EFFECT OF TIME-BASED HOT AIR DRYING METHOD ON CHEMICAL COMPOSITION OF *JATROPHA ZEYHERI* TEA

by

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TABLE OF CONTENTS

DECLARATION

I 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 material contained herein has been duly acknowledged.

…… ……..

Signature Date

Mutshekwa, N (Mr)

ACKNOWLEDGMENTS

Firstly, I would like to thank the Almighty God for His wisdom and guidance throughout my life and studies. For Him nothing is impossible (Luke 1:37). May His Name be praised, now and forever. Amen.

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DEDICATION

This work is dedicated to my mother Thifhelimbilu Gladys Makuya. She played a vital role in my upbringing and my studies.

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Tea is one of the most popular consumed beverages in the world, which has beneficial properties such as anti-oxidization, anti-carcinoma and preventing arteriosclerosis. The major essential components of catechins present in tea leaves, includes epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C). Influence of time-based hot air drying method on chemical composition of the *Jatropha zeyheri* Sond, widely consumed in rural communities of Zebediela (Khureng village), Limpopo Province, South Africa, was investigated. Four treatments, namely; 0, 24, 48, and 72 hours, were arranged in completely randomised design (CRD), replicated five times. The study demonstrated that drying significantly increased total phenolic content, total antioxidant capacity and tannin content. It also demonstrated that drying significantly increased minerals elements; Mg, K, P, S, Al, Co, Mn, Si and Zn content and decreased Na, Ca and Ni and Zn quantities. Sodium-potassium ratio was very low across drying periods. Drying time did not significantly influence proximate chemicals; energy, protein, carbohydrates, ash and fibre content. Moisture and fat were significantly increased by drying period. Results of the study suggested that time-based hot air drying method improved the chemical composition of *J. zeyheri,* which has the potential of enhancing nutrition in marginal rural communities of Limpopo Province.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

Tea is one of the most popular and pleasant consumed beverages in the world, which has not only a popular flavour but also beneficial properties such as anti-oxidization, anti-carcinoma and preventing arteriosclerosis (du Toit *et al.*, 2001). It is widespread for its aroma, taste, beneficial effects and safe drink that is enjoyed every day by hundreds of millions of people across all the continents (Khan and Mukhtar, 2007). Tea polyphenols constitute the major portion of the soluble ingredients and are the essential components of tea, which have physiological functions. Tea catechins are the primary polyphenols in tea, and accounts for 75-80% of soluble ingredients and get oxidized to form theaflavins and thearubigins during fermentation (Muthumani and Kumar, 2007). These thearubigins are responsible for colour, body and taste while theaflavins content in tea determines the briskness, brightness and quality of the liquor (Venkateswaran *et al.*, 2002). Hence, any increase in tea phenolic compounds could improve the quality and therapeutic value of tea (Erickson, 2003).

During drying, the colour change associated with the development of characteristic aroma takes place (Dermience *et al.*, 2014). There is an increasing interest in green tea catechins as protective agents against free radicals and cardiovascular diseases (Imai and Nakachi, 1995). The major essential components of catechins present in tea leaves include epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), gallocatechin (GC) and catechin (C). In which the EGCG comprises over 50% of the content of tea catechins (Dufresne and Farnworth, 2000).

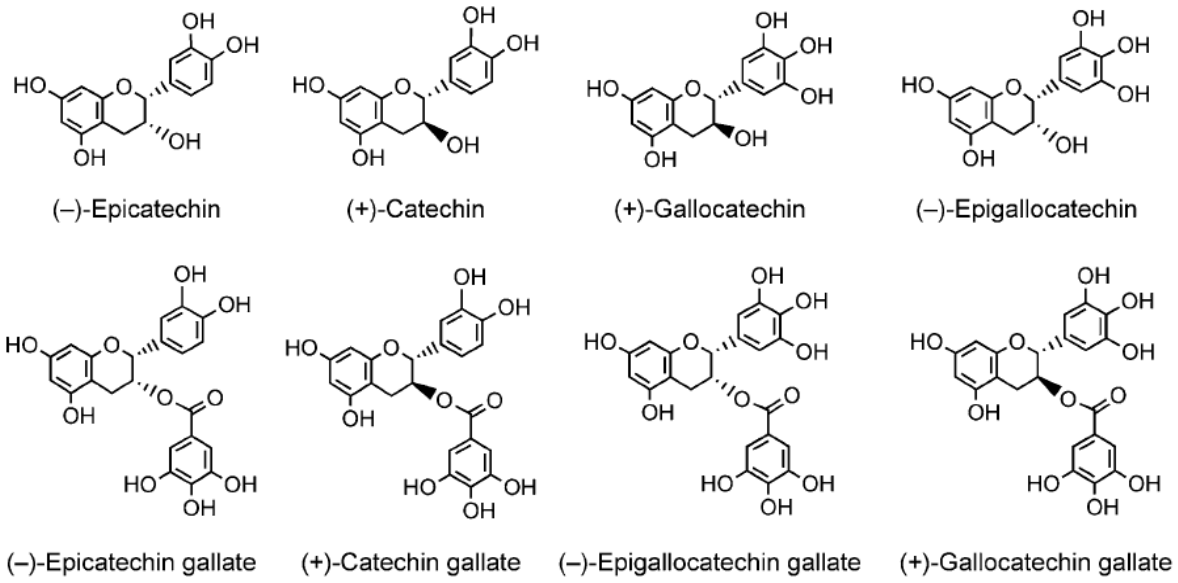

Figure 1.1 Eight major catechins found in tea (Yashin *et al.,* 2015).

EGCG is the major constituent of tea catechins, accounting for approximately 40% of the total polyphenols in green tea (Singh *et al.*, 2014; Stoner and Mukhtar, 1995). There are many components in tea, which directly influences the flavour, taste and colour qualities of tea beverages such as volatile components, tea polyphenols, which are thermally unstable and may degrade during thermal extractions (Singh *et al.*, 2014).

Jatropha zeyheri Sond*,* is a succulent perennial, monoecious, densely hairy herb producing stems up to 30 cm long that are usually erect but are sometimes prostrate (Arnold *et al.*, 2002). It belongs to the Euphorbiaceae family, which is a large family of flowering plants with about 300 genera and 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. Some are succulent and resemble cacti because of convergent evolution (Di Cesare *et al.*, 2003). Many members are important sources of food; others are useful for their waxes and oils and as a source of medicinal drugs; dangerous for their poisonous fruits, leaves, or sap; or attractive for

their colourful bracts (leaf like structures located just below flower clusters) or unusual forms. Although species of the family grow throughout the world, except in cold alpine or arctic regions, most of them are found in temperate and tropical regions. The family consists of annual and perennial herbs and woody shrubs or trees in tropical areas. *Jatropha zeyheri* is widely distributed throughout the eastern parts of South Africa and neighbouring countries, such as Botswana, Zimbabwe, Northern South Africa and Swaziland (Van Wyk, 2008).

Jatropha zeyheri is an indigenous plant known as “Sefapabadia” amongst Sepedi speaking people and also known as “Xidomeja” or “Mudomeja” among Xitsonga speaking people of South Africa. It is used to treat sexually transmitted and urinary tract infection by traditional healers (Van Wyk and Gericke, 2000). Van Wyk and Gericke (2000) reported that an infusion of the rhizome of *J. zeyheri* is used to treat menstrual pains, irregular periods, and is also believed to improve foetal development during pregnancy. *Jatropha zeyheri* leaves has been used before by different ethnic groups, for example in Zebediela (Khureng village). *Jatropha zeyheri* leaves are believed to have aphrodisiac properties. The Pedi speaking people use the extract from the soaked roots and leaves as a medicine to cure wounds, abnormal menstruation and foetus growth problem. The dried leaves are boiled and the extract is drunk with sugar as a tea beverage (Van Wyk and Gericke, 2000).

