor *et al.*, 2011). Hand-plucked teas were very rich in their green leaf biochemical precursors and had higher contents of made-tea quality constituents than mechanical plucked teas (Wright, 2005).

Pruning is an agronomic practice that comprises of selective elimination of certain plant parts (Yilmaz *et al*., 2004). Tea plants are usually pruned to eliminate unwanted and diseased branches, for rejuvenation of the plant and to obtain healthier and better quality tea (Yilmaz *et al*., 2004). Pruning prior to harvest has great outcome on plant productivity and quality. Phytochemicals such as total phenolic content were found to increase in the first year after pruning (Yilmaz *et al*., 2004). All the pigment contents of black tea, with the exception of chlorophyll were higher in pruned tea leaves than un-pruned (Maudu *et al*., 2010). Maudu *et al*. (2010) also reported higher total phenolic content in black tea. In contrast, Maudu *et al*. (2010) reported highest total phenolic content and total tannin content in un-pruned tea leaves of *bush tea (A. phylicoides).*

2.2.2 Post-harvest factors affecting tea quality

i) Drying: It is one of the common methods used in tea processing and it consists of different techniques. The techniques have an impact on the nutritional value, taste, colour, texture and economic value of tea (Roslan *et al*., 2020). Drying basically modifies the physical micro structure and chemical structure of plant tissues (Roslan *et al*., 2020). There is breakage of cell wall and creation of many micro-cavities. Cellular deformation is primarily driven by the lower moisture content in the plant due to drying (Roslan *et al*., 2020). The drying process breaks down cellular constituents leading to increased release of active compounds from the food matrix (Roshanak *et al*., 2016). There are differences with retaining of phytochemicals due to nature of plants bioactive compounds during drying. Some of the plant materials undergo oxidation that can destroy heat sensitive active ingredients (Roshanak *et al*., 2016). Consequently, plant materials undergo enzymatic degradation under certain drying conditions (Roshanak *et al*., 2016). A high temperature can inactivate plant phenolic oxidase (PPO) (Roshanak *et al*., 2016). Additionally, the chemical conservation of phenolic compounds to quinone's is catalysed by PPO and can lead to enzymatic browning and loss of phenolic and fresh plant materials (Roshanak *et al*., 2016). The chemical oxidation of phenolic compounds to phenoxyl radicals is catalysed by plant polyphenol oxidase (PPO) with hydrogen peroxide. Inability of the drying method to inactivate enzymes such as PPO causes oxidation of chlorophyll (Li *et al*., 2018). Generally, oxidation or pyrolysis reactions during drying affect the chemical component of plant materials.

Drying has profound influence on the quality of a product and its value. It represents 30-50% of total cost in a dried plant (Roshanak *et al*., 2016). The various techniques

used for drying may either be of technical or natural. Technical ones are in use of auxiliary energy from the dryers. The natural method dries without auxiliary energy and includes methods such as drying in the field or in sheds (Müller and Heindl, 2006). However, optional combination of the dryer design, operational method, drying temperature and quality maintenance are vital during drying (Müller and Heindl, 2006). To some extent they have an influence on the appearance of tea and the preservation of its unstable components. Thus, depending on the drying temperature used and hence the length of time required to achieve constant weight, drying can cause loss in water soluble carbohydrates due to respiration (Müller and Heindl, 2006). Drying plant materials at high temperatures results in the formation of indigestible protein carbohydrates complexes called the Millard products (Müller and Heindl, 2006). Depending on the method in use, drying can oxidise and destroy heat sensitive polyphenols causing tea to lose most of its antioxidant properties (Müller and Heindl, 2006). The effect of a particular drying method on the retention of active ingredient quality is not predictable and depends on the compounds and the specific plant involved (Müller and Heindl, 2006). Various drying methods such as shade drying, sun drying, oven drying and freeze drying are reviewed in detail below.

Shade drying: Shade drying is one of the most common drying methods as it is operationally simple and inexpensive (Mbondo *et al*., 2018). During shade drying, plant materials are subjected to drying where there is efficient air flow and no sunlight exposure. The dried materials are usually placed in open trays for better aeration. Shade drying is usually beneficial for the preservation of sun-unstable components (Mbondo *et al*., 2018). However, it is a slow process, which usually allows inherent metabolic processes of the plant to continue after harvest (Mbondo *et al*., 2018).

Shade drying as a slow process, its slower rate of drying promotes loss of non-structural carbohydrates, loss of volatile organic substrates and protein degradation (Mbondo *et al*., 2018). The loss of these cellular components results in dried substrates with a higher concentration of cell wall components (Mbondo *et al*., 2018). However, low temperatures can protect against degradation of active components. This may lead to adverse effects of the dried plant materials, such as colour changes or loss of active ingredients (Mbondo *et al*., 2018). Roshanak *et al.* (2016) reported that shade drying of green tea resulted in lower TPC as compared to oven drying. Mathivha and Mudau (2017) reported that shade drying retained the highest TPC on bush tea (*A. phylicoides*). According to Rababah *et al.* (2015), shade drying also reported the highest TFC in mint leaves *(Mentha. spicata*). During shade drying there might be colour change of leaves, which could be a favourable quality attribute for tea production.

Sun-drying: It is an indigenous drying method whereby auxiliary energy from the sunrays is used specifically as heat requirement to reduce the moisture content in the leaves (Brennand, 1994). The solar radiation heats up the leaves as well as the surrounding air and thus increases the rate of water evaporation (Brennand, 1994). Consequently, the method leads to leaf morphology deformation as it reduces the moisture content. The drying conditions affect the epidermal surfaces and cause shrinkage of granular trachoma in the leaves (Brennand, 1994). This is usually a result of increased drying temperature from the sun. These suggest that drying affects the physical structure of cell wall, which might lead to breakage of plant tissues. However, the UV radiation has beneficial effect on secondary metabolism processes in plant (Brennand, 1994). Most of the active ingredients in medicinal plants are secondary

metabolites. Many studies suggested that enhanced UV radiation could induce secondary metabolism process and therefore increase active ingredient contents in medicinal plants (Brennand, 1994). On the contrary, higher drying temperature tends to reduce the yields and phytochemicals in plants (Ahmed *et al*., 2019). To dry in the sun, hot, dry, breezy days are best with minimum temperature ranges of 30°C - 50°C to protect sensitive phytochemicals (Ahmed *et al*., 2019). It takes several days to dry plant materials out-of-doors, due to uncontrollable weather. Humidity below 60% is best for sun drying (Ahmed *et al*., 2019). Screens need to be safe for contact with food. The best screens are stainless steel, teflon coated fiberglass or plastic (Ahmed *et al*., 2019). Sun dried leaves of shell ginger tea (*Alpinia zerumbet*) had the lowest TPC as compared to other drying methods. Losses in antioxidant potential of heat-treated samples have been attributed to thermal degradation of phenolic compounds (Chan *et al*., 2009).

Oven-drying: Oven drying is a thermo gravimetric method, in which the sample is dried for a defined period of time at constant temperature (Ahmed, 2011). The oven-dried samples are heated by convection. This means the samples are at the same temperature as the drying oven (Ahmed, 2011). The sample heats up and dries by absorbing infrared radiation from the heating element (Ahmed, 2011). The sample's temperature and drying time depends on its absorption characteristics, which has an influence on the economic parameters, such as drying capacity, energy requirement and plant quality (Ahmed, 2011). The oven drying method is effective in shortening the drying time and guarantees the stability of drying temperature. However, high and prolonged temperatures might result in the loss of heat sensitive phytochemicals (Müller and Heindl, 2006). Some phytochemicals decompose rapidly when exposed

to intense temperature (Müller and Heindl, 2006). This explains that there is thermal degradation of phenolic compounds, which follows first order kinetics, in which the degradation rate depends on the temperature, the amount of soluble solids and the pH (Müller and Heindl, 2006). Stability of phenolic compounds depends on the source, from which they have been extracted (Müller and Heindl, 2006). However, most studies reported that the higher the temperature, the faster the degradation rate of total phenolic compounds (Muller *et al*., 2007). This behaviour is typically of anthocyanins, which present slow hydrolysis of the glycosidic bond in position three and opening of the ring to produce colourless chalcones (Muller *et al*., 2007). Consequently, low drying temperatures between 30 and 50°C are recommended to protect sensitive active ingredients, but the decelerated drying process causes a low capacity of drying installations (Müller and Heindl, 2006). To achieve increased dryer capacity, drying temperature should be chosen without reducing the quality of the product. Maximum allowable temperatures depend mainly on the chemical composition of the active ingredients and in respect of the medicinal plant species. According to Müller and Heindl (2006), different plant species revealed that no general recommendations about drying temperature can be made, but that each species has to be investigated individually. Roshanak *et al.* (2016) reported that oven drying retained the highest TPC and TFC on *C. assamica*. According to Chan *et al.* (2009) oven drying had the highest content of TPC on ginger leaves. Alternatively, the oven drying method had the lowest content of TFC in *M. spicata* (Rababah *et al*., 2015).

Freeze-drying: Lyophilization or freeze drying is a process, in which water is frozen, followed by its removal from the sample, initially by sublimation, and then by desorption (Gaidhani *et al*., 2015). It is used to maintain materials for prolonged

storage periods in dry state. It is central to the protection of materials, which require low moisture content in order to ensure stability and require a sterile and gentle preservation process (Gaidhani *et al*., 2015). Freeze drying has been used in a number of applications for many years, most normally in the food and pharmaceutical industries (Gaidhani *et al*., 2015). During the process of freeze drying, leaves are usually dried without being harmed. The leaves are frozen and dried under vacuum, without being allowed to thaw out (Gaidhani *et al*., 2015). Freeze dried leaves of black tea (*C. sinensis*) maintained the highest antioxidant activity compared to other drying methods (Roslan *et al.*, 2020). Mathivha and Mudau (2017) reported that freeze drying retained the highest TPC on *A. phylicoides*. The bioactive compounds retention in freeze drying depends on the variety, post-harvest factors, and other factors of the tea leaves (Mathivha and Mudau, 2017).

ii) Storage: The conditions and time of storage are essential for retaining of phytochemicals and antioxidant activity of tea leaves. Tea leaves have a considerably long shelf-life due to their low moisture content (Kosińska and Andlauer, 2014). Chinese pu-erh tea and old oolong tea (*Camellia sinensis*) long storage is even necessary for the development of desirable taste and aroma (Kosińska and16 Andlauer, 2014). However, for other tea types, storage for an extended period can lead to loss of quality of the product (Thomas *et al*., 2008). It was reported that tea catechins are not stable during long-term storage (Thomas *et al*., 2008). Storage of black tea (*C. sinensis*) for up to 12 months can affect theaflavins and thearubigins content (Thomas *et al*., 2008). Factors such as light, oxygen and temperature affect stability of bioactive compounds during storage. Furthermore, storage stability of tea depends on the packaging material used (Thomas *et al*., 2008). It was reported that

cold storage at 4°C of tea beverages in polyethylene terephthalate (PET) bottles ensures a slower decrease in catechins content in white, black, and green teas (Thomas *et al*., 2008).

2.3 Chemical compositions of tea quality

2.3.1 Phytochemicals and antioxidant activity

Phytochemicals are substances produced naturally by plants, and these substances have biological activity (Mendoza and Silva, 2018). They provide desirable health benefits and reduce the risk of major chronic diseases (Mendoza and Silva, 2018). Phytochemicals also help plants in defence against fungi, bacteria, plant virus infections, attack of pests and protect them from environmental hazards (Mendoza and Silva, 2018). There are different categories of phytochemicals, which include phenolic compounds, anthocyanin, carotenoids, tannins, flavonoids, glycosides and carotenoids (Altemimi *et al*., 2017).

Phenolic compounds: Phenolic compounds are secondary metabolites that are abundant in plants and plants derived foods and beverages (Altemimi *et al*., 2017). Phenolic compounds arise biogenetically from either the shikimate or phenylpropanoid pathway, which results in simple phenols or both (Lattanzio, 2013). It produces monomeric or polymeric phenols that fulfil a very broad range of physiological roles in plants (Lattanzio, 2013). Plant phenols are considered to have a key role as defence compounds against environmental stresses, such as high light, low temperatures, pathogen infection, herbivores, and nutrient deficiency (Lattanzio, 2013). They can lead to an increased production of free radicals and other oxidative species in plants (Lattanzio, 2013). Phenolic compounds are the most widely distributed secondary

metabolites, ubiquitously present in the plant kingdom (Lattanzio, 2013). The phenolic compound in tea includes catechins, theaflavins, tannins and flavonoids (Chan *et al*., 2009). A study carried out by Yang and Liu (2013) reported that green tea leaves had the highest phenolic content as compared to black tea (*C. sinensis*). Moreover, study by Gololo *et al.* (2016) reported that the phenolic content on leather leaf barleria (*Barleria dinteri*) was higher than that of brandy bush (*Grewia flava*). Phenolic compounds are great parameters of quality for tea and they concentrations vary with plant varieties.

Total flavonoid contents: Flavonoids are a group of phenolic compounds with numerous sub-classes namely anthocyanidins, flavanones, flavanols, flavones, flavonols and isoflavones (Panche *et al*., 2016). The categories are according to the oxidation level of the central heterocyclic ring (Panche *et al*., 2016). Analysis of the diet is simplified by converting the glycosides to 25-30 aglycones, but only a few are relevant to tea (Panche *et al*., 2016). The most common subclasses of flavonoids in tea are the flavanols (primarily catechins) and flavanols (such as quercetin). Also present in tea, but at significantly lower concentrations, are phenolic acids such as gallic acid and cinnamic acid esters of quinic acid (Panche *et al*., 2016). Flavonoids are associated with a broad spectrum of health-promoting effects and are an essential component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al*., 2016). This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (Hodgson and Croft, 2010). Flavonoids are responsible for the astringency and pigmentation of most tea (Panche *et al*., 2016). Sukrasno *et al*., (2011) reported concentration of flavonoids in wild cosmos (*Cosmos caudutus)*.

Total tannin contents: Tannins are astringent large polyphenolic compound containing carboxyl form strong complexes with proteins and other macromolecules (Gelaw *et al*., 2012). The astringency from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (Gelaw *et al*., 2012). Their main biological role in plants is related to protection against infection insects or animal herbivory (Izawa *et al*., 2010). They are divided into two groups, hydrolysable tannins and condensed tannins (Akiyama *et al*., 2001). Hydrolysable tannins re hydrolysed by weak acids or weak base to produce carbohydrate and phenolic acids. Condensed tannins are flavonoid units that are joined by carbon-carbon bonds, which are not susceptible to being cleaved by hydrolysis (Gelaw *et al*., 2012). Tea tannins are potential indicators of health benefits in tea due to their antioxidants and anti-inflammatory effects (Gelaw *et al*., 2012).

Antioxidant activity: Antioxidants are a group of naturally bound chemicals that constrain oxidative stress in biological systems (Sharma *et al*., 2012). Antioxidants contain properties that can scavenge radicals such as reactive oxygen species and reactive nitrogen species (Sharma *et al*., 2012). These species can damage the DNA and lead to excess oxidation of lipids and protein cells (Sharma *et al*., 2012). Plants are abundant sources of naturally producing antioxidants. The arubigins, epicatechins, and catechins are the main antioxidants present in tea plants (Vishnoi *et al*., 2018). Diet derived antioxidants in tea are particularly important as they protect cells from the free radicals that can damage and lead to blood clot formation, cardiovascular disease, cancer and neurodegenerative diseases (Rietveld and Wiseman, 2003). According to Vishnoi *et al*. (2018) green tea (*C. sinensis*) had high amount of antioxidants, which

result from its higher content of catechins as compared black tea or oolong tea (*C. sinensis*).

2.3.2 Mineral elements

Mineral elements are non-plant synthesized chemical substances that are absorbed from the external environment by the roots and they are vital for completion of a plant life cycle (Malongane *et al*., 2020). Fairweather-Tait and Cashman (2015), reported that essential mineral elements are well characterized for physiological functions in the human body, which is contrary to non-essential mineral elements. There is large variation in the mineral composition of tea species, part of which is due to differences in plant species and other agronomic and post-harvest practices (Malongane *et al*., 2020). Rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia species*) contains minerals such as potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), sodium (Na), manganese (Mn), zinc (Zn), copper (Cu), boron (B), iron (Fe), sulphur (S), selenium (Se) and chromium (Cr) in addition to other bioactive (Malongane *et al*., 2020). The consumption of these teas is therefore regarded as an important source of minerals, which exert positive biological effect in humans and contribute to tea quality. Inadequate absorption of essential minerals by the tea plant will have vast effect on the quality of tea (Malongane *et al*., 2020).

2.4 Work not done on the research problem

The effect of harvesting seasons and drying methods on the quality of *J. zeyheri* indigenous tea has not been yet documented. Therefore, the researcher intended to investigate the effect of harvesting seasons and drying methods on phytochemicals

and antioxidant activity, essential and non-essential mineral elements of *J. zeyheri* tea leaves.

CHAPTER 3
EFFECT OF HARVESTING SEASONS AND DRYING METHODS ON PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT ACTIVITY OF *JATROPHA ZEYHERI* TEA LEAVES

3.1 Introduction

Different teas are harvested in different seasons throughout the year. As plants undergo various harvesting seasons, they are exposed to different environmental and climatic conditions for example different temperature levels. The conditions bring about changes in the chemical, physical, physiological and sensory characteristics of plants throughout the seasons (Wahba *et al*., 2017). Transformation of chemical constituents in plants that occur during different harvesting seasons have major influence on certain factors such as taste, flavour, aroma, colour, appearance and overall quality of tea (Ahmed *et al*., 2019). The quality of tea is also influenced by processing techniques such as cutting, rolling, fermentation and drying, which are very important in the preservation of the natural health promoting properties (Singh *et al*., 2014).

Phytochemicals are important natural bioactive compounds and are widely recognized for their health benefits (Saxena *et al*., 2013). The phytochemicals in tea are highly dominant for their medicinal importance (Mahomoodally, 2013). Tea consists of various phytochemicals such as flavonoids, tannins, phenols and others. Total phenolic and flavonoid contents play important role in food and beverage due to their contribution to taste, astringency, colour and health promoting properties (Oliveira *et al*., 2014). Tannins are astringent, bitter plant polyphenols that are present in many plant foods especially in black tea, which tend to have bitter taste (Oliveira *et al*., 2014). Antioxidants are one of the principal ingredients that protect food quality by preventing

oxidative deterioration of lipids, which help retain nutritional quality of plants (Sharma *et al*., 2012). Antioxidants provide some functions such as those affecting duodenum, colon, skin, lung, breast, oesophageal, pancreatic and prostate cancer (Suganuma *et al*., 1999).

Jatropha zeyheri is mostly used for nutritional and medicinal purpose, the leaves are brewed and consumed as tea beverage as it is a rich source of phytochemicals and essential mineral elements. The effect of harvesting seasons and drying methods on phytochemical and antioxidant activity of *J. zeyheri* tea leaves has not yet been documented. Therefore, the objective of this study was to determine the effect of harvesting seasons and drying methods on total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC) and antioxidants activity (AA) of *J. zeyheri* tea leaves.

3.2 Materials and methods

3.2.1 Description of the study site

Mature leaves of *J. zeyheri* were harvested in the wild during autumn, winter and summer seasons at Khureng village, Lepelle-Nkumpi Municipality (24°33’53”S, 29°23’4”E), in Limpopo Province, South Africa. Khureng village is characterised by semi-arid climate with summer, autumn and winter (Table 3.1). Materials were transported to Limpopo Agro-Food Technology Station (LATS), where the experiment was conducted.

Table 3.1 Weather data during harvesting seasons			
	Autumn	Winter	Summer
Temperature	25°C	20°C	30°C
Rainfall	120-300 mm/pa	0-120 mm/pa	300-600 mm/pa
Relative Humidity	60%	56%	64%

3.2.2 Treatments and research design

The study was laid out in 3 × 4 factorial experiment arranged in a randomised completely block design (RCBD) with 9 replications. The first factor was 3 harvesting seasons (autumn, winter and summer), whereas the second factor comprised of different drying methods (shade, sun, oven and freeze-drying).

3.2.3 Procedures

Matured leaves of *J. zeyheri* were harvested in the wild during the morning of autumn, winter and summer seasons (Figure 3.1). Thereafter, the leaves were transported to LATS where they were cleaned from soil particles and dirt before being subjected to different drying methods (Figure 3.2).

Shade-drying: Harvested leaves were spread into plastic trays (66,04 cm x 56 cm), occasionally turned to allow rapid drying. They were placed indoor at 24°C and 17°C day and night temperatures, respectively. The prepared materials were left under the shade for 7, 8 and 6 days in autumn, winter and summer harvesting seasons, respectively, to dry until the moisture content reduced to 10% (Figure 3.2 A).

Sun-drying: Harvested leaves were placed inside plastic trays (66,04 cm x 56 cm), occasionally turned and left to dry under direct exposure to sunlight at approximately 25°C, 20°C, 29°C day temperatures in autumn, winter and summer harvesting seasons, respectively. At sunset the materials were covered and removed to ensure that they were not moistened by dew. The leaves were dried for 6, 7, 5 days in autumn, winter and summer harvesting seasons, respectively, until the moisture content was reduced to 10% (Figure 3.2 B).

Oven-drying: Harvested leaves were oven dried at 60°C for 24 hours until the moisture content was reduced to10% (Figure 3.2 C).

Freeze-drying: Harvested leaves were arranged uniformly inside the table top freeze dryer (Ilshin Lab Co. Ltd, USA) and allowed to dry for 3 days at –45°C. A maximum of five trays were used simultaneously and subjected to lyophilizadtion, whereby water is frozen, followed by its removal from the leaves, initially by sublimation, and then by desorption (Figure 3.2 D). The dried leaves were ground through 1 mm sieve using grinder (MF 10 basic micro fine grinder drive, IKA-Werke, USA) at LATS prior analysis.

Figure 3.1: *Jatropha zeyheri* harvesting seasons, A). Autumn, B). Winter and C). Summer.

Figure 3.2: *Jatropha zeyheri* drying methods, A) Shade-drying, B) Sun-drying, C) Oven-drying and D) Freeze-drying

3.2.4 Data collection

Approximately, 1 g of ground powdered plant materials were extracted with 10 mL of acetone. The filtrates were filtered into pre-weighed vials and the solvents were evaporated at room temperature (24°C). The mass extracted was determined and samples were reconstituted in acetone to a final concentration of 10 mg/mL for subsequent assays.

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

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

Determination of total flavonoids content: Determination of total flavonoids was done using the aluminum chloride colorimetric assay by (Zhishen *et al*., 1999). Approximately, 1 mL of extract was diluted with 4 mL of distilled water followed by addition of 0.3 mL of 5% sodium nitrite. After 5 minutes of incubation, 0.3 mL of 10% aluminum chloride was added. This was followed by addition of 2 mL of 1 mol Sodium hydroxide after incubation for another 5 minutes. 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. The standard was prepared using a serial dilution of quercetin (0 to 500 µg/mL) in place of the extract. The total flavonoid content was expressed as mg of QE/g of extract.

Determination of total tannins content: The Folin-Ciocalteu assay was used to determine the total tannin content of the plant extracts. In a volumetric flask (10 mL) a volume of 0.1 mL of the plant extract was mixed with 7.5 mL of distilled water, into which 0.5 mL of the Folin-Ciocalteu phenol reagent was added. Approximately, 1 mL of 35% solution of Sodium carbonate was added and the mixture was diluted with 10 mL of distilled water. The mixture was then shaken well and incubated in dark environment at room temperature (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.

3.2.5 Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistix 10.0 software per season. When the treatments were significant at the probability level of 5% and the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Waller-Duncan Multiple Range Test ($P \leq 0.05$). Unless otherwise stated, only treatments that are significant at the probability level of 5% were discussed.

3.3 Results

Harvesting seasons had highly significant ($P \leq 0.01$) effects on TPC, TFC, TTC and AA, contributing 68, 86, 80 and 65% in TTV, respectively (Table 3.2). Drying methods had highly significant effects on TPC, TFC, AA contributing, 18, 10 and 18% in TTV, respectively, whereas drying methods had no significant effect on total tannin content (TTC) (Table 3.2). Interaction of drying methods and harvesting seasons had highly significant effects on TPC and AA contributing 10 and 14% in TTV, respectively, whereas TFC was significantly ($P \leq 0.05$) affected contributing, 2% in TTV (Table 3.2). However, TTC was not affected by the interaction between harvesting seasons and drying methods (Table 3.2).

The three harvesting seasons had different distributions of TPC, TFC, TTC and AA (Table 3.3). Summer harvesting season accounted for the highest TPC (1.4397^{a} ± 0.1962 mg GAE/g) and AA (4.2052^{a} ± 0.1811 mg GAE/g), whereas autumn had the highest TFC (0.8850^{a} ± 0.0477 mg QE/g) and winter had the highest TTC (0.5460^{a} ± 0.0558 mg GAE/g) (Table 3.3). The lowest contents of TPC (0.5064^{b} ± 0.0229 mg GAE/g) and AA (2.6352^{c} ± 0.1940 mg GAE/g) were reported in winter, however summer and autumn had the lowest contents of TFC (0.2925^{c} ± 0.0348 mg QE/g) and TTC (0.1848^{b} ± 0.0167 mg GAE/g) respectively (Table 3.3).