Most of these indigenous/herbal [rooibos (*Aspalathus linearis*), bush tea (*Athrixia phylicoides*), green tea (*Camellia sinensis*) and honeybush tea (*Cyclopia species*)] plants are still used by local rural communities as medicine, food and for making beverages. Previous studies on indigenous plant species focused mainly on their

medicinal potential (Rampedi, 2010). In view of the global and local beverages industry research or survey, indigenous plant species may have commercial development potential on beverage making. There is insufficient documented information on herbal plants for commercial beverage production (Aloys and Angeline, 2009).

Indigenous people in Limpopo Province of South Africa harvest dried leaves of *J. zeyheri* and utilize them as indigenous tea infusion. This is contrary to what is practiced in the tea industry, for old tea leaves are regarded as agricultural waste and believed to have lost important tea compounds due to sink-source nutrient re-allocation (Yasari *et al.*, 2009). Drying methods are known to have variable effects on antioxidant properties of tea leaves (Chan *et al.*, 2009). The need to appropriate hot-air drying duration with minimal effects on chemical composition of *J. zeyheri* tea infusion is important. Currently, there is no information regarding the *J. zeyheri* leaf chemical composition in South Africa. Therefore, the study investigated effect of time-based hot air drying method on tea chemical compounds of *J. zeyheri* (Kaya *et al.*, 2007).

1.2 Problem Statement

Drying methods are known to have variable effects on antioxidant properties of tea leaves. The need to appropriate time-based hot air drying duration with minimal effects on antioxidant properties of *J. zeyheri* tea is essential. Currently, there is no information regarding the *J. zeyheri* leaf chemical composition. Therefore, the researcher proposed to investigate effect of time-based hot air drying method on the tea phenolic compounds, mineral elements and proximate composition of the tea.

1.3 Rationale of the study

The communities around Zebediela (Khureng village) harvest *J. zeyheri* leaves when they are already dry and make tea. This is contrary to what is practiced in the tea industry, for old tea leaves are regarded as agricultural waste and believed to have lost important tea compounds due to sink-source nutrient re-allocation (Yasari *et al.*, 2009). There is need to quantify chemical composition of *J. zeyheri* tea leaves that are still green and leaves that are harvested being dry from *J. zeyheri* plant. The researcher seeks to investigate effect of time-based hot air drying method on chemical composition of *J. zeyheri* tea leaves under varying time regimes.

1.4 Aim

To investigate effect of time-based hot air drying method on chemical composition of *J. zeyheri* tea.

1.5 Objectives

1.5.1 To determine effect of time-based hot air drying method on total polyphenols, antioxidants and tannins of *J. zeyheri* leaves.

1.5.2 To determine effect of time-based hot air drying method on mineral elements of *J. zeyheri* leaves.

1.5.3 To determine effect of time-based hot air drying method on proximate composition of *J. zeyheri* leaves.

1.6 Format of mini-dissertation

The mini-dissertation was designed using the Senate-approved format of the University of Limpopo. Subsequent to the description and detailed outlining of the research

problem (Chapter 1), work done and the work not done on the research problem were reviewed (Chapter 2). Then, each of the three objectives would constitute a separate Chapter (Chapters 3-5). In the final chapter (Chapter 6), results from all chapters would be summarised and integrated to provide the significance of the results and recommendations with respect to future research and then culminated in an overall conclusion of the study. The Harvard referencing style, as approved by the University senate, was adopted in this mini-dissertation.

CHAPTER 2
LITERATURE REVIEW

2.1 Background of drying

The preservation of foods by drying is the time-based and common method used by the food processing industry (Mudau and Ngezimana, 2014). The main function of drying is to lower the water activity of the product. Consequently, to inhibit the growth of microorganisms and decrease chemical reactions in order to prolong the shelf-life and reducing the post-harvest decays of the product at room temperature (Praveen Kumar *et al.*, 2005). The choice of drying method depends on the harvesting time of a plant material, plant organ and the active ingredients to be preserved (Praveen Kumar *et al.*, 2005). Processes of drying can be categorized according to the application of water-removing method as (i) osmotic dehydration (ii) mechanical dewatering and (iii) thermal drying (Rahman and Saidur, 2016). Under osmotic dehydration processes, a solution is applied to remove water whereas in mechanical dewatering application of physical force is necessary (Baysal *et al.*, 2003).

In thermal drying process, a gaseous and void medium are utilized to remove water from the food product. Thermal drying can be subdivided into three categories (a) air drying (b) low air environment drying and (c) modified atmosphere drying (Van Wyk, 2015). There are factors that need to be considered before any type of drying process can be utilized. These include (i) the type of product to be dried (ii) expected characteristics of the finished product (iii) permissible temperature tolerance (iv) the product tolerance to heat (v) required pre-treatments (vi) capital and processing costs and (vii) environmental factors (Díaz-Maroto *et al.*, 2002; Kaya *et al.*, 2007).

2.1.1 Oven-drying

Oven-drying falls under the category of air-drying methods and under this process; the atmosphere is used as the drying medium and heat as different modes. The drying medium, hot air, is allowed to pass over the product that has been placed in open trays. The rate of drying under oven drying conditions depends on temperature, humidity, air velocity and distribution pattern, air exchange, product geometry and properties and thickness (Porter and Murray, 2001). Generally, the hotter the air temperature applied, the faster the rate at which drying process occurs. Similarly, the higher the air velocity the higher the drying rate; the lower the air humidity, the higher the drying rate. Relative humidity (measure of dryness) found to be lower when the temperature is raised (Ratti, 2001).

The advantage of oven-drying method is that all the trays get equal heat and therefore the drying is uniform. It is also faster than sun-drying or using a food dryer. Drying also produces concentrated form of food and drying inhibits microbial growth autolytic enzymes (Shadung *et al.*, 2012a). After the drying period, most nutrients are retained (Parrott, 1994). It has been widely used to inactivate enzymes in green tea processing in China. However, this method induces unstable quality in green tea owing to the uncontrolled temperature during processing (Cañumir *et al.*, 2002).

2.1.2 Sun-drying

Under sun-drying process, foods are directly exposed to the sun by placing them on the land or left hanging in the air. The main disadvantages of this type of drying are (i) contamination from the environment (ii) product losses and contaminations by insects and birds (iii) floor space requirements (iv) inability of users to control temperature

fluctuations and (v) bad odour. In general the rate of drying is low, with increasing risks of spoilage during the drying process (Mudau and Ngezimana, 2014). When the climate is not particularly suitable for air drying or better quality is desired, mechanical air drying is mainly used. However, sun-drying is the cheapest method of drying foods. It is used to preserve most grains, vegetables, fruits and other agricultural products in developing countries (Latapi and Barrett, 2006).

2.1.3 Solar-drying

Solar-drying is the extension of sun drying and utilizes radiation energy derived from the sun. it is a process that does pollute and utilizes renewable energy. The main disadvantages of solar drying are (i) the rate of drying can be easily controlled (ii) contamination by microorganisms and insect infestation (iii) the need for large areas of space and (iv) high labour inputs (Yaldýz and Ertekýn, 2001).