The four drying methods had different distributions of TPC, TFC and AA (Table 3.4). Oven drying method accounted for the highest TPC (1.2767^{a} ± 0.2243 mg GAE/g) and TFC (0.7139^{a} ± 0.0816 mg QE/g), whereas freeze drying method had low TPC (0.5313^{b} ± 0.0737 mg GAE/g) and TFC (0.4379^{c} ± 0.0519 mg QE/g). In contrast freeze drying recorded the highest AA (4.2069^{a} ± 0.1587 mg GAE/g), whereas sun drying had the lowest AA (3.0285^{c} ± 0.2884 mg GAE/g) (Table 3.4).

Interaction of harvesting seasons and drying methods had resulted in different distributions of TPC, TFC and AA (Table 3.5). On the interaction of autumn and drying methods, autumn and oven drying accounted for the highest TPC (0.6243^{d} ± 0.0319 mg GAE/g), TFC (1.1752^{a} ± 0.1158 mg QE/g) and AA (3.9581^{bc} ± 0.1413 mg GAE/g), however autumn and freeze drying had the lowest contents of TPC (0.4326^{d} ± 0.0372 mg GAE/g) and TFC (0.7124^{bcd} ± 0.0572 mg QE/g) while autumn and sun drying had the lowest AA (3.3953^{bcd} ± 0.2739 mg GAE/g). Interaction of winter and oven drying had the highest TPC (0.6243^{d} ± 0.0319 mg GAE/g), whereas winter and shade drying had the highest TFC (0.6522^{cd} ± 0.0759 mg QE/g). In contrast, winter and freeze

drying had the lowest TPC ($0.4326^{d} \pm 0.0360$ mg GAE/g) and TFC ($0.3880^{efg} \pm 0.0484$ mg QE/g). Winter and freeze drying had the highest AA ($3.9996^{bc} \pm 0.2374$ mg GAE/g), whereas winter and sun drying had the lowest AA ($1.2540^{g} \pm 0.0573$ mg GAE/g). Interactively, summer and oven drying had the highest TPC ($2.5813^{a} \pm 0.4114$ mg GAE/g) and TFC ($0.4204^{ef} \pm 0.0754$ mg QE/g), whereas summer and freeze drying had the lowest TPC ($0.7288^{cd} \pm 0.2070$ mg GAE/g) and TFC ($0.2131^{g} \pm 0.0677$ mg QE/g). In contrast, summer and freeze drying had the highest AA ($5.0400^{a} \pm 0.0091$ GAE/g) while summer and oven drying had the lowest AA ($3.2819^{de} \pm 0.2548$ mg GAE/g).

Table 3.2 Partitioning mean sum of squares for total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC) and antioxidant activity (AA) on harvesting seasons and drying methods of *Jatropha zeyheri* leaves (n = 108).

		TPC (mg GAE/g)		TFC (mg QE/g)		TTC (mg GAE/g)		AA (mg GAE/g)	
Source	DF	MSS[y]	%[z]	MSS	%	MSS	%	MSS	%
Replication	8	0.3876	2	0.01994	1	0.09578	6	0.7776	2
Harvesting seasons	2	10.4527	68***	3.18499	86***	1.30224	80***	24.1582	65***
Drying methods	3	2.7708	18***	0.36752	10***	0.11633	7[ns]	6.7029	18***
Drying × Season	6	1.5374	10***	0.09873	2**	0.03278	2[ns]	5.0669	14***
Error	88	0.3212	2	0.04444	1	0.07142	5	0.5143	1
Total	107	15.4697	100	3.71512	100	1.61855	100	37.2199	100

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05, [ns]Non-significant at P ≥ 0.05

[y]MSS = Mean Sum of Squares.

[z]TTV (%) = Percentage of Total Treatment Variation

Table 3.3 Response of total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC) and antioxidant activity (AA) to harvesting seasons of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	TPC (mg GAE/g) Variable[y]	TFC (mg QE/g) Variable	TTC (mg GAE/g) Variable	AA (mg GAE/g) Variable
Autumn	$0.5064^{b} \pm 0.1242$	$0.8850^{a} \pm 0.0477$	$0.1848^{b} \pm 0.0167$	$3.8257^{b} \pm 0.1027$
Winter	$0.5064^{b} \pm 0.0229$	$0.5427^{b} \pm 0.0330$	$0.5460^{a} \pm 0.0558$	$2.6352^{c} \pm 0.1940$
Summer	$1.4397^{a} \pm 0.1962$	$0.2925^{c} \pm 0.0348$	$0.4689^{a} \pm 0.0513$	$4.2052^{a} \pm 0.1811$

[y] Column means ± SE (Standard error) followed by the same letter were not different ($P \leq 0.05$) according to Waller-Duncan Multiple Range test.

Table 3.4 Response of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) to drying methods of *Jatropha zeyheri* leaves (n = 108).

Drying methods	TPC (mg GAE/g)	TFC (mg QE/g)	AA (mg GAE/g)
	Variable[y]	Variable	Variable
Shade	0.7213[b] ± 0.1306	0.6077[ab] ± 0.0622	3.6253[b] ± 0.2231
Sun	0.7409[b] ± 0.1976	0.5342[bc] ± 0.5001	3.0285[c] ± 0.2884
Oven	1.2767[a] ± 0.2243	0.7139[a] ± 0.0816	3.3607[bc] ± 0.1654
Freeze	0.5313[b] ± 0.0737	0.4379[c] ± 0.0519	4.2069[a] ± 0.1587

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 3.5 Interactive effects of harvesting seasons and drying methods on total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	Drying methods	TPC (mg GAE/g) Variable[y]	TFC (mg QE/g) Variable	AA (mg GAE/g) Variable
Autumn	Shade	$0.4968^{d} \pm 0.0527^{z}$	$0.8610^{b} \pm 0.0834$	$3.3953^{bcd} \pm 0.2739$
	Sun	$0.4721^{d} \pm 0.0376$	$0.7914^{bc} \pm 0.0290$	$3.8283^{bcd} \pm 0.1745$
	Oven	$0.6243^{d} \pm 0.0319$	$1.1752^{a} \pm 0.1158$	$3.9581^{bc} \pm 0.1413$
	Freeze	$0.4326^{d} \pm 0.0372$	$0.7124^{bcd} \pm 0.0572$	$3.5810^{cd} \pm 0.2187$
Winter	Shade	$0.4968^{d} \pm 0.0527$	$0.6522^{cd} \pm 0.0759$	$2.4449^{f} \pm 0.1211$
	Sun	$0.4721^{d} \pm 0.0358$	$0.5844^{de} \pm 0.0369$	$1.2540^{g} \pm 0.0573$
	Oven	$0.6243^{d} \pm 0.0319$	$0.5461^{de} \pm 0.0697$	$2.8422^{ef} \pm 0.3209$
	Freeze	$0.4326^{d} \pm 0.0360$	$0.3880^{efg} \pm 0.0484$	$3.9996^{bc} \pm 0.2374$
Summer	Shade	$1.1703^{bc} \pm 0.3505$	$0.3099^{fg} \pm 0.0751$	$4.4957^{ab} \pm 0.3387$
	Sun	$1.2786^{b} \pm 0.2958$	$0.2267^{fg} \pm 0.0426$	$4.0032^{bc} \pm 0.4298$
	Oven	$2.5813^{a} \pm 0.4114$	$0.4204^{ef} \pm 0.0754$	$3.2819^{de} \pm 0.2548$
	Freeze	$0.7288^{cd} \pm 0.2070$	$0.2131^{g} \pm 0.0677$	$5.0400^{a} \pm 0.0091$

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

3.4 Discussion.

3.4.1 Effect of harvesting seasons on phytochemical constituents and antioxidant activity

Different harvesting seasons (autumn, winter and summer) had an effect on TPC, TFC, TTC and AA of *J. zeyheri* tea leaves. Similar results were reported on wild bush tea (*A. phylicoides* DC) (Mudau *et al*., 2008), ceylon tea (*C. sinensis*) (Jayasekera *et al*., 2014), Leather leaf (*Barleria dinteri*) and wild currant tea (*Grewia flava*) (Gololo *et al*., 2016). The changes in temperatures and wind patterns associated with seasons affect precipitation and thereby plant growth, flowering stages, fruiting and phytochemical composition of plants (Mudau and Makunga, 2018). Seasonal change exposes plants to distinct environmental factors that contribute to variable phytochemical accumulation (Mudau and Makunga, 2018). Temperature, rainfall, humidity and other factors have effect on phytochemical and antioxidant syntheses in plants (Zargoosh *et al*., 2019). The syntheses of phytochemicals are also influenced by the physiological stages of the plant at that sea3son and their response to environmental stress (Tasiu, 2019). Different phytochemicals possess different biological activities and they are accumulated in various amounts during different seasons. This influences both primary and secondary plants metabolites, which profiles tea quality, flavour, taste and aroma (Mudau and Makunga, 2018).

Summer retained higher TPC of *J. zeyheri* tea leaves and winter had the lowest content. Similar trends were observed on AA of *J. zeyheri* tea leaves, where summer had the highest AA and winter had the lowest. In summer most plants develop new leaf growth, thus the fresh leaves of plants are reported to be high in the concentration of phenolic and AA, depending on plant species (Ercisli *et al.,* 2008). The retention of

higher TPC in summer season in plant leaves may be because seasonal changes promote different stresses in plants (Roshanak *et al*., 2016). The thermal stress during summer season induces the production of antioxidant properties (Roshanak *et al*., 2016). It also influences the production of phenolic compounds (Roshanak *et al*., 2016). Results of this study agree with those on *C. sinensis (*Erturk *et al*., 2010), *A. phylicoides DC* (Mudau *et al*., 2008), Labrador tea (*Rhododendron tomentosum*) (Black *et al*., 2011) and *J. lagarinthoides* (Gololo *et al*., 2016), where TPC and AA were higher in summer season as compared to other seasons. Contrary results were reported on *A. phylicoides (L.)* (Mudau *et al*., 2008), *G. flava* (Gololo *et al*., 2016) and on black tea (*C. sinensis*) (Owuor *et al*., 2011) where TPC and AA were lowest in summer as compared to other seasons.

Autumn had high TFC in *J. zeyheri* tea leaves, whereas, summer had low phytochemical. Autumn season has low average rainfall as compared to summer season. According to Sharma *et al.* (2012) the biosynthesis of flavonoids is increased during periods of less rainfall or drought as well as low temperatures. Flavonoids are highly synthesised during plant stress periods. The transcriptions of various genes that are involved in flavonoid biosynthesis are stimulated by water deficit as a response to plants oxidative stress (Sharma *et al*., 2012). The variation of TFC more especially under field conditions may also be caused by genetic and other agronomic factors (Sharma *et al*., 2012). The results of this study agree with those reported on *C. sinensis* (Owuor *et al*., 2011), *C. sinensis var. assamica* (Yao *et al*., 2005) where TFC was high in autumn and winter as compared to summer. In contrast, Gololo *et al*. (2016) reported high TFC during summer season on *G. flava*, *B. dentri* and *Jatropha lagarinthodes* as compared to colder seasons.

Winter had the highest TTC of *J. zeyheri* tea leaves, whereas summer and autumn reported the lowest content. The accumulation of TTC in plants is mostly induced by environmental stress perceived by the plant (Zhang *et al*., 2019). *Jatropha zeyheri* could have reported high accumulation of TTC in winter because of unfavourable environmental conditions for plant growth and defence. Winter seasons consist of low average rainfall as compared to autumn/summer seasons. Water scarcity and low temperature increases tannin accumulation in plants, the tannin serve as defence mechanism for the plant (Zhang *et al*., 2019). During summer season there may be low TTC because growth conditions are presumably closer to optimal, the decrease in tannin production could be a result of the plant specially allocating other biomolecules for growth (Zhang *et al*., 2019). Similar results were reported by Mudau *et al*. (2007) on *A. phylicoides* were winter had high TTC as compared to summer. In contrast, Chiu (1990) reported high TTC during summer season on Pauching tea (*C. sinensis*).

3.4.2 Effect of drying methods on phytochemical constituents and antioxidant activity

Drying methods play important roles in the processing of medicinal plants. They could either lead to the conservation or loss of the bioactive compounds and their associated antioxidant capacity (Müller and Heindl, 2006). After drying, the structure of a leaf is more open as the cell membranes are raptured, allowing for easier penetration of extraction solvents for extraction of bioactive compounds (Tau, 2018). However, the bioactive compound either could be protected or destroyed depending on the drying method, temperature and the specific duration used, (Tau, 2018; Mbondo *et al*., 2018).

In the current study, different drying methods had an effect on TPC, TFC and AA of *J. zeyheri* leaves. Similar results were reported on green tea leaves (*Camelia assamica*) (Roshanak *et al*., 2016), vitex tea (*Vitex negundo* and *Vitex trifolia*) (Chong and Lim, 2011), tea plant (*Camelia senensis*) (Roslan *et al*., 2020), Shell ginger tea (*Alpinia zerumbet*) (Chan *et al*., 2009) and on Mediterranean herbs (*Salvia officinalis*, *Melissa officinals*, *Thymus vulgaris, Mentha spicata*) (Rababah *et al*., 2015). However, in this study, TTC was not affected by drying methods. Similar results were reported on bush tea (*Athrixia phylicoides*) where tannin content was not affected by different drying methods (Mudau and Ngezimana, 2014). Contrary results were observed on fresh moringa (*Moringa oleifera),* where tannin content was increased by drying methods (Mbah *et al*., 2012). The drying conditions affect the retention of phytochemicals as they modify the physical and chemical micro structure of plant tissues (Lewicki and Pawak, 2003). Extend and direction of these changes depends on the mode of drying, which may cause numerous breaking of the cell wall (Lewicki and Pawak, 2003). Some of the plant materials undergo oxidation that can destroy heat sensitive active ingredients during drying (Saifullah *et al*., 2019). Plant materials undergo enzymatic degradation at certain drying conditions and heat sensitivity nature, which leads to increased extraction yields or alternatively reduced extraction yields (Saifullah *et al*., 2019).

Oven drying had reported the highest TPC in the dried plant material, whereas freeze drying had reduced the phytochemical. Similar findings were observed by Roshanak *et al*. (2016) on *C. assamica* were TPC was high under oven drying, whereas shade drying resulted in low content. Chan *et al*. (2009) also reported high TPC in oven drying as compared to shade drying on (*A. zerumbet*) leaves*,* torch ginger *(Etlinger aelatior*)

leaves, turmeric (*Curcuma longa*) leaves and aromatic ginger (*Kaempferia. galanga*) tea leaves. In addition, Rababah *et al*. (2015) also reported high TPC after oven drying on *S. officinals*. Contrasting results were observed on *A. phylicoides,* where freeze drying and shade drying contained the highest TPC (Mudau and Ngezimana, 2014). During drying some plant materials undergo oxidation that can destroy the plants heat sensitive active ingredients. Consequently, the heat sensitivity nature could either lead to increased extraction yields or alternatively reduced extraction yields. This outlines that the TPC of *J. zeyheri* in this study was not heat sensitive; or rather the used drying method increased the extraction of the TPC. The heat treatment as result of thermal drying methods such as oven drying, help the enzyme catalyse the oxidation of phenolic compounds in highly reactive quinone's (Orphanides *et al*., 2013). Total phenolic content loss after freeze drying may be attributed to inability of the drying temperature to activate the plant phenolic oxidase enzyme that is responsible for the conservation of phenolic compounds to quinone's (Lim and Murtijaya, 2007; Roshanak *et al*., 2016). During various drying methods, the physical micro structure and the chemical structure of the plant are modified. The low TPC after freeze drying might be due to alterations in the chemical structures of polyphenols during drying, which cannot be extracted or determined by the methods (Lewicki and Pawak, 2003).

Oven drying had the highest TFC and freeze drying had lower content. Similar results were observed by Roshanak *et al*. (2016), whereby oven drying retained high TFC as compared to freeze drying on *C. assamica*. In contrast, Rababah *et al.* (2015) reported low TFC in oven dried leaves of *S. officinalis*, *T. vulgaris*, *M. officinalis* and *M. spicata* as compared to shade drying. In addition, Sukrasno *et al.* (2011) reported that oven-drying had low TFC on herbal tea (*Cosmos caudatus)* as compared to shade drying.

Oven drying degrades L-ascorbic acid, which affects cell wall integrity and causes leakage of some flavonoid compounds that are easily extractable (Roshanak *et al*., 2016). The ability of oven drying to retain more flavonoids as compared to other drying methods on *J. zeyheri* leaves may also be attributed to the oven drying temperature used, which was at 60°C. The drying temperature activates plant phenolic oxidase (PPO), which catalyzes the oxidation of phenolic compounds into high reactive quinone's (Roshanak *et al*., 2016). Oven drying at 60 and 100 degrees revealed the highest radical scavenging activity which is due to more oxidation and polymerisation by enzymes derived from tea leaves (Sukrasno *et al*., 2011). Consequently, Sukrasno *et al*. (2011) reported that keeping temperature at optimal level causes an increase in activity of PPO enzyme to degrade flavonoids, resulting in increased TFC after oven drying. Maximum allowable temperatures depend mainly on the chemical composition of the active ingredients of the plant species under consideration (Sukrasno *et al*., 2011). Therefore, the low TFC after freeze drying might be due to intense breakage of the cellular constituents by the drying method. This may have caused an effect on the cell wall integrity and caused migration of flavonoids. Additionally, the break-down of cellular constituents can lead to the accelerated release of TFC from the food matrix (Sukrasno *et al*., 2011).

Antioxidant activity was high in freeze dried leaves of *J. zeyheri* and lower in sun dried leaves. Results of this study agree with those on *C. sinesis* (Roslan *et al*., 2020), *A. phylicoides* (Mudau and Ngezimana, 2014), *V. negundo* (Chong and Lim, 2012), *T. vulgaris* (Rababah *et al*., 2015) and *M. spicata* (Orphandies *et al*., 2013) where AA was high in freeze drying compared to oven drying methods. Acontrasting study was reported on gale of the wind tea leaves (*Phyllanthus amarus*), whereby oven drying

had the highest AA (Lim and Murtijaya, 2007). There are heat sensitive antioxidants such as tocopherols, ascorbic acid, carotenoids and plant phenolic that are present in some tea plants (Shofian *et al*., 2011). These AA are highly preserved by freeze drying, which dehydrate plant materials through sublimation of ice crystals with no heat treatment. Consequently, prolonged thermal treatment such as sun-drying may have significant effect on the loss of most natural antioxidants (Rababah *et al*., 2015). Oven drying may also deactivate enzymes and degrade plant antioxidants. It may be possible that initial enzymatic degradation of antioxidant compounds where there was slow heat transfer in sun drying resulted inefficient denaturation of the enzyme involved (Chong and Lim, 2012).

3.4.3 Interaction of harvesting seasons and drying methods on phytochemical and antioxidant activity

Interaction between harvesting seasons and drying methods had an effect on TPC, TFC and AA of *J. zeyheri* tea leaves. Various leaves of tea plants consist of vast array of phytochemicals that were synthesised during different harvesting seasons (Gololo *et al*., 2016). During drying, there are alterations of plant bioactive compounds. There are heat sensitive compounds, which can oxidise and be destroyed by drying heat (Roslan *et al*., 2020). This results in changes in the aroma, appearance, flavour, taste, colour of the tea leaves, which affects overall tea quality (Roslan *et al*., 2020). The retention or loss of phytochemicals depends on the amount accumulated per season and the drying methods used. The interaction of leaves harvested in summer and oven dried, resulted in high TPC, whereas the interaction of winter harvested leaves and freeze dried reduced the phytochemical. The biosynthesis of phenolic compounds in tea shoots of most tea plants can be effectively induced by stronger sunlight and length

of daytime that is highly present during the summer season (Roshanak *et al*., 2016). The PPO that catalyses phenolic compounds is usually activated by the amount of oven drying temperatures similarly to the one used in this study (Roshanak *et al*., 2016). The low concentration of TPC in leaves of *J. zeyheri* harvested in winter and freeze dried may be because, during winter most tea leaves are not green and fresh, which attribute to low concentration of phenolic content (Ahmed *et al*., 2019). Additionally, the biosynthesis of TPC in tea plants is induced by sunlight concentration, therefore lower sunlight concentration that is present in winter could have result in reduced accumulation of the phytochemical (Sun *et al*., 2017). Freeze drying is a non-thermal drying technique, it has lower temperature that might not be able to deactivated degraded enzymes that induce phenolic content thus resulting in lower TPC of freeze dried leaves (Erturk *et al*., 2010). Erturk *et al*. (2010) reported that summer retained high TPC on *C. sinensis*. Roshnaka *et al.* (2016) reported high TPC after oven drying on *C. assamica*. In contrast, Gololo *et al*. (2016) reported high TPC during autumn on *G. flava*. Additionally, Mudau and Ngezimana (2014) reported that freeze drying retained high TPC on *A. phylicoides*.

The interaction of leaves harvested in autumn and oven dried, resulted in high TFC, whereas the interaction of summer harvested leaves and freeze dried reduced the phytochemical amount. Flavonoids protect plants from different biotic and abiotic stresses (Panche *et al*., 2016). The higher concentration during autumn may be plant response to drought stress conditions due to low moisture content in autumn as compared to summer. Oven drying is effective in preserving flavonoids content, the heating temperature is known to decrease the enzyme activity of flavonoids to degrading enzyme polyphenol oxidase, which results in an increase in the TFC in plant

leaves (Rababah *et al*., 2015). The low content of TFC in summer and freeze drying may have resulted because the biosynthesis of flavonoid slows during winter, which allows the accumulation of epigallocatechin gallate (EGC) in the fresh leaves during summer season (Rababah *et al*., 2015). Freeze drying might have caused migration of the cell wall integrity that leads to less flavonoid retention. Rababah *et al* (2015) reported high TFC in autumn on *C. sinensis*. Roshnaka *et al* (2015) reported that oven drying retained high TPC on *C. assamica*. Similar differential results were reported on *G. flava*, *B. dentri* and *J. langarinthodes* where TFC were high in summer (Gololo *et al*., 2016). In addition, summer harvesting season retained high TFC in *S. officinalis* (Rababah *et al*., 2015).

The interaction of leaves harvested in summer and freeze dried, resulted in high AA, whereas the interaction of winter harvested leaves and sun dried reduced the phytochemical. Non-thermal drying which includes freeze drying exhibits much stronger activity in the DPPH during freeze drying process (Singh *et al*., 2009). Freezing could lead to the development of ice crystals within the leaves tissue matrix and relating in greater rapturing of cell structure for better solvent accessibility and compounds extraction (Singh *et al*., 2009). In contrast, the lowest antioxidant reported in leaves harvested in winter and sun dried may be because high temperature or intense thermal processing might cause significant loss in antioxidant that are found in natural plants as well as deactivate enzyme and degrade phytochemicals (Rabbah *et al*., 2015). The results of this study agree with those reported by Erturk *et al* (2010) on *C. sinensis* where summer had the highest AA. Additionally, in a study carried out by Roslan *et al*. (2020), freeze drying retained high AA as compared to other drying methods. The same results were reported by (Black *et al*., 2011) with summer having

high AA on *R. tomentosum.* Additionally, Chan *et al* (2009) observed that freeze drying also retained high AA on *A. zerumbet*. In contrast Roshanak *et al* (2016) reported high AA after oven drying on green tea *C. sinensis*. The retention of AA and phytochemicals depend on the different plant species involved (Müller and Heindl, 2006).

3.5 CONCLUSION

In conclusion, the results of this study suggest that harvesting of *J. zeyheri* leaves should be done during the summer season and oven-dried for improved accumulation of phytochemicals. However, for improved accumulation of AA the leaves should be subjected to freeze-drying. The interaction of summer harvesting season and oven/freeze-drying are more appropriate for the preservation of selected phytochemicals and AA of *J. zeyheri* tea leaves, which will improve health properties of the tea.

CHAPTER 4
EFFECT OF HARVESTING SEASONS AND DRYING METHODS ON ESSENTIAL AND NON-ESSENTIAL MINERAL ELEMENTS OF *JATROPHA ZEYHERI* TEA LEAVES

4.1 Introduction

Teas are rich sources of mineral elements and some of the mineral elements are essential for functioning of the human body, whereas others are harmful if they are consumed beyond their daily intake (Ercisli *et al*., 2008). There are 28 mineral elements in tea leaves but potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and sulphur (S) are the most important minerals in tea leaves (Zhen, 2002). The available mineral elements at optimum levels serve for medicinal purpose for example, reducing blood cholesterol levels. Although tea may contain heavy metals such as aluminum (Al), arsenic (As), cobalt (Co), chromium (Cr), nickel (Ni) and lead (Pb) which may be toxic to human health (Ercisli *et al*., 2008; Zhao *et al.,* 2017). Both essential and non-essential mineral elements are absorbed from the plant roots, which may be exposed to external environmental conditions (Ercisli *et al*., 2008). The external environmental conditions includes harvesting seasons, which have irregularities of certain factors such as changes in the rainfall patterns, temperature, and water retention capacity of the soil (Zhao and Zhao, 2019). Irregularities of these factors cause different absorptive capabilities of minerals by the roots throughout various seasons (Zhao *et al*., 2017).

Consequently, different tea processing methods result in changes on the nutritive profile of plants, which includes changes in minerals constituents. Drying methods have a major impact on the nutritive degradation and retention of tea leaves (Roslan *et al*., 2020). During drying, mineral elements can be retained or destroyed depending

on the plant species involved. The knowledge of both micronutrients and toxic elements content in beverages is important (Salahinejad and Aflaki, 2010). The effect of harvesting seasons and drying methods on the mineral composition of *Jatropha zeyheri* tea leaves has not yet been documented. Therefore, the objective of this study was to determine the effect of harvesting seasons and drying methods on essential and non-essential mineral elements of *J. zeyheri* tea leaves.