2.1.4 Freeze-drying

Freeze-drying fall under the low air environment drying methods as explained in the above paragraph. Under freeze drying, a frozen food material is subjected to a pressure below 610 Pa at 0 ℃, heated to cause ice sublimation to vapour (Rahman and Saidur, 2016). It is used for high quality dried products that contain heat sensitive components such as vitamins, antibiotics, and microbial culture (Martínez-Las Heras *et al.*, 2014). The procedure prolongs the shelf-life longevity by preventing microbial growth and retarding lipid oxidation. The absence of air and low temperature prevents deterioration due to oxidation or chemical modification of the product. It also gives very porous products that results in high rehydration rates (Dorta *et al.*, 2012). Freeze-drying is an excellent recommended drying method for quality preservation of active ingredients

intended for pharmaceutical uses. However, its energy consumption is excessive when compared with those in other drying methods (Ratti, 2001).

2.1.5 Microwave-drying

Microwave-drying is a relatively new alternative drying method to the conventional drying. It is rapid, more uniform, energy efficient, space utilization, prevents food decomposition and appears to have a high potential for the agricultural products processing (Vadivambal and Jayas, 2007). Microwaves have a key advantage of heating over conventional heating by microwaves energy. The great interest in this technology is due to the high capacity of penetration of these waves, that heat not only on the surface but also inside the food. This speed up the drying process and can improve the quality of the final product (Contreras *et al.*, 2008). It is widely used in the food industry for its reduced processing time and costs, enhancing product uniformity and yields, improving unique micro-structure and protecting food from surface browning and crusting (Acierno *et al.*, 2004). It was found that the quality of tea was improved by using the microwave technology for food product enzyme inactivation and dehydration (Gulati *et al.*, 2003).

2.2 Chemical composition of tea

The chemical composition of tea is the most important factor that determines the price of the tea for the market (du Toit *et al.*, 2001). Tea quality is a complex that includes major constituents of tea, which are: polyphenols, catechins, caffeine, amino acids, carbohydrates, protein, chlorophyll, volatile compound, fluoride, minerals and other undefined compounds (Cabrera *et al.*, 2003). Flavanol is one of the important polyphenols in the tea in which catechins are predominant. Tea quality index [(EGCG +

ECG)/EGC] has been found to be directly related to the sensory properties of green tea (Chen *et al.*, 2003). It has been reported that amino acids, known as thiamine is the most abundant contained in the tea (Hu *et al.*, 2001).

2.3 Health benefits of tea

Polyphenols in green tea act as antioxidant and may actually inhibit the growth of existing cancer cells (Ho and McKay, 2000). EGCG, is one of the constituents of polyphenols which are antimutagenic and anti-infammatory by intercepting carcinogenic agents and by reducing oxidant species before they damage the DNA, not only inhibits an enzymes required for cancer cell growth, but also kills cancer cells with no effect on healthy cells (Erickson, 2003).

Many studies have demonstrated that the major polyphenols that are found in teas are antimutagenic, antidiabetic, antioxidant, antibacterial, anti-inflamatory, antitumor, hypocholesterolemic, and cancer prevention (Chantre and Lairon, 2002). There is little information on *J. zeyheri* leaf chemical composition to date as a tea infusion (Mudau *et al.*, 2006).

CHAPTER 3
INFLUENCE OF TIME-BASED HOT AIR DRYING METHOD ON TOTAL POLYPHENOLS, TOTAL ANTIOXIDANTS AND TANNINS OF *JATROPHA ZEYHERI* LEAVES

3.1 Introduction

Teas are known to be rich in various antioxidant polyphenols such as catechins, theaflavins, and thearubigins, and other nutrients including amino acids and bioactive carbohydrates (Dias *et al.*, 2013; Higdon and Frei, 2003). Tea polyphenols are well known for their antioxidant properties with catechins being the most abundant polyphenol in fresh leaves of tea, whereas, theaflavins are orange-red compounds responsible for the astringent taste and coppery colour of black tea. Theaflavins and thearubigins are sometimes called tannins and responsible for the darker colour of black tea and more heavily-oxidized oolong teas (Łuczaj and Skrzydlewska, 2005). High tannin containing teas are referred as tannic. Tannins are water-soluble polyphenols that are present in many plant foods, especially black tea, which tend to have a bitter flavour and astringent properties. The antioxidant and antimicrobial activities of tannin are well documented in literature (Higdon and Frei, 2003).

There is a need to appropriate time-based hot air drying duration with minimal effects on total phenolic content, total antioxidant capacity and tannin content of *J. zeyheri* tea. Currently, there is no information regarding the *J. zeyheri* leaf chemical composition in South Africa. The objective of this study was to investigate effect of time-based hot air drying method on tea phenolic compounds.

3.2 Materials and methods

3.2.1 Experimental site and plant collection

Fully developed green leaves were sampled randomly at Zebediela (Khureng village), (24°33’53”S, 29°23’4”E) in Limpopo Province, South Africa. Khureng village is characterised by semi-arid climate, with rainfall of less than 400 mm per annum. The area is predominately clay with bushveld vegetation. Samples were transported to Limpopo Agro-Food Technology Station (LATS) laboratory for analysis.

3.2.2 Experiment design, treatments and procedures

Four treatments, namely; 0, 24, 48, and 72 hours, were arranged in completely randomised design (CRD), replicated five times. Total phenolic, total antioxidant capacity and tannin content were determined using Folin-Ciocalteu, phosphate-molybdate and Vanillin-HCl method, respectively (Waterman and Mole, 1994).

3.2.3 Data collection

Amount of 1-gram dry plant sample plus 25 mL of 100% methanol were incubated at room temperature (25 ℃) for 60 minutes with vortexing every 15 minutes. After separation by centrifuge at 2000 rpm for 10 minutes, the supernatant were decanted into a 50 mL conical tube. The extraction procedure was repeated by adding 15 mL of methanol to the residues which were vortex every 10 minutes for 30 minutes. After extraction the supernatant were combined into one 50 mL conical tube and total extraction volume was adjusted to 40 mL with cold methanol. The dilution of the extract was done by mixing 1 mL of extract and 4 mL of methanol (Hlahla *et al.*, 2010).

Determination of polyphenols: Total phenolic content (TPC) were determined according to the method of Folin-Ciocalteau (Wang *et al.*, 2011). Briefly, tea infusion (0.02 mL) were diluted with 1.58 mL of deionized water, followed by 0.1 mL of Folin-Ciocalteau reagent. A volume of 0.3 mL of 7.5% sodium carbonate were then added. Mixture were allowed to stand at room temperature for 30 minutes before measuring the absorbance at 765 nm. The phenolic content were compared to a gallic acid standard curve. The total phenolic content of the samples were expressed as milligrams garlic acid equivalent (GAE) per 100 mg of dry material (Hlahla *et al.*, 2010).

Determination of antioxidant activity: The reaction were based on reduction of Mo (VI) to Mo (V) by extract and subsequence formation of green phosphate- Mo (V) complex at acid pH. A volume of 0.1 mL of plant extract were added into 1 mL of reagent solution (0.6M H_2SO_4, 28mM Na_2PO_4, 4mM ammonium molybdate) pre-heated to 95 ℃. The reaction mixture were incubated at 95 ℃ for 90 minutes, thereafter; absorbance was read at 695 nm. Garlic acid solutions in the range of 0 to 2.5 mg/mL were used as standard and results expressed as mg of garlic acid equivalent (GAE) per 100 mg of dry material (Mittal *et al.*, 2014).