4.2 Materials and methods

4.2.1 Description of the study site

Mature leaves of *J. zeyheri* were harvested in the morning during autumn, winter and summer seasons at Khureng village, Lepelle-Nkumpi Municipality (24°33’53”S, 29°23’4”E), in Limpopo Province, South Africa. The weather data is as described in Chapter 3. The harvesting of leaves was done as previously described in Chapter 3.

4.2.2 Treatments and research design

The study was laid out in 3 × 4 factorial experiments arranged in a randomised completely block design (RCBD) with nine (9) replications. The first factor was three harvesting seasons (autumn, winter and summer), whereas the second factor comprised of different drying methods (shade-drying, sun-drying, oven-drying and freeze-drying). All the procedures were done as previously described in Chapter 3.

4.2.3 Data collection

Approximately, 1 g of the ground powdered plant materials were extracted with 10 mL of acetone. The filtrates were filtered into pre-weighed vials and the solvents were evaporated at room temperature (24°C). The mass extracted was determined and

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

A microwave digestion system (PerkinElmer, Titan MPS, USA) was used to prepare the samples. Approximately, 0.5 g of each sample was weighed and 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 for 20 minutes. The solution was transferred into 50 ml centrifuge tubes and diluted with deionized water to top up to 50 ml. The extracts were stored in a cold room at 5°C prior mineral elements analysis. The essential mineral elements, calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorus (P) and zinc (Zn) and non-essential mineral elements, silver (Ag), aluminum (Al), sodium (Na) and lead (Pb) were determined using Inductive Coupled Plasma Emission (ICPE-9000, Shimadzu, Japan).

4.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using Statistix 10.0 software. When the treatments were significant at the probability level of 5% and the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Waller-Duncan Multiple Range Test ($P \leq 0.05$). Unless otherwise stated, only treatments that are significant at the probability level of 5% were discussed.

4.3 Results

Harvesting seasons had a highly significant ($P \leq 0.01$) effect on essential mineral elements, Ca, Cu, Fe, K, Mn, Ni and P contributing 14, 50, 25, 42, 53, 74 and 49% in TTV, respectively (Table 4.1 and 4.2), whereas the treatment had significant ($P \leq 0.05$) effect on Zn contributing 38% in TTV (Table 4.1 and 4.2). However, harvesting seasons had no significant effect on Mg (Table 4.1 and 4.2). Drying methods had highly significant effects on Ca, Fe, Mg, Mn and P contributing 57, 37, 57, 25 and 19% in TTV, respectively (Table 4.1 and 4.2), whereas K was significantly affected, contributing 13% in TTTV (Table 4.1 and 4.2). However, no significant effect was observed on Cu, Ni and Zn (Table 4.1 and 4.2). Interaction of harvesting seasons and drying methods had highly significant effects on Ca, Cu, Fe, K, Mn, P and Zn contributing 25, 26, 30, 32, 20, 24 and 48% in TTV, respectively (Table 4.1 and 4.2), whereas the treatment had significant ($P \leq 0.05$) effect on Mg contributing 14% in TTV (Table 4.1 and 4.2). However, no significant effect was observed on Ni (Table 4.1 and 4.2).

Harvesting seasons had a highly significant ($P \leq 0.01$) effect on non-essential mineral element Al, contributing 38% in TTV (Table 4.3), whereas the treatment had significant ($P \leq 0.05$) effect on Na contributing 39% in TTV (Table 4.3). However, harvesting seasons had no significant effects on Ag and Pb. Drying methods had highly significant effect on Al and Pb contributing 29 and 42% in TTV, respectively (Table 4.3), whereas no significant effects were observed on Ag and Na (Table 4.3). Interactive effect of harvesting seasons and drying methods had highly significant effects on Al and Pb contributing 28 and 35% in TTV, respectively (Table 4.3), whereas Ag was significant

and contributed 32% in TTV (Table 4.3). However, no significant effect was observed on Na (Table 4.3).

The three harvesting seasons had different distributions of essential mineral elements in the leaves of *J. zeyheri* tea plant (Table 4.4). Leaves harvested in autumn accounted for the highest content of Ca ($120.12^{a} \pm 1.9053$ mg/kg), K ($73.658^{a} \pm 1.1242$ mg/kg), and Mn ($4.3817^{a} \pm 0.0763$ mg/kg) (Table 4 and 4.5.), whereas leaves harvested in winter had the highest Cu ($-0.6992^{b} \pm 0.0052$ mg/kg) (Table 4.4). However, summer harvested leaves had the highest Fe ($3.0528^{a} \pm 0.3981$ mg/kg), Ni ($0.3321^{a} \pm 0.0375$ mg/kg), P ($17.506^{a} \pm 0.4327$ mg/kg) and Zn ($0.1511^{a} \pm 0.0233$ mg/kg) (Table 4.4 and 4.5). In contrast the lowest content of Ca ($112.75^{b} \pm 2.8797$ mg/kg), Cu ($-0.6305^{a} \pm 0.0244$ mg/kg), K ($69.367^{b} \pm 0.8756$ mg/kg) and Mn ($3.7036^{b} \pm 0.1093$ mg/kg) was reported in summer harvested leaves, whereas the lowest Fe ($2.1075^{b} \pm 0.0619$ mg/kg) and P ($14.210^{c} \pm 0.4690$ mg/kg) content was reported in autumn harvested leaves (Table 4.4). Leaves harvested in winter had the lowest Ni ($0.1864^{b} \pm 0.0123$ mg/kg) and Zn ($0.0863^{b} \pm 0.0110$ mg/kg).

Harvesting seasons had different distributions of non-essential mineral elements in the leaves of *J. zeyheri* tea plant (Table 4.6). Leaves harvested in summer accounted for the highest content of Al ($10.094^{a} \pm 0.3877$ mg/kg) and Na ($2.1778^{a} \pm 0.0614$ mg/kg), whereas leaves harvested in autumn reported the lowest content of Al ($8.839^{b} \pm 0.0921$ mg/kg) and winter harvested leaves had the lowest content of Na ($1.9383^{b} \pm 0.07887$ mg/kg).

The four different drying methods had different distributions of essential mineral elements of *J. zeyheri* tea leaves (Table 4.7 and 4.8). Freeze dried leaves accounted for the highest Ca (127.22^{c} ± 2.5844 mg/kg), Mn (4.4352^{a} ± 0.0835 mg/kg) and P (16.678^{a} ± 0.7553 mg/kg) content, whereas sun dried leaves had the highest Fe (3.5237^{a} ± 0.5050 mg/kg) content. Shade dried leaves reported the highest content of K (73.581^{a} ± 0.8706 mg/kg) and Mg (34.307^{a} ± 0.4831 mg/kg) contents. In contrast, sun dried leaves reported the lowest Ca (109.11^{c} ± 1.6919 mg/kg), K (69.893^{b} ± 1.0257 mg/kg), Mg (31.615^{c} ± 0.3135 mg/kg), and Mn (3.7559^{c} ± 0.8861 mg/kg) (Table 4.7 and 4.8). The lowest content of Fe (2.0574^{b} ± 0.0856 mg/kg) and P (14.148^{b} ± 0.4441 mg/kg) were reported in oven dried leaves. Drying methods had effect on non-essential mineral elements of *J. zeyheri* tea leaves (Table 4.9). Sun dried leaves accounted the highest content of Al (9.9678^{a} ± 0.4284 mg/kg), whereas freeze dried leaves had the highest Pb content (2.1367^{a} ± 0.0382 mg/kg). In contrast, the lowest content of Al (8.3919^{c} ± 0.1696 mg/kg) and Pb (1.9111^{b} ± 0.0581 mg/kg) were reported on oven dried leaves.

Interaction of harvesting seasons and drying methods had different distributions of essential mineral elements of *J. zeyheri* tea leaves (Table 4.10 and 4.11). The interaction of autumn harvested leaves and freeze dried had the highest content of Ca (134.33^{a} ± 2.3214 mg/kg), Mg (33.400^{bc} ± 0.6218 mg/kg), P (16 689^{bcd} ± 1.2311 mg/kg) and Zn (0.1838^{ab} ± 0.0450 mg/kg), whereas sun dried leaves had the highest Cu (-0.7126^{b} ± 0.0029 mg/kg). However, the highest Fe (2.2533^{b} ± 0.1193 mg/kg), K (80.400^{a} ± 1.4666 mg/kg) and Mn (4.7289^{a} ± 0.1353 mg/kg) contents were reported on the interaction of leaves harvested in autumn and oven dried. In contrast, leaves harvested in autumn and sun dried had the lowest Ca (112.92^{c} ± 3.3982 mg/kg), Fe

(2.0022^{b} ± 0.1623 mg/kg), K (67.356^{de} ± 2.0823 mg/kg), Mg (31.478^{d} ± 0.7926 mg/kg) and Mn (3.8633^{cd} ± 0.1474 mg/kg) contents, whereas autumn harvested leaves and freeze dried had the lowest content of Cu (-0.6762^{b} ± 0.0049 mg/kg). In addition, the lowest contents of P (12.463^{e} ± 0.5516 mg/kg) and Zn (0.0684^{c} ± 0.0112 mg/kg) were reported on autumn harvested leaves and shade dried. The interaction of winter harvested leaves and shade dried accounted for the highest Ca (122.11^{b} ± 2.2818 mg/kg), K (75.156^{b} ± 2.0909 mg/kg), Mg (34.222^{b} ± 0.6309 mg/kg) and Mn (4.4600^{ab} ± 0.1617 mg/kg) contents. Winter harvested leaves and sun dried had the highest content of Cu (-0.7091^{b} ± 0.0172 mg/kg), Fe (2.6311^{b} ± 0.0925 mg/kg) and P (18.467^{ab} ± 2.3318 mg/kg). Additionally, leaves harvested in winter and freeze dried had the highest Zn content (0.1838^{ab} ± 0.0450 mg/kg). In contrast, the lowest contents of Ca (111.63^{c} ± 2.8276 mg/kg), Mg (31.856^{cd} ± 0.4343 mg/kg) and Mn (3.9511^{cd} ± 0.1361 mg/kg) were reported in leaves harvested in winter and sun dried, whereas leaves harvested in winter and oven dried had the lowest Cu (-0.6833^{b} ± 0.0047 mg/kg) content. However, Fe (2.1089^{b} ± 0.1366 mg/kg) and Zn (0.0539^{c} ± 0.0124 mg/kg) contents were low on leaves harvested in winter and shade dried. Additionally, winter harvested leaves and freeze dried reported the lowest K (72.089^{bc} ± 0.6883 mg/kg) and P (13.111^{e} ± 0.6332 mg/kg) contents.

The interaction of leaves harvested in summer and freeze dried reported the highest contents of Ca (134.78^{a} ± 3.4512 mg/kg), Mn (4.6100^{a} ± 0.0747 mg/kg) and P (20.233^{a} ± 0.6890 mg/kg), however leaves harvested in summer and oven dried had the highest content of Cu (-0.7254^{b} ± 0.0098 mg/kg). Additionally, summer harvested leaves and sun dried had higher Fe (1.7211^{b} ± 0.0485 mg/kg) content, whereas summer harvested leaves and shade dried had high K (64.022^{e} ± 2.0419 mg/kg), Mg (36.256^{a}

± 0.2631 mg/kg) and Zn (0.2517^{a} ± 0.0564 mg/kg) contents. In contrast, the interaction of leaves harvested in summer and oven dried had the lowest contents of Ca (95.00^{d} ± 2.6805 mg/kg), Fe (1.7211^{b} ± 0.0485 mg/kg), K (64.022^{e} ± 2.0419 mg/kg), Mn (3.0133^{f} ± 0.1670 mg/kg), P (14.633^{cde} ± 0.4640 mg/kg) and Zn (0.0736^{c} ± 0.0144 mg/kg), whereas leaves harvested in summer and shade dried had the lowest Cu (-0.5554^{a} ± 0.1870 mg/kg). Similarly, leaves harvested in summer and sun dried had lower Mg (31.511^{d} ± 0. 3611 mg/kg) content.

Interaction of harvesting seasons and drying methods had different distributions of non-essential mineral elements of *J. zeyheri* tea leaves (Table 4.12). The interaction of leaves harvested in autumn and freeze dried accounted for the highest Ag (07463^{a} ± 00480 mg/kg), Al (9143^{b} ± 01170 mg/kg), and Pb (22389^{ab} ± 0.0487 mg/kg) contents. The lowest content of Ag (0.6301^{c} ± 0.0063 mg/kg) was reported in autumn harvested leaves and shade dried, however, the interaction of autumn harvested leaves and sun dried had the lowest Al (8.426^{bc} ± 0.1753 mg/kg) and Pb (1.9589^{d} ± 0.0539 mg/kg) contents. The interaction of winter harvested leaves and shade dried reported the highest content of Ag (0.6788^{abc} ± 0.0264 mg/kg) and Pb (2.0856^{abcd} ± 0.0516 mg/kg), whereas winter harvested leaves and sun dried had the highest content of Al (9.273^{b} ± 0.4394 mg/kg). In contrast the lowest contents of Ag (0.6209^{c} ± 0.0065 mg/kg), Al (8.621^{bc} ± 0.3038 mg/kg), and Pb (1.9244^{d} ± 0.0254 mg/kg) were reported in the interaction of winter harvested leaves and the respective drying methods sun, oven and freeze drying respectively. The interaction of summer harvested leaves and respective drying methods, shade, sun, freeze drying reported the highest the highest content of Ag (0.7186^{ab} ± 0.0362 mg/kg), Al (12.204^{a} ± 0.7636 mg/kg) and Pb (1.6778^{e} ± 0.0739 mg/kg), respectively. Similarly, the lowest contents of Ag (0.6363^{c} ± 0.0194

mg/kg), Al (7.652^{c} ± 0.2188 mg/kg) and Pb (1.6778^{e} ± 0.0739 mg/kg) were reported on the interaction of summer harvested leaves and oven dried (Table 4.12).

Table 4.1 Partitioning mean sum of squares of essential nutrient elements calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg) on harvesting seasons and drying methods of *Jatropha zeyheri* leaves (n = 108).

		Ca (mg/kg)		Cu (mg/kg)		Fe (mg/kg)		K (mg/kg)		Mg (mg/kg)	
Source	DF	MSS[y]	%[z]	MSS	%	MSS	%	MSS	%	MSS	%
Replication	8	89.64	2	0.00395	4	1.7403	5	45.768	9	3.9294	6
Harvesting seasons	2	493.44	14***	0.05049	50***	8.7928	25***	219.458	42***	11.0078	17^{ns}
Drying methods	3	2033.55	57***	0.01360	14^{ns}	12.8813	37***	69.810	13**	36.6402	57***
D × S	6	902.14	25***	0.02685	26***	10.8143	30***	167.315	32***	9.2680	14**
Error	88	82.81	2	0.00646	6	1.0600	3	20.113	4	4.0341	6
Total	107	3601.58	100	0.10135	100	35.2887	100	522.464	100	64.8795	100

***Treatments effects were highly significant at P ≤ 0.01, [ns] non-significant at P ≥ 0.05.

[y]MSS = Mean Sum of Squares.

[z]TTV (%) = Percentage of Total Treatment Variation.

Table 4.2 Partitioning mean sum of squares of essential nutrient elements manganese (Mn), nickel (Ni), Phosphorus (P) and Zinc (Zn) on harvesting seasons and drying methods of *Jatropha zeyheri* tea leaves (n = 108).

		Mn (mg/kg)		Ni (mg/kg)		P (mg/kg)		Zn (mg/kg)	
Source	DF	MSS[y]	%[z]	MSS	%	MSS	%	MSS	%
Replication	8	0.07241	1	0.02041	7	8.6376	4	0.00186	2
Harvesting seasons	2	4.69325	53***	0.22638	74***	97.8919	49***	0.04029	38**
Drying methods	3	2.20687	25***	0.02275	7ns	37.8969	19***	0.00440	4ns
D × S	6	1.78999	20***	0.01493	5ns	47.5171	24***	0.05013	48***
Error	88	0.15048	1	0.02128	7	8.6238	4	0.00881	8
Total	107	8.91300	100	0.30575	100	200.5673	100	0.10549	100

***Treatments effects were highly significant at P ≤ 0.01, ns non-significant at P ≥ 0.05.

[y]MSS = Mean Sum of Squares.

[z]TTV (%) = Percentage of Total Treatment Variation.

Table 4.3 Partitioning mean sum of squares for non-essential nutrient elements silver (Ag), aluminium (Al), sodium (Na), lead (Pb), on harvesting season and drying methods of *Jatropha zeyheri* tea leaves (n = 108).

		Ag (mg/kg)		Ni (mg/kg)		Na (mg/kg)		Pb (mg/kg)	
Source	DF	MSS[y]	%[z]	MSS	%	MSS	%	MSS	%
Replication	8	0.00060	1	1.5085	3	0.09563	6	0.06586	9
Harvesting seasons	2	0.01381	27^{ns}	17.7614	38***	0.59658	39**	0.05793	9^{ns}
Drying methods	3	0.01389	28^{ns}	13.6170	29***	0.30652	20^{ns}	0.28524	42***
D × S	6	0.01581	32**	13.1436	28***	0.33268	22^{ns}	0.23941	35***
Error	88	0.00594	12	1.0790	2	0.18979	13	0.03278	5
Total	107	0.05005	100	47.1095	100	1.52120	100	0.68122	100

***Treatments effects were highly significant at P ≤ 0.01, ^{ns} non-significant at P ≥ 0.05.

[y]MSS = Mean Sum of Squares.

[z]TTV (%) = Percentage of Total Treatment Variation

Table 4.4 Response of essential mineral elements, calcium (Ca), copper (Cu), iron (Fe), potassium (K), to harvesting seasons of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)
	Variable[y]	Variable	Variable	Variable
Autumn	$120.12^{a} \pm 1.9053$	$-0.6907^{b} \pm 0.0040$	$2.1075^{b} \pm 0.0619$	$73.658^{a} \pm 1.1242$
Winter	$115.79^{b} \pm 1.8889$	$-0.6992^{b} \pm 0.0052$	$2.3300^{b} \pm 0.0701$	$73.628^{a} \pm 0.7923$
Summer	$112.75^{b} \pm 2.8797$	$-0.6305^{a} \pm 0.0244$	$3.0528^{a} \pm 0.3981$	$69.367^{b} \pm 0.8756$

[y] Column means ± SE (Standard error) followed by the same letter were not different ($P \leq 0.05$) according to Waller-Duncan Multiple Range test.

Table 4.5 Response of essential mineral elements, manganese (Mn), nickel (Ni), phosphorus (P) and zinc (Zn) to harvesting seasons of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	Mn (mg/kg)	Ni (mg/kg)	P (mg/kg)	Zn(mg/kg)
	Variable[y]	Variable	Variable	Variable
Autumn	$4.3817^{a} \pm 0.0763$	$0.2049^{b} \pm 0.0135$	$14.210^{c} \pm 0.4690$	$0.1043^{b} \pm 0.0146$
Winter	$4.2578^{b} \pm 0.0835$	$0.1864^{b} \pm 0.0123$	$15.742^{b} \pm 0.7527$	$0.0863^{b} \pm 0.0110$
Summer	$3.7036^{b} \pm 0.1093$	$0.3321^{a} \pm 0.0375$	$17.506^{a} \pm 0.4327$	$0.1511^{a} \pm 0.0233$

[y] Column means ± SE (Standard error) followed by the same letter were not different ($P \leq 0.05$) according to Waller-Duncan Multiple Range test.

Table 4.6 Response of non-essential mineral elements aluminium (Al) and sodium (Na) to harvesting seasons of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	Al (mg/kg)	Na (mg/kg)
	Variable[y]	Variable
Autumn	8.839[by] ± 0.0921[z]	1.9761[ab] ± 0.0786
Winter	8.920[b] ± 0.1455	1.9383[b] ± 0.07887
Summer	10.094[a] ± 0.3877	2.1778[a] ± 0.0614

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 4.7 Response of essential mineral elements, calcium (Ca), iron (Fe), potassium (K), to different drying methods of *Jatropha zeyheri* tea leaves (n = 108).

Drying methods	Ca (mg/kg)	Fe (mg/kg)	K (mg/kg)
	Variable[y]	Variable	Variable
Shade	119.11^{by} ± 1.4415^{z}	2.1289^{b} ± 0.0680	73.581^{a} ± 0.8706
Sun	109.11^{c} ± 1.6919	3.5237^{a} ± 0.5050	69.893^{b} ± 1.0257
Oven	109.43^{c} ± 2.9833	2.0574^{b} ± 0.0856	72.819^{a} ± 1.6349
Freeze	127.22^{c} ± 2.5844	2.2770^{b} ± 0.0566	72.578^{a} ± 0.7423

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 4.8 Response of essential mineral elements, magnesium (Mg), manganese (Mn), and phosphorus (P) to different drying methods of *Jatropha zeyheri* tea leaves (n = 108).

Drying methods	Mg (mg/kg)	Mn (mg/kg)	P (mg/kg)
	Variable[y]	Variable	Variable
Shade	$34.307^{a} \pm 0.4831$	$4.2141^{b} \pm 0.0898$	$15.806^{a} \pm 0.5582$
Sun	$31.615^{c} \pm 0.3135$	$3.7559^{c} \pm 0.8861$	$16.644^{a} \pm 0.8861$
Oven	$32.500^{bc} \pm 0.5027$	$4.0522^{b} \pm 0.1654$	$14.148^{b} \pm 0.4441$
Freeze	$33.444^{ab} \pm 0.2769$	$4.4352^{a} \pm 0.0835$	$16.678^{a} \pm 0.7553$

[y] Column means ± SE (Standard error) followed by the same letter were not different ($P \leq 0.05$) according to Waller-Duncan Multiple Range test.

Table 4.9 Response of non-essential mineral elements aluminium (Al) and lead (Pb) to different drying methods of *Jatropha zeyheri* tea leaves (n = 108).

Drying methods	Al (mg/kg)	Pb (mg/kg)
	Variable[y]	Variable
Shade	9.7263[ay] ± 0.3141[z]	2.0885[a] ± 0.0294
Sun	9.9678[a] ± 0.4284	1.9774[b] ± 0.0369
Oven	8.3919[c] ± 0.1696	1.9111[b] ± 0.0581
Freeze	9.0519[b] ± 0.0664	2.1367[a] ± 0.0382

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 4.10 Response of interactive effect of harvesting seasons and drying methods on essential mineral elements, calcium (Ca), copper (Cu), iron (Fe), potassium (K), of *Jatropha zeyheri* tea leaves (n = 108).

Harvesting seasons	Drying methods	Ca (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	K (mg/kg)
		Variable[y]	Variable	Variable	Variable
Autumn	Shade	$116.78^{bc} \pm 2.100^{z}$	$-0.6894^{b} \pm 0.0080$	$2.0467^{b} \pm 0.1422$	$73.289^{bc} \pm 1.2653$
	Sun	$112.92^{c} \pm 3.3982$	$-0.7126^{b} \pm 0.0029$	$2.0022^{b} \pm 0.1623$	$67.356^{de} \pm 2.0823$
	Oven	$116.44^{bc} \pm 2.7137$	$-0.6844^{b} \pm 0.0094$	$2.2533^{b} \pm 0.1193$	$80.400^{a} \pm 1.4666$
	Freeze	$134.33^{a} \pm 2.3214$	$-0.6762^{b} \pm 0.0049$	$2.1278^{b} \pm 0.0411$	$73.589^{b} \pm 1.8345$
Winter	Shade	$122.11^{b} \pm 2.2818$	$-0.7002^{b} \pm 0.0066$	$2.1089^{b} \pm 0.1366$	$75.156^{b} \pm 2.0909$
	Sun	$111.63^{c} \pm 2.8276$	$-0.7091^{b} \pm 0.0172$	$2.6311^{b} \pm 0.0925$	$73.233^{bc} \pm 1.6771$
	Oven	$116.86^{bc} \pm 5.7580$	$-0.6833^{b} \pm 0.0047$	$2.1978^{b} \pm 0.1827$	$74.033^{b} \pm 1.6431$
	Freeze	$112.56^{c} \pm 2.7341$	$-0.7040^{b} \pm 0.0598$	$2.3822^{b} \pm 0.0733$	$72.089^{bc} \pm 0.6883$
Summer	Shade	$118.44^{bc} \pm 2.9633$	$-0.5554^{a} \pm 0.1870$	$2.2311^{b} \pm 0.0635$	$72.300^{bc} \pm 0.9443$
	Sun	$102.79^{d} \pm 0.8709$	$-0.5636^{a} \pm 0.0623$	$5.9378^{a} \pm 1.1551$	$69.089^{cd} \pm 0.9107$
	Oven	$95.00^{d} \pm 2.6805$	$-0.7254^{b} \pm 0.0098$	$1.7211^{b} \pm 0.0485$	$64.022^{e} \pm 2.0419$
	Freeze	$134.78^{a} \pm 3.4512$	$-0.6774^{b} \pm 0.01259$	$2.3211^{b} \pm 0.1405$	$72.056^{bc} \pm 1.4022$

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

Table 4.11 Response of interactive effect of harvesting seasons and drying methods on essential mineral elements, magnesium (Mg), manganese (Mn), phosphorus (P), and zinc (Zn) of *Jatropha zeyheri* leaves (n = 108).