Determination of tannins: The reaction contained 1 mL of methanol extract, 5 mL vanillin reagent (4% HCL in methanol and 0.5 mL vanillin in methanol) previously placed at 30 ℃. Blank solution contained 4% HCL in methanol replacing vanillin reagent. The reactants were maintained at 30 ℃ and absorbance read at 500 nm after 20 minutes. Catechin were used as appropriate standard and results expressed as mg of catechin equivalent (CE) per 100 mg of dry material (Hlahla *et al.*, 2010).

3.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA), and data analyses were done using Statistical Analysis System (SAS) General Linear Model procedure. Least Significant Difference (LSD) test were used to identify differences among the means at (P<0.05) (Statistical Analysis System (SAS) Institute Inc. New York, 2003).

3.3 Results

Table 3.1 Effect of time-based hot air drying on total phenolic, total antioxidant capacity and tannin content of *Jatropha zeyheri* tea.

Time (Hours)	Total phenolic content (mg GAE/100 mg DM[x])	Total antioxidant capacity (mg GAE/100 mg DM[x])	Tannin content (mg CE/100 mg DM[x])
0	3.95b	0.63b	4.61c
24	3.05c	0.97a	11.52a
48	3.14c	1.02a	11.60a
72	4.96a	1.15a	8.57b

*Mean values with different letter within a column are significantly different (P<0.05).

[x]DM = Dry material.

Table 3.2 Comparison of total phenolic, antioxidants capacity and tannin content in teas.

Tea type	TPC (mg GAE/100 mg DM[x])	Total antioxidants (mg GAE/100 mg DM[x])	Tannins content (mg CE/100 mg DM[x])	References
J. zeyheri	3.1	1.02	11.6	This study
Bush tea	3.4	ND	0.90	(Hlahla *et al.*, 2010).
Green tea	11.40	8.38	ND	(Chan *et al.,* 2009).
Black tea	8.40	5.23	ND	(Chan *et al.,* 2009).
Bush tea	ND	ND	2.81	(Negukhula, 2010).
Green tea	3.16	ND	ND	(Bizuayehu *et al.*, 2016).
Bush tea	0.56	ND	0.03	(Mudau and Ngezimana, 2014).

ND = not determined (i.e. values were not available determined in standards used for analysis of *J. zeyheri* tea in order to compare values with similar standards).

Total phenolic, total antioxidant capacity and tannin content were significantly increased by 63%, 82%, and 152%, respectively when subjected to different drying period (Table 3.1). Relative to bush and black tea, *J. zeyheri* had the highest tannin content of 11.6 mg CE/100 mg DM compared to 0.90 mg CE/100 mg DM (Hlahla *et al.*, 2010), 2.81 mg CE/100 mg DM (Negukhula, 2010) and 0.03 mg CE/100 mg DM (Mudau and Ngezimana, 2014), respectively (Table 3.2). The study showed that *J. zeyheri* had antioxidant capacity of 1.02 mg GAE/100 mg DM compared to the 8.38 mg GAE/100 mg DM and 5.23 mg GAE/100 mg DM of green and black tea, respectively (Chan *et al.,* 2009) (Table 3.2). The total phenolic content of 3.1 mg GAE/100 mg DM of *J. zeyheri* was similar to the total phenolic content of bush and green tea (Bizuayehu *et al.*, 2016; Hlahla *et al.*, 2010) (Table 3.2). The green tea is still the highest with the total phenolic

content value of 11.40 mg GAE/100 mg DM, followed by black tea with 8.40 mg GAE/100 mg DM (Chan *et al.,* 2009) (Table 3.2). Whereas, bush tea is the lowest with the total phenolic content value of 0.56 mg GAE/100 mg DM (Mudau and Ngezimana, 2014) (Table 3.2).

3.4 Discussion

The increased total phenolic content in *J. zeyheri* due to drying process makes it a potential tea infusion like bush, green and black tea. Phenolic compounds are the most active antioxidant derivatives in plants (Bors *et al.*, 2001). They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates (Maillard *et al.*, 1996). This study demonstrated that there was a close relationship between total phenolic content and antioxidant capacity. These findings were in line with what has been reported in the study by (Roy *et al.*, 2007) on the total phenolic content of different foods.

The increased antioxidant activity observed was previously reported by Tomaino *et al.* (2005), where it was concluded that drying processes could improve the properties of naturally occurring antioxidants. The change in antioxidant activity has been attributed to the fact that many antioxidant phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers (Niwa and Miyachi, 1986). If this bonding is not strong, drying treatment could liberate and activate low-molecular-weighted natural antioxidants in plants (Jeong *et al.*, 2004; Zhou and Han, 2006). Antioxidants also known as oxidation inhibitors protect food materials against autoxidation process that is initiated by free radicals which initiate further oxidation reactions (Diplock, 1994; Fang *et al.*, 2002).

Free radicals attack nucleic acids, changing their structure and genetic code of cells resulting in them being mutagenic, teratogenic and carcinogenic (Aruoma *et al.*, 1996). They also induce changes in polypeptide chains of proteins, resulting in cross-linking and also form insoluble polymers responsible for aging in humans. Addition of antioxidants in human diets is important for the suppression of free radicals that result in the prevention of cancer, aging and cardiovascular diseases that are common in rural communities of South Africa (Aruoma, 1994; Aruoma *et al.*, 1996).

In conclusion, the rural communities consuming *J. zeyheri* infusion could benefit health wise from total phenolic content, antioxidant capacity and tannin content if they could increase the processing period as recommended in this study.

CHAPTER 4
INFLUENCE OF TIME-BASED HOT AIR DRYING METHOD ON MINERAL ELEMENTS COMPOSITION OF *JATROPHA ZEYHERI* LEAVES

4.1 Introduction

Tea is second to water as the most consumed drink in the world. Black tea, which is mostly produced in India, Sri Lanka, Kenya and many other countries, is the most popular type of tea (Tiwari *et al.*, 2005). Different types of green teas are produced in China and Japan (Dermience *et al.*, 2014). China also produces black teas and several other types such as oolong tea, pu-erh tea and kombucha tea. Due to high anti-oxidative power, tea has been reported to reduce alzheimer (McKenzie *et al.*, 2010; Zuo *et al.*, 2002), blood pressure (Yokogoshi *et al.*, 1995) and prevent obesity (Kim *et al.*, 2009). Tea has been reported in other studies to alleviate minor maladies such as headaches and pains (Dermience *et al.*, 2014). The presence of various minerals in tea plant leaves, represent an important form of ingesting bio-essential elements in the tea industry.

Mineral elements are found in nearly all foods, but their concentration in plant sources vary substantially depending on the local soil conditions (Tsuji *et al.,* 2016). Mineral elements are categorized into macro elements and micro elements based on their concentration in the body. Macro elements are the main cellular and structural building materials and also play vital role in osmotic pressure, acid/base regulation (Kohlmeier, 2015; McKenzie *et al.*, 2010). Micro elements play a vital role in the metabolic functions and are major components of vitamins, hormones, enzymes (Berto *et al.*, 2015; Pereira and Dantas, 2016). Unlike organic food components, minerals cannot be destroyed by heat and oxidation processes (Mosele *et al.*, 2011; Watzke, 1998). The absorption of

minerals depends on their chemical form, the presence of ligands and the redox activity of the food (Ersoy and Özeren, 2009).