Harvesting seasons	Drying methods	Mg (mg/kg)	Mn (mg/kg)	P (mg/kg)	Zn (mg/kg)
		Variable[y]	Variable	Variable	Variable
Autumn	Shade	$32.444^{bcd} \pm 0.9483$	$4.4444^{ab} \pm 0.0761$	$12.463^{e} \pm 0.5516$	$0.0684^{c} \pm 0.0112$
	Sun	$31.478^{d} \pm 0.7926$	$3.8633^{cd} \pm 0.1474$	$14.044^{de} \pm 0.6243$	$0.0737^{c} \pm 0.0131$
	Oven	$32.367^{bcd} \pm 0.7498$	$4.7289^{a} \pm 0.1353$	$13.644^{e} \pm 0.6675$	$0.0914^{c} \pm 0.0176$
	Freeze	$33.400^{bc} \pm 0.6218$	$4.4900^{ab} \pm 0.0735$	$16.689^{bcd} \pm 1.2311$	$0.1838^{ab} \pm 0.0450$
Winter	Shade	$34.222^{b} \pm 0.6309$	$4.4600^{ab} \pm 0.1617$	$17.222^{bc} \pm 0.7049$	$0.0539^{c} \pm 0.0124$
	Sun	$31.856^{cd} \pm 0.4343$	$3.9511^{cd} \pm 0.1361$	$18.467^{ab} \pm 2.3318$	$0.0889^{c} \pm 0.0180$
	Oven	$32.944^{bcd} \pm 0.8623$	$4.4144^{ab} \pm 0.1045$	$14.167^{de} \pm 1.0959$	$0.1228^{bc} \pm 0.0112$
	Freeze	$32.778^{bcd} \pm 0.1665$	$4.2056^{bc} \pm 0.2148$	$13.111^{e} \pm 0.6332$	$0.0797^{c} \pm 0.0348$
Summer	Shade	$36.256^{a} \pm 0.2631$	$3.7378^{de} \pm 0.0636$	$17.733^{ab} \pm 0.3536$	$0.2517^{a} \pm 0.0564$
	Sun	$31.511^{d} \pm 0.3611$	$3.4533^{e} \pm 0.0218$	$17.422^{b} \pm 0.7025$	$0.2002^{ab} \pm 0.0552$
	Oven	$32.189^{cd} \pm 1.0578$	$3.0133^{f} \pm 0.1670$	$14.633^{cde} \pm 0.4640$	$0.0736^{c} \pm 0.0144$
	Freeze	$34.156^{b} \pm 0.4628$	$4.6100^{a} \pm 0.0747$	$20.233^{a} \pm 0.6890$	$0.0790^{c} \pm 0.0098$

[y] Column means ± SE (Standard error) followed by the same letter were not different ($P \leq 0.05$) according to Waller-Duncan Multiple Range test.

Table 4.12 Response of interactive effect of harvesting seasons and drying methods on non-essential mineral elements, silver (Ag), aluminium (Al) and lead (Pb) on *Jatropha zeyheri* leaves (n = 108).

Harvest seasons	Drying methods	Ag (mg/kg)	Al (mg/kg)	Pb (mg/kg)
		Variable[y]	Variable	Variable
Autumn	Shade	$0.6301^{cy} \pm 0.0063^{z}$	$8.886^{b} \pm 0.1943$	$1.9989^{d} \pm 0.0443$
	Sun	$0.6378^{c} \pm 0.0042$	$8.426^{bc} \pm 0.1753$	$1.9589^{d} \pm 0.0539$
	Oven	$0.6328^{c} \pm 0.0065$	$8.902^{b} \pm 0.1807$	$2.0700^{bcd} \pm 0.0819$
	Freeze	$0.7463^{a} \pm 0.0480$	$9.143^{b} \pm 0.1170$	$2.2389^{ab} \pm 0.0487$
Winter	Shade	$0.6788^{abc} \pm 0.0264$	$8.952^{b} \pm 0.2139$	$2.0856^{abcd} \pm 0.0516$
	Sun	$0.6209^{c} \pm 0.0065$	$9.273^{b} \pm 0.4394$	$1.9511^{d} \pm 0.0820$
	Oven	$0.6527^{bc} \pm 0.0198$	$8.621^{bc} \pm 0.3038$	$1.9856^{d} \pm 0.1007$
	Freeze	$0.6294^{c} \pm 0.0060$	$8.833^{b} \pm 0.1080$	$1.9244^{d} \pm 0.0254$
Summer	Shade	$0.7186^{ab} \pm 0.0362$	$11.341^{a} \pm 0.6240$	$2.1811^{abc} \pm 0.0417$
	Sun	$0.6902^{abc} \pm 0.0397$	$12.204^{a} \pm 0.7636$	$2.0222^{cd} \pm 0.0574$
	Oven	$0.6363^{c} \pm 0.0194$	$7.652^{c} \pm 0.2188$	$1.6778^{e} \pm 0.0739$
	Freeze	$0.6927^{abc} \pm 0.0220$	$8.833^{b} \pm 0.0928$	$2.2467^{a} \pm 0.0526$

[y] Column means ± SE (Standard error) followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

4.4 DISCUSSION

4.4.1 Effect of harvesting seasons on essential and non-essential mineral elements.

Overall variation in mineral elements by different harvesting seasons may be attributed to variations in environmental variables such as temperature, humidity, light, drought, salinity, parasitic plants, soil microbes and rainfall (Kumar *et al*., 2017). In some plants, high water uptake increases nutrients uptake as it enhances root physiological functions that facilitate nutrient absorption (Carvalho and Foulkes, 2018). Low amount of rainfall or drought can cause less mineral elements absorption and transportation due to poor stomata functioning, which can result in nutrient deficiency. Consequently, high amounts of rainfall or flooding can result in soil degradation and leaching of mineral elements. In some plants, the active process of nitrate and calcium uptake can be retarded by high root temperature (Carvalho and Foulkes, 2018). Parasitic plants compete with main plants for nutrient uptake, their presence affect quantity of nutrients that could be absorbed by plants. Moreover, mineral contents in leaves also change seasonally. During winter harvesting season, cold restricts majority of plants from water and nutrient uptake by roots due to stomata closure (Fahad *et al*., 2017). This causes lowered mineral uptake that could also interfere with the contents of some trace metals such as Fe, Mn and Cu that are significant to the photosynthetic electron transfer (Fahad *et al*., 2017). However, during warmer season there may be optimal mineral uptake. Cold also has the ability to affect the plant to perform changes in specific elements as part of acclimation process.

Harvesting seasons had an effect on essential (Ca, Fe, K, Mn and P) and non-essential (Cu, Ni and Zn) mineral elements, of *J. zeyheri* leaves. The results of this study agree with those reported on black tea (*Camellia sinensis*) (Zhao *et al*., 2017),

mint tea (*Mentha longifolia*) (Ahmad *et al*., 2011), derepazari tea (*C sinensis var. sinensis*) (Ercisli *et al*., 2008) and mulberry leaves (*Morus alba L*) (Levickienė *et al*., 2018). However, in this study, Mg was not affected by harvesting seasons. According to Guo *et al*. (2016) absorption of Mg by plants may be affected by the cultivar and nature of the soil as an exchange complex, as well as heavy presence of K in the soil. Consequently, Mg deficiency can be induced not only by low Mg concentration in the soil, but also by high concentration of other metals during the harvesting season (Guo *et al*., 2016). Contrasting results were observed on green tea leaves (*C. sinensis*) (Demir and Bostan, 2018) and yerba mate tea leaves (Ilex *paraguariensis*) where Mg was affected by harvesting seasons (Bastos *et al.,* 2018).

In the current study, Ca, K, and Mn were mostly dominant in the autumn season. Calcium has been reported to be an essential requirement for chilling induced stomata closure. The autumn season is chilly, the high calcium might be plant response to mediate stress response during chilling injury, acclimation to stress and protection of leaves to dehydration (Waraich *et al*., 2012). Calcium absorption is mostly facilitated by moisture content, the autumn season might have had adequate moisture retained in the soil that allowed easy Ca mobility. Potassium plays a crucial role in survival of plants under environmental stress, it enhances turgidity and activation of enzymes under stress conditions (Waraich *et al*., 2012). The high presence of K during the autumn season might be response chilling stress and low moisture availability endured by the plant as compared to summer harvesting season. High availability of Mg has been reported mostly during colder and drier growing seasons hence its high accumulation in autumn in the leaves of *J. zeyheri* as compared to summer harvesting season. Summer harvesting season retained the highest level of Fe, Ni, P and Zn.

Ahmad *et al*. (2011), reported that Fe and Zn components are bio-molecules and also participate in a number of metabolic reactions as co-factors. Their requirements in plants are relatively higher during the period of fast growth, which occurs in summer (Khan and Mukhtar, 2013; Ahmad *et al*., 2011). The high accumulation of P in summer maybe because P is an integral component of a number of macro-molecules such as proteins and nucleic acids, their concentration could be higher in plants at maturity level. Leaves harvested in winter did not retain high concentrations of most mineral elements, this may be because during winter most plants including *J. zeyheri* plant undergo a dormant stage.

Harvesting seasons affected two non-essential mineral elements Al and Na of *J. zeyheri* leaves. Similar results were reported on Longjing tea *(C. sinensis)* and Shu Cha Zao (*C. sinensis*) Chinese teas (Pan *et al*., 2015). The main sources of heavy metals in plants are their growth media, nutrients, agro-inputs, elemental absorption, and translocation in plants and soil, which vary seasonally (Kumar *et al*., 2017). Metallic constituents of tea leaves normally differ according to the type of tea and its geological source (AL-Oud, 2003). In this study, Ag and Pb were not affected by harvesting seasons. Silver is mostly present in contaminated water and may permeate into fields through fertilization and irrigation (Guo *et al*., 2016). The absorption of the heavy metals from the soil depends on the contamination, of which throughout the seasons there may have been low accumulation to the plants as they were harvested in an open field.

Summer harvesting seasons had the highest content of Al and Na. High heavy metal concentration during summer may be attributed to high rainfall and temperature that

result in increased metal toxicity (Sharma *et al*., 2007). Similar findings were reported on Assam tea (*Camellia assamica*) were summer harvesting season had high concentration of Al (Peng *et al*., 2018). The high concentration of Al in summer season may be a result of higher plant metabolism or increased transpiration in summer compared to autumn (Peng *et al*., 2018). The high content of Na in summer may be a result of high amount of rainfall in the summer season, which usually concentrates Na that was readily available in the soil.

4.4.2. Effect of drying methods on essential mineral elements of *Jatropha zeyheri* tea leaves.

Drying methods had effects on essential mineral elements (Ca, Fe, K, Mg, Mn and P) of *J. zeyheri* leaves. Similar results were reported on *J. zeyheri* tea leaves (Mutshekwa, 2017; Mutshekwa *et al*., 2019), green tea (*C. sinensis*) (Roshanak *et al*., 2016), stinging nettle leaves (*Urtica dioica*) (Shonte *et al*., 2020). Plants generally respond differently to drying techniques. Various drying techniques affect bioactive compounds in plants differently. Furthermore, the effect of drying on nutritional components of the plant depends on the sensitivity of the nutrients. The drying methods chosen might have had a major impact on nutrient degradation and retention (Shonte *et al*., 2020). In this study Cu, Ni and Zn were not affected by drying methods. Contrary results were reported on *B. alba* where different drying methods had effect on Zn (Oni *et al*., 2015). The drying of plants nutritional components depends on the sensitivity of the heavy metals to the drying technique.

In this study, freeze-drying and shade-drying retained the highest content of Ca, Mn, P, K, and Mg as compared to sun-drying and oven-drying. During freeze drying, very

few chemical changes occur, whereas oven drying can cause faster degradation of colour and loss of primary metabolites (Shonte *et al*., 2020). In general, low temperature of the freeze drying process it slows down degradation reactions and preserves the nutrient content of food more efficiently than oven or solar drying. According to Oni *et al*. (2015), heat alone is capable of destroying the nutritional component of perishables including medicinal plants because many of the nutritional factors found in plants are liable to heat. This outlines that the above-mentioned elements are sensitive to heat. The results of this study agree with those reported stinging nettle leaves tea (*U. dioica*) (Shonte *et a*l., 2020) where non thermal drying retained more nutrients. Contrary results were observed on rosemary tea leaves (*Rosmarinus officinalis L*) where the highest content of nutrients was retained by oven drying (Arslan and Ozcan, 2008). The effect of the drying methods to various nutrients depends on the sensitivity of the nutrients to oxygen, pH, and light (Shonte *et al*., 2020). This outlines that the leaves are not heat sensitive (Arslan and Ozcan, 2008). In this study, Fe was retained by sun drying, this may be because that the bioactive nature of the element allowed the convective energy of sun drying to cause more increase in the solubility of the element than in freeze and shade drying (Arslan and Ozcan, 2008). Similar results were reported on congo bololo (*Vernonia amygdalina)* whereby the highest content of Fe was retained by sun dried leaves as compared to other drying methods (Garba and Oviosa, 2019).