Drying is a preserving method that plays an important role in tea processing. Drying may also influence nutritional value of many tea products either through chemical modifications or direct loss of minerals (Crowley and O'Mahony, 2016; Jangam *et al.*, 2016). The objective of the study was to determine effects of time based hot air drying methods on mineral elements composition of *J. zeyheri* leaves.

4.2 Materials and methods

4.2.1 Experimental site and plant collection

Experimental site and plant collection were as explained under Chapter 3.

4.2.2 Experimental design, treatments and procedures

Four treatments, namely; 0, 24, 48, and 72 hours, were arranged in completely randomised design (CRD), replicated five times. About 0.1 g dried and ground sample was put into 50 mL conical tube and 40 mL 5% of HNO_3 was added. Samples were incubated at 95 ℃ for 60 minutes, cooled and filtered using Whatman filter paper 185 mm diameter. Mineral elements concentrations were determined using multi-type Inductively Coupled Plasma Atomic Emission Spectrometer (ICPE-9000 AES, Shimadzu, Japan) (Wheal and Palmer, 2010).

4.2.3 Data collection

The analysis of mineral elements such as Mg, Na, K, P, S and Ca and trace elements Al, Co, Mn, Ni, Si and Zn were determined using multi-type Inductively Coupled Plasma

Atomic Emission Spectrometer (ICPE-9000 AES, Shimadzu, Japan) (Huang and Schulte, 1985). For analysis of macro and trace elements, the instrument was calibrated using standard solutions of multi-elements of trace elements (concentration range 0.1 to 1 ppm) and macro (concentration range 10 to 50ppm). The concentration of elements in the samples was determined from the linear calibration of the standard and expressed as parts per million (ppm).

4.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA), and data analyses were done using SAS General Linear Model procedure. Least Significant Difference (LSD) test was used to identify differences among the means at ($P<0.05$) (Statistical Analysis System (SAS) Institute Inc. New York, 2003).

4.3 Results

Table 4.1 Effect of time-based hot air drying on the mineral elemental composition of *Jatropha zeyheri* tea.

Time (Hours)	Mineral elements units (mg/L)											
	Mg	Na	K	P	Ca	S	Al	Co	Mn	Ni	Si	Zn
0	8.95b	1.06a	18.96b	1.6b	33.7a	1.3bc	0.5b	0.0b	0.45b	0.12a	0.27b	0.09b
24	15.5a	0.00b	23.1ab	1.6b	15.8c	0.7c	0.5b	0.2b	0.00b	0.00b	0.00b	0.00c
48	15.3a	0.00b	22.92b	4.4b	17.1bc	3.1b	1.0b	0.1b	0.26b	0.00b	0.13b	0.03bc
72	15.2a	0.00b	27.72a	8.4a	22.1b	5.9a	2.1a	0.8a	1.91a	0.00b	0.74a	0.20a

*Mean values with different letter within a column are significantly different (P<0.05).

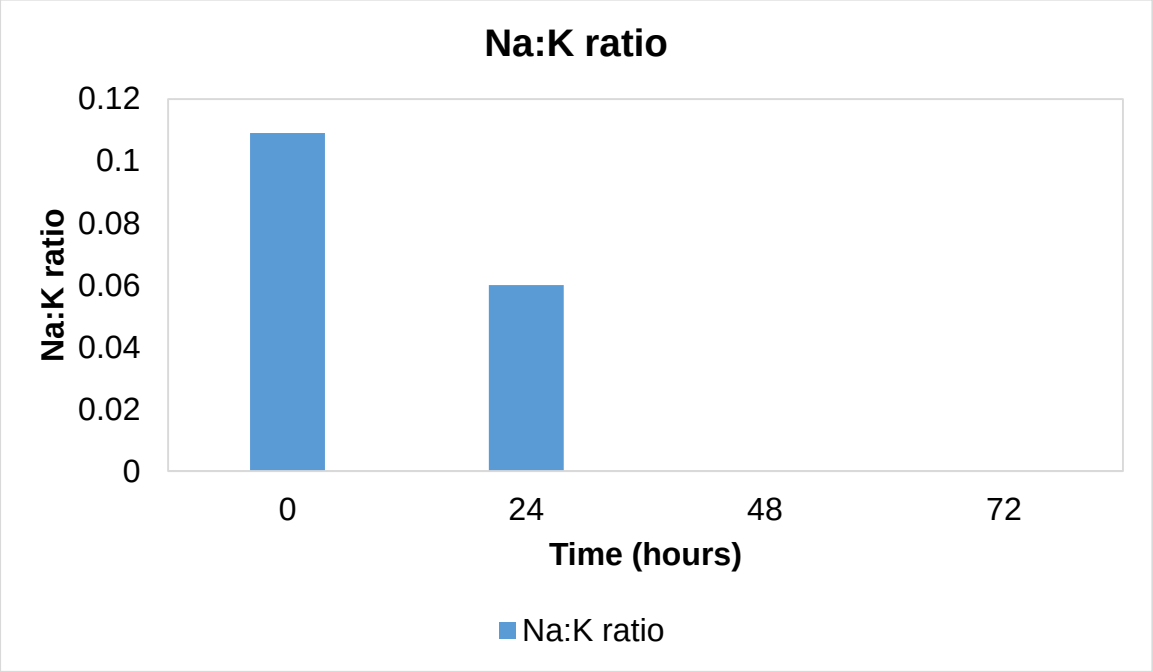

Figure 4.1 *Jatropha zeyheri* Na:K ratio based on drying period.

Table 4.2 Comparison of macro elements in teas.

Tea type	Macro elements (mg/L)					Reference
	Mg	Na	K	P	Ca	
J. zeyheri	15.5	1.06	27.72	8.37	33.7	This study
Black tea	4.42	0.47	143.89	4.61	0.0	(Özcan *et al.*, 2008)
Green tea	6.54	1.23	141.13	3.3	0.0	(Özcan *et al.*, 2008)

Table 4.3 Comparison of micro elements in teas.

Tea type	Micro elements (mg/L)					Reference
	Al	Co	Mn	Ni	Zn	
J. zeyheri	2.10	0.78	1.91	0.12	0.20	This study
Black tea	6.02	0.00	1.56	0.04	0.04	(Özcan *et al.*, 2008)
Green tea	1.20	0.01	1.75	0.05	0.16	(Özcan *et al.*, 2008)

The study demonstrated that drying significantly increased Mg, K, P, S, Al, Co, Mn, Si and Zn content by 73%, 46%, 425%, 743%, 320%, 700%, 635%, 469% and 567%, respectively and decreased Na, Ca and Ni quantities by 100%, 113% and 100%, respectively (Table 4.1). Sodium-potassium ratios were very low across drying periods (Figure 4.1).

Relative to black and green tea, *J. zeheri* had high content of Mg, P and Ca (Table 4.2). The study showed that magnesium content of *J. zeyheri* were 15.5 mg/L higher than

4.42 mg/L and 6.54 mg/L of black and green tea, respectively (Özcan *et al.*, 2008) (Table 4.2). Phosphorus content of *J. zeyheri* were 8.37 mg/L higher compared to 4.61 mg/L and 3.3 mg/L of black and green tea, respectively (Table 4.2). Calcium content of *J. zeyheri* were 33.7 mg/L higher compared to 0.00 mg/L and 0.00 mg/L of black and green tea, respectively (Table 4.2). Study recorded the highest calcium content when compared with the findings by Özcan *et al.* (2008), that observed zero values for calcium content in black and green teas. High calcium in diets is known to restrict lead uptake (Sikorski, 2006) and calcium is also known to regulate endo- and exo-enzymes and blood pressure (Hofmeyr *et al.*, 2015; McIntyre *et al.*, 2016; Shehata, 2016).