4.4.3. Effect of drying methods on non-essential mineral elements of *Jatropha zeyheri* tea leaves

In the current study, drying methods had effects on non-essential mineral elements (Al and Pb) of *J. zeyheri* leaves. Similar results were observed on basil tea (*O. basilicum L.*) (Özcan *et al*., 2005), congo bololo (*V. amygdalina*) (Garba and Oviosa, 2019). The changes in the concentrations of heavy metals are dependent on the method and the drying temperature and how they affect the sensory and nutritious qualities of the final products (Arslan and Ozcan, 2008). In this study, Ag and Na were not affected by drying methods. Similar results were reported in rosemary tea leaves (*R. officinalis L*) whereby non-essential mineral elements of the dried samples were not statistically significant (Arslan and Ozcan, 2008). The drying process can have a major impact on the degradation of minerals.

Sun-dried and freeze-dried leaves retained the highest content of Al and Pb, as compared to shade-dried and oven-dried leaves of *J. zeyheri* tea leaves. The ability of freeze dried leaves to retain high content of Pb may be because Pb is a heat liable element, thus the bioactive content of the mineral is depreciated in heat drying methods. Aluminium was more retained by sun dried leaves, this maybe be because the convective style of energy and wave strength of the sun drying method caused more increase in the solubility of the elements than the freeze drying. The results of this study agree with those on V *amygdalina* where shade dried leaves reported the highest content of Pb (Garba and Oviosa, 2019). Contrary results were observed on basil (*O. basilicum* L.) where oven dried leaves retained non-essential elements (Özcan *et al*., 2005).

4.4.4 Interactive effect of harvesting seasons and drying methods on essential mineral elements activity of *Jatropha zeyheri* tea leaves.

The interaction between harvesting seasons and drying methods had an effect on Ca, Cu, Fe, K, Mg, Mn, P and Zn essential mineral elements. Mineral elements are absorbed from the soil through plant roots. The absorptive capability of minerals by roots is affected by various factors such as light temperature and rainfall, which alter seasonally. The variation in environmental factors results in variation in the absorbed mineral content in dried leaves. Similar results were reported on black tea (*C. sinensis*) (Zhao *et al*., 2017), kuntze tea (C. *sinensis L*) (Han *et al*., 2007), mint tea (*Mentha longifolia*) (Ahmad *et al*., 2011). In this study, Ni was not affected by the interaction of harvesting seasons and drying methods. This may be because there was no hype accumulation or presence of Ni during harvesting seasons. Consequently, it's present in low contents that made the element not to be retained by the various drying methods.

The interaction of autumn harvesting season with drying methods reported that autumn and freeze dried leaves retained the highest content on the essential mineral elements Ca, Mg, P and Zn. This may be because of the relationship between plant water content and the respective seasons that have an effect on the concentration of mineral element (Smith, 1978). Autumn season is a warm season as compared to winter with adequate amount of rainfall that may be able to concentrate mineral absorption as compared to winter season. Freeze drying has been documented as one of the non-thermal drying methods that are sufficient for the retention of most of the mineral elements. The lyophilisation process in freeze drying retains much of its cellular integrity, preserving both nutritional values (Sharma *et al*., 2012). This outlines

that the bioactive molecules of the listed minerals they may be sensitive to heat or light. Interaction of autumn and oven drying had the highest content of Fe, K, and Mn. In general, the interaction of autumn harvested leaves and sun dried reported the lowest content of most essential mineral elements because the bioactive nature of most of the mineral elements outlines that they are sensitive to heat.

4.4.5 Interactive effect of harvesting seasons and drying methods on non-essential minerals elements of *Jatropha zeyheri* tea leaves.

Interaction between harvesting seasons and drying methods had effect on Ag, Al and Pb non-essential mineral elements. Specific plant species can absorb and hyper accumulate metal contaminants and/or excess nutrients in harvestable root from the growth substrate (Tangahu *et al*., 2011). Heavy metals are present in soil, air and water and can easily enter into the plant (Tangahu *et al*., 2011). In this study, Na was no affected by the interaction of harvest season and drying methods. The interaction of autumn harvested leaves and drying methods reported that the highest content of Ag, Al and Pb was retained by autumn and freeze drying. Sun dried leaves reported the lowest content of Al and Pb. The interaction of leaves harvested in winter and shade dried had the highest content of Ag and Pb. Leaves harvested in winter and sun, oven and freeze dried had the lowest contents of Ag, Al, and Pb respectively. The results of this study conclude that the retention of mineral elements is mostly achieved during autumn harvesting season and freeze drying, which indicate that the mineral elements may be highly sensitive to heat.

4.5 CONCLUSION

The interactive effect of harvesting seasons and drying methods on essential and non-essential mineral elements of *J. zeyheri* leaves demonstrated that the leaves are a rich source of mineral elements which will contribute to quality of the tea. However, concentrations of essential mineral elements were mostly retained by leaves harvested in autumn and summer. In contrast, leaves harvested in winter had the highest content of heavy metals. Aluminium was mostly dominant in the leaves as compared to other selected heavy metals. Freeze dried and shade-dried leaves retained most of the mineral elements, which highlight that most of the elements in *J. zeyheri* may be heat sensitive.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

The study assessed the effect of harvesting seasons and drying methods on phytochemicals, antioxidant activity and mineral elements of *J. zeyheri* leaves. Harvesting seasons had an effect on phytochemicals and antioxidant activity of *J. zeyheri* leaves. Antioxidant activity was mostly abundant in all the harvesting seasons and different drying methods used on the leaves. However, leaves harvested in summer and oven-dried retained the highest contents of the phytochemicals. In contrast leaves harvested in summer harvesting season and freeze-dried reported the highest antioxidant activity. Majority of the mineral elements were reduced after being subjected to oven and sun-drying methods used on leaves. In contrast the mineral elements were more retained on selected harvesting seasons after being subjected to shade and freeze drying methods used on leaves.

5.2 Significance of findings

The results of this study indicated that harvesting the leaves of *J. zeyheri* during summer season retained higher concentration of total phenolic contents and antioxidant activity, which could contribute towards better health effects. Harvesting of tea leaves during winter increased the tannin content of tea, resulting in high astringency, which might affect consumer taste. Additionally, oven-drying and freeze-drying reported better retention of chemical compositions of *J. zeyheri* leaves, which could be used by small scale farmers and rural dwellers. The study further demonstrated that *J. zeyheri* is a rich source of several mineral elements, leaves harvested in summer retained most of the mineral elements. However, majority of the

selected mineral elements were reduced after sun and oven drying methods used on leaves, as a result of heat sensitivity trait. Shade and freeze drying maintained high retention of the mineral elements in the harvested leaves.

5.3 Recommendations

Tea quality is an important attribute within the tea industry. In this study, one of the major factors affecting tea quality, which is harvesting seasons, indicated that there is low retention of selected phytochemicals in winter and autumn. It may be important to carry out further study outlining this factor. This may also be important in case of domestication of *J. zeyheri* plant. Furthermore, the study demonstrated the presence of non-essential mineral elements, further study should be carried out to investigate the presence of non-essential mineral elements.

5.4 Conclusions

In conclusion, *J. zeyheri* leaves are rich in antioxidant activity, which is of great importance for tea quality and medicinal use. High amount of total phenolic content and antioxidant activity was retained by summer harvesting season. Furthermore, oven-drying method retained most phytochemicals except antioxidant activity, which was mostly retained by freeze-drying method. Mineral elements were mostly retained by freeze drying, demonstrating that oven and freeze drying could help to increase the quality of *J. zeyheri* tea leaves. Therefore, the presence of total phenolic content, total flavonoid content, mineral elements and antioxidant activity indicated that *J. zeyheri* leaves are of good tea quality and medicinal importance. The study concluded that harvesting seasons and drying methods improved the development *of J. zeyheri* tea leaves.

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APPENDICES

Appendix 3.1 Analysis of variance for total phenolic content to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	3.1008	0.3876		
Drying	3	8.3124	2.7708	8.63***	0.0000
Season	2	20.9054	10.4527	32.54***	0.0000
D × S	6	9.2243	1.5374	4.79***	0.0003
Error	88	28.2666	0.3212		
Total	107	69.8095			

Appendix 3.2 Analysis of variance for total flavonoid content to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.1595	0.01994		
Drying	3	1.1026	0.36752	8.27***	0.0001
Season	2	6.3700	3.18499	71.67***	0.0000
D × S	6	0.5924	0.09873	2.22	0.0483
Error	88	3.9108	0.04444		
Total	107	12.1352			

Appendix 3.3 Analysis of variance for total antioxidant activity to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	6.221	0.7776		
Drying	3	20.109	6.7029	13.03***	0.0000
Season	2	48.316	24.1582	46.97***	0.0000
D × S	6	30.401	5.0669	9.85***	0.0000
Error	88	45.263	0.5143		
Total	107	150.310			

Appendix 4.1 Analysis of variance for Ag to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.00482	0.00060		
Drying	3	0.04167	0.01389	2.34	0.0788
Season	2	0.02762	0.01381	2.33	0.1036
D × S	6	0.09487	0.01581	2.66	0.0202
Error	88	0.52237	0.00594		
Total	107	0.69135			

Appendix 4.2 Analysis of variance for Al to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	12.068	1.5085		
Drying	3	40.851	13.6170	12.62	0.0000
Season	2	35.523	17.7614	16.46	0.0000
D × S	6	78.862	13.1436	12.18	0.0000
Error	88	94.955	1.0790		
Total	107	262.259			

Appendix 4.3 Analysis of variance for Ca to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	717.1	89.64		
Drying	3	6100.6	2033.55	24.56	0.0000
Season	2	986.9	493.44	5.96	0.0037
D × S	6	5412,8	902.14	10.89	0.0000
Error	88	7287.7	82.81		
Total	107	20505.2			

Appendix 4.4 Analysis of variance for Cu to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.03156	0.00395		
Drying	3	0.04079	0.01360	2.10	0.1053
Season	2	0.10097	0.05049	7.81	0.0008
D × S	6	0.16112	0.02685	4.16	0.0010
Error	88	0.56852	0.00646		
Total	107	0.90297			

Appendix 4.5 Analysis of variance for Fe to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	13.922	1.7403		
Drying	3	38.644	12.8813	12.15	0.0000
Season	2	17.86	8.7928	8.30	0.0005
D × S	3	0.04079	0.01360	2.10	0.1053
Error	88	93.280	1.0600		
Total	107	228.317			

Appendix 4.6 Analysis of variance for K to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	366.14	45.768		
Season	2	438.92	219.458	10.91	0.0001
Drying	3	209.43	69.810	3.47	0.0195
D × S	6	1003.89	167.315	8.32	0.0000
Error	88	1769.92	20.113		
Total	107	3788.30			

Appendix 4.7 Analysis of variance for Mg to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	31.435	3.9294		
Drying	3	109.921	36.6402	9.08	0.0000
Season	2	22.016	11.0078	2.73	0.0708
D × S	6	55.608	9.2680	2.30	0.0416
Error	88	355.001	4.0341		
Total	107	573.980			

Appendix 4.8 Analysis of variance for Mn to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.5793	0.07241		
Season	2	9.3865	4.69325	31.19	0.0000
Drying	3	6.6206	2.20687	14.67	0.0000
D × S	6	10.7399	1.78999	11.90	0.0000
Error	88	13.2419	0.15048		
Total	107	40.5683			

Appendix 4.9 Analysis of variance for Na to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.7650	0.09563		
Season	2	1.1932	0.59658	3.14	0.0480
Drying	3	0.9196	0.30652	1.62	0.1916
D × S	6	1.9961	0.33268	1.75	0.1182
Error	88	16.7017	0.18979		
Total	107	21.5755			

Appendix 4.10 Analysis of variance for Ni to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.16331	0.02041		
Drying	3	0.06826	0.02275	1.07	0.3664
Season	2	0.45275	0.22638	10.64	0.0001
D × S	6	0.08956	0.01493	0.70	0.6492
Error	88	1.87281	0.02128		
Total	107	2.64668			

Appendix 4.11 Analysis of variance for P to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	69,10	8,6376		
Drying	3	113.69	37.8969	4.39	0.0063
Season	2	195.78	97.8919	11.35	0.0000
D × S	6	285.10	47.5171	5.51	0.0001
Error	88	758.90	8.6238		
Total	107	1422.58			

Appendix 4.12 Analysis of variance for Pb to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.52687	0.06586		
Season	2	0.11587	0.05793	1.77	0.1768
Drying	3	0.85571	0.28524	8.70	0.0000
D × S	6	1.43644	0.23941	7.30	0.0000
Error	88	2.88495	0.03278		
Total	107	5.81983			

Appendix 4.13 Analysis of variance for Zn to harvesting seasons and drying methods of *Jatropha zeyheri* leaves.

Source	DF	SS	MS	F	P
Replication	8	0.01485	0.00186		
Drying	3	0.01319	0.00440	0.50	0.6840
Season	2	0.08057	0.04029	4.57	0.0129
D × S	6	0.30077	0.05013	5.69	0.0000
Error	88	0.77538	0.00881		
Total	107	1.18477			