4.4 Discussion

Macro elements are elements, which the body need in quantities greater than 100 milligram per day. These elements make up more than 99% of the mass of human bodies. The human body need macro elements such as; calcium, magnesium phosphorus, sulphur, potassium and sodium in order to function properly (Liu *et al.*, 2008). The study showed Sodium-potassium ratios were very low across drying periods (Figure 4.1). This is good from nutrition point of view. Zhou and Han (2006), in their study showed that the intake of sodium chloride and diets with high Na:K ratio have been related to the incidence of hypertension (Hofmeyr *et al.*, 2015).

Trace elements are vital micro elements that are found in minute quantities, within the human body. These are found in such small quantities, that they could be detected by spectrographic methods or by using radioactive elements (Dermience *et al.*, 2014). Relative to black and green tea, *J. zeyheri* had high content of Co, Mn, Ni and Zn (Table 4.3). The study showed that cobalt content of *J. zeyheri* were 0.78 mg/L higher than

0.00 mg/L and 0.01 mg/L of black and green tea, respectively (Özcan *et al.*, 2008) (Table 4.3). Manganese content of *J. zeyheri* were 1.91 mg/L higher compared to 1.56 mg\L and 1.75 mg/L of black and green tea, respectively (Table 4.3). Nickel content of *J. zeyheri* were 0.12 mg/L higher compared to 0.04 mg/L and 0.05 mg/L of black and green tea, respectively (Table 4.3). Zinc content of *J. zeyheri* were 0.20 mg/L higher than 0.04 mg/L and 0.16 mg/L of black and green tea, respectively (Özcan *et al.*, 2008) (Table 4.3). In conclusion, drying period increased Mg, K, P, S, Al, Co, Mn, Si and Zn content and inversely decreased Na, Ca, and Ni quantities.

CHAPTER 5
INFLUENCE OF TIME-BASED HOT AIR DRYING METHOD ON PROXIMATE COMPOSITION OF *JATROPHA ZEYHERI* LEAVES

5.1 Introduction

The preservation of foods by drying is the time-honoured and most common method used by humans and the food processing industry (Mudau and Ngezimana, 2014). Drying processes can be broadly classified, based on the water-removing method applied as a (i) thermal drying (ii) osmotic dehydration and (iii) mechanical dewatering (Rahman and Saidur, 2016). Drying reduces the water activity, thus preserving foods by avoiding microbial growth and deteriorative chemical reactions. It is also important to maximize microorganism and enzyme inactivation for preventing spoilage and enhancing safety and reduced the components responsible for the deterioration of the dried foods (Díaz-Maroto *et al.*, 2002).

Drying process has detrimental effects that are desirable or undesirable, depending on the purpose of the drying process (Ratti, 2001). *Jatropha zeyheri* food composition data is extremely needed for the development of food composition tables, balanced nutrients, quantification of nutritional adequacy of diets of individuals and populations and food industry introduction (Figuerola *et al.*, 2005). Food proximate composition includes moisture content, fats, carbohydrates, crude fibre, protein and ash. Moisture content is important as an agent in chemical reactions and is a factor in the perishability and preservation of foods (Mosele *et al.*, 2011). Therefore, the objective of the study was to determine effects of time-based hot air drying method on proximate composition of *J. zeyheri* leaves.

5.2 Materials and methods

5.2.1 Experimental site and plant collection

Experimental site and plant collection were as explained under Chapter 3.

5.2.2 Experiment design, treatments and procedures.

Four treatments, namely; 0, 24, 48, and 72 hours, were arranged in completely randomised design (CRD), replicated five times. After drying, samples were ground to pass through 1 mm sieve using grinder (MF 10 basic micro fine grinder drive, IKA-Werke). Powdered samples were used as is for proximate analysis.

5.2.3 Data collection

The proximate parameters: Carbohydrates were determined by calculation [i.e. 100-(Protein + Fibre + Fat + Ash + Moisture)] (Eyeson and Ankrah, 1975). Ash were determined using (Muffle furnace, Gravimetric Method at 550 ℃) and protein by dumas methods using (Leco Truspec N). Total fats were determined using (Leco TFE 2000). Dietary fiber determined using (Fiber tec). Moisture content determined using (Max 50) and Energy determined using (Leco AC 600) (Horwitz, 2003; Hussain *et al.*, 2011).

5.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) and data analyses were done using Statistical Analysis System (SAS) General Linear Model procedure. Least Significant Difference (LSD) test were used to identify differences among the means at ($P<0.05$) (Statistical Analysis System (SAS) Institute Inc. New York, 2003).

5.3 Results

Table 5.1 Effect of time-based hot air drying method on the proximate chemical composition of *Jatropha zeyheri* tea.

Time (hours)	Energy Kj/100g	Protein	Carbohydrate	Moisture	Ash	TF[x]	TDF[y]
		g/100g					
0	773.659	18.751	20.690	7.946	10.3	2.788	39.578
24	764.232	18.884	18.34	8.948	10.3	3.552	39.998
48	770.212	18.472	21.894	7.802	9.73	2.270	39.832
72	786.532	18.896	21.838	7.088	10.7	2.542	38.910

*Mean values with different letter within a column are significantly different (P<0.05).

[x]TF = Total Fat

[y]TDF = Total Dietary Fibre

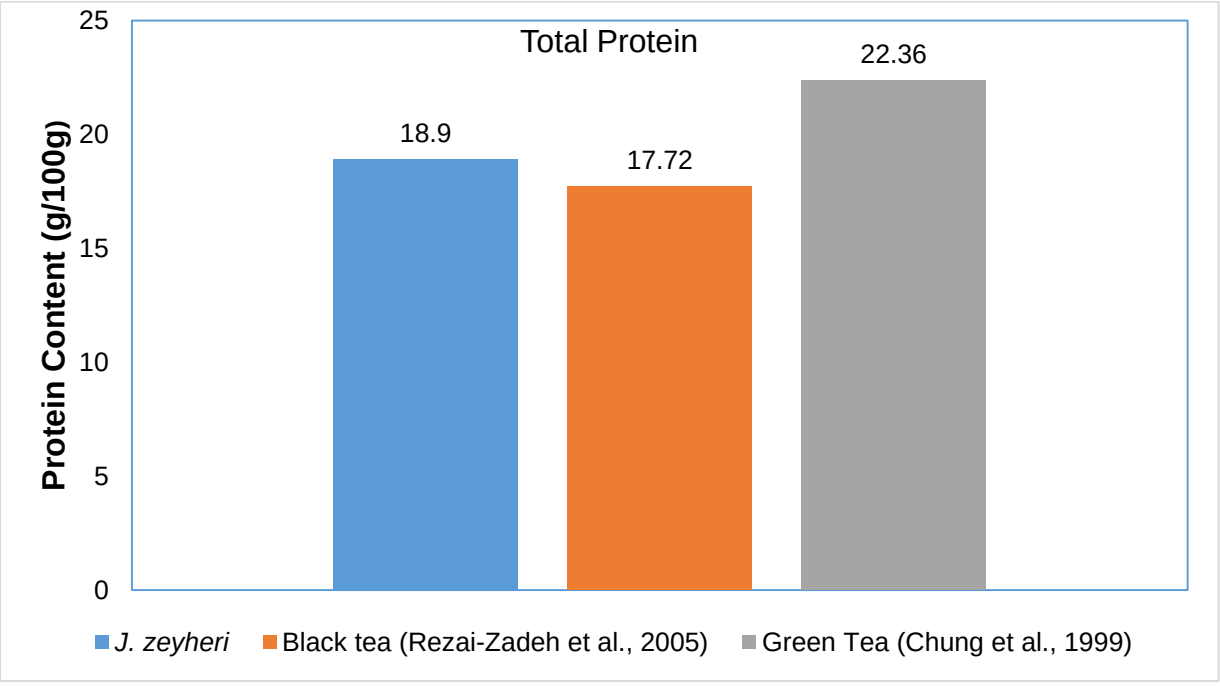

Figure 5.1 Total protein of *Jatropha zeyheri,* black and green tea.

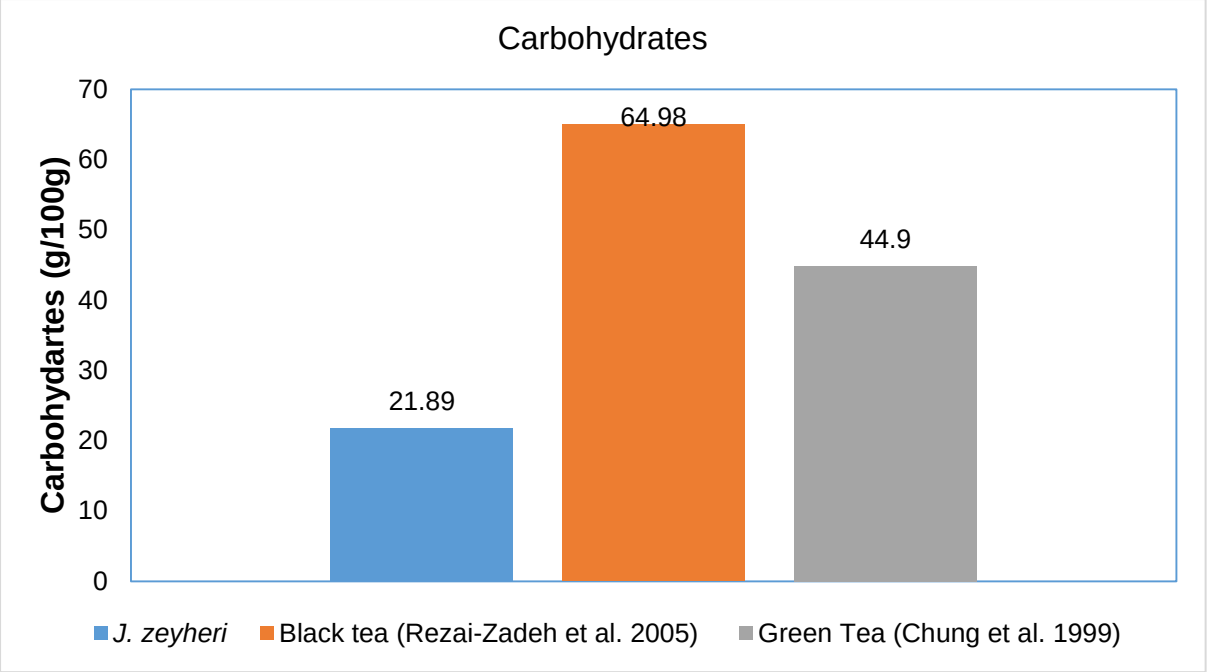

Figure 5.2 Carbohydrates of *Jatropha zeyheri*, black and green tea.

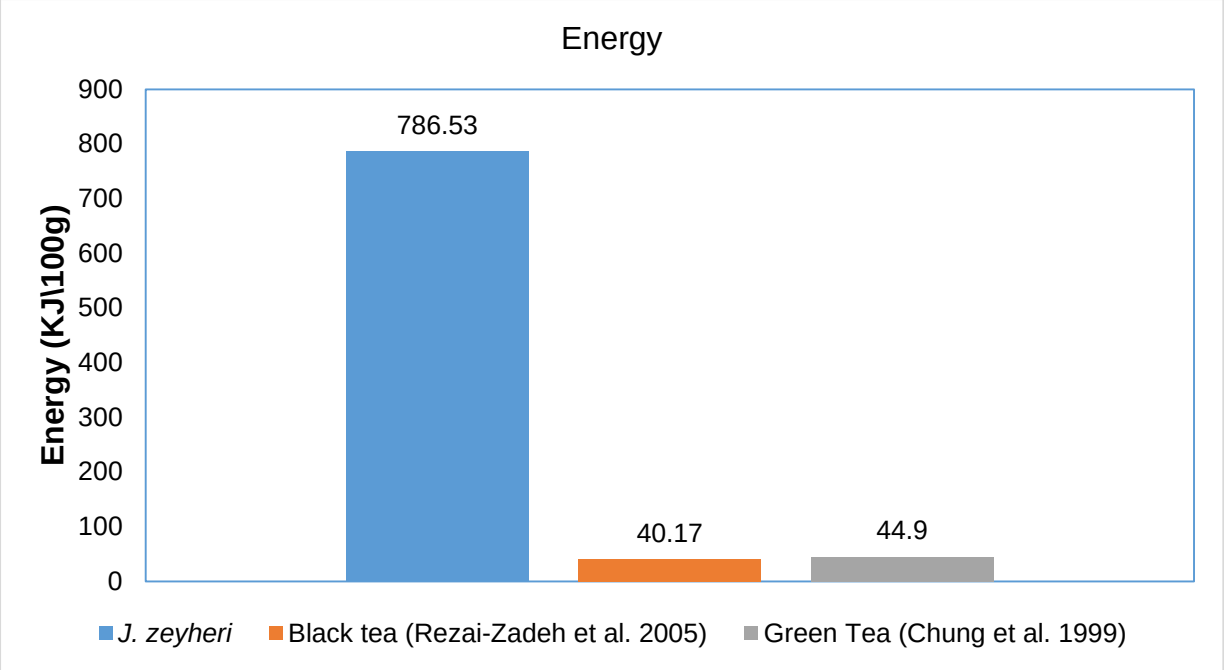

Figure 5.3 Energy content of *Jatropha zeyheri*, black and green tea.

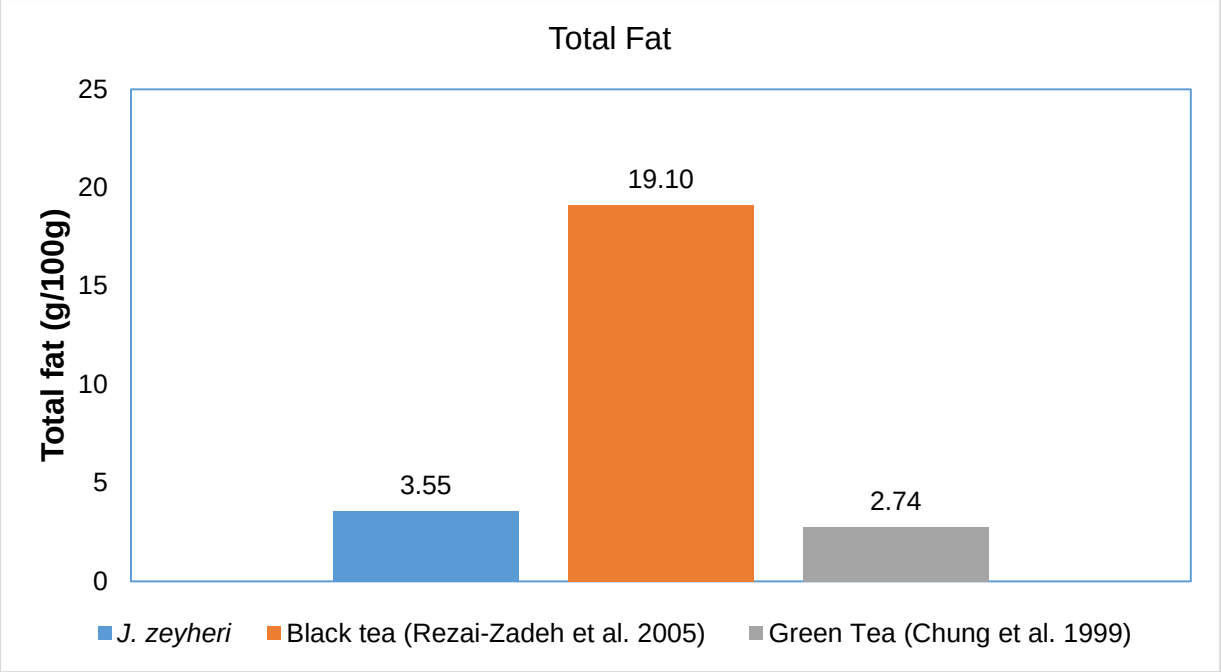

Figure 5.4 Total fat of *Jatropha zeyheri,* black and green tea.

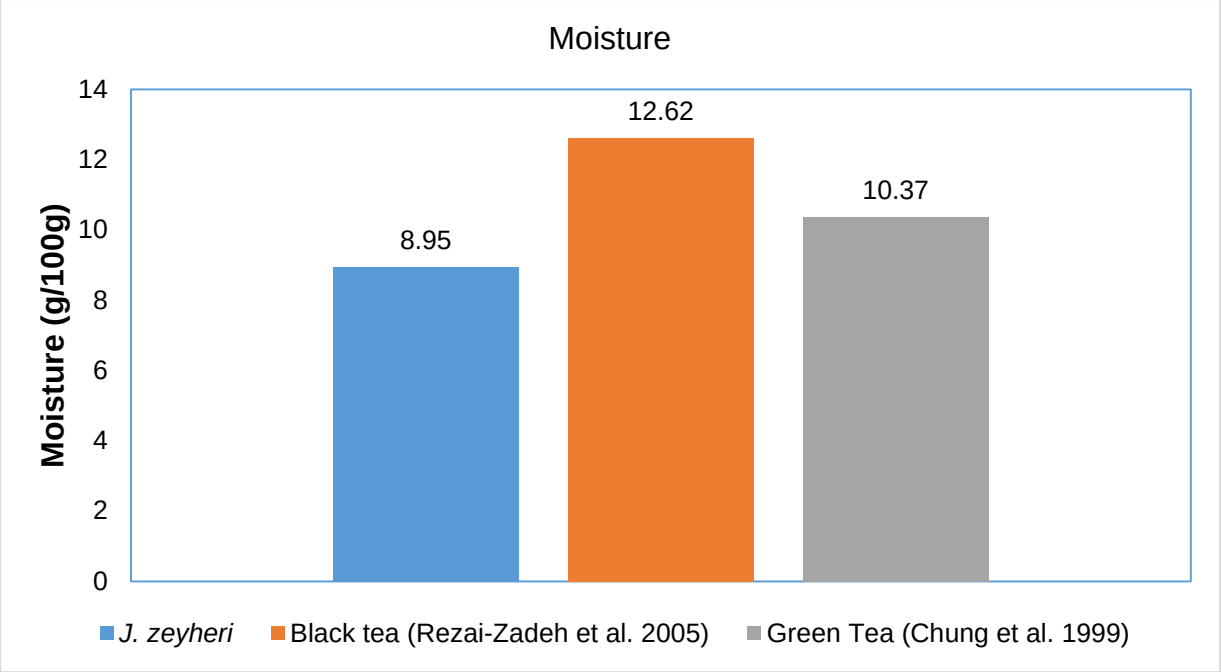

Figure 5.5 Moisture content of *Jatropha zeyheri*, black and green tea.

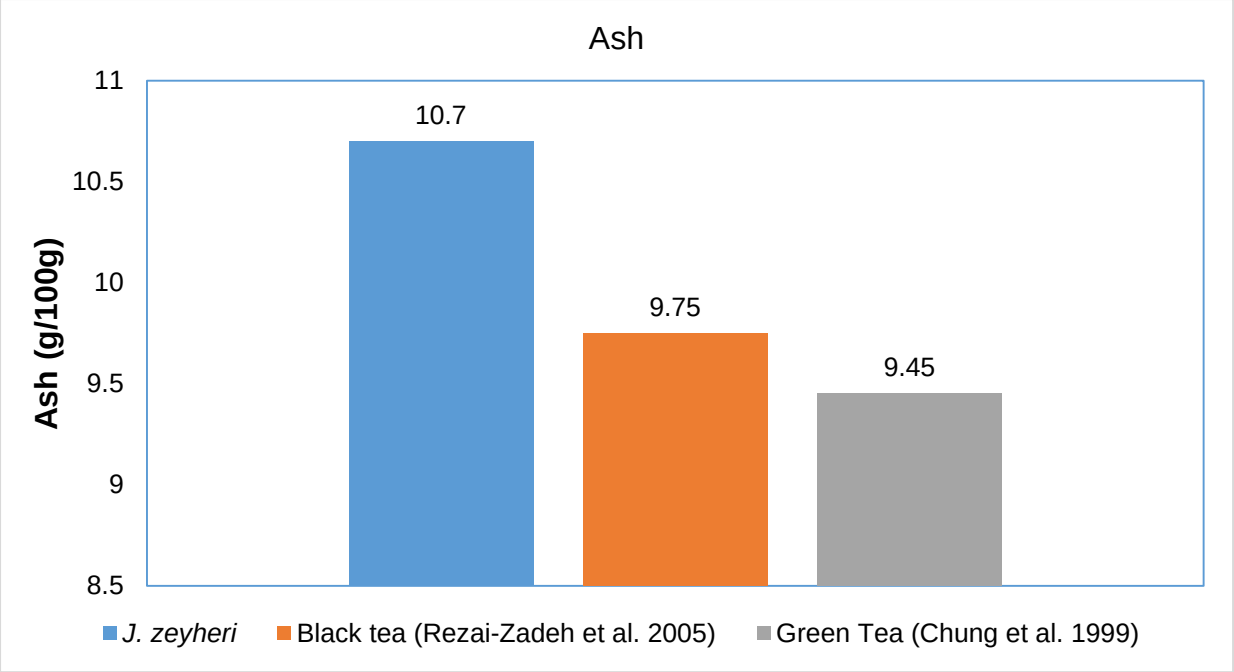

Figure 5.6 Ash content of *Jatropha zeyheri,* black and green tea.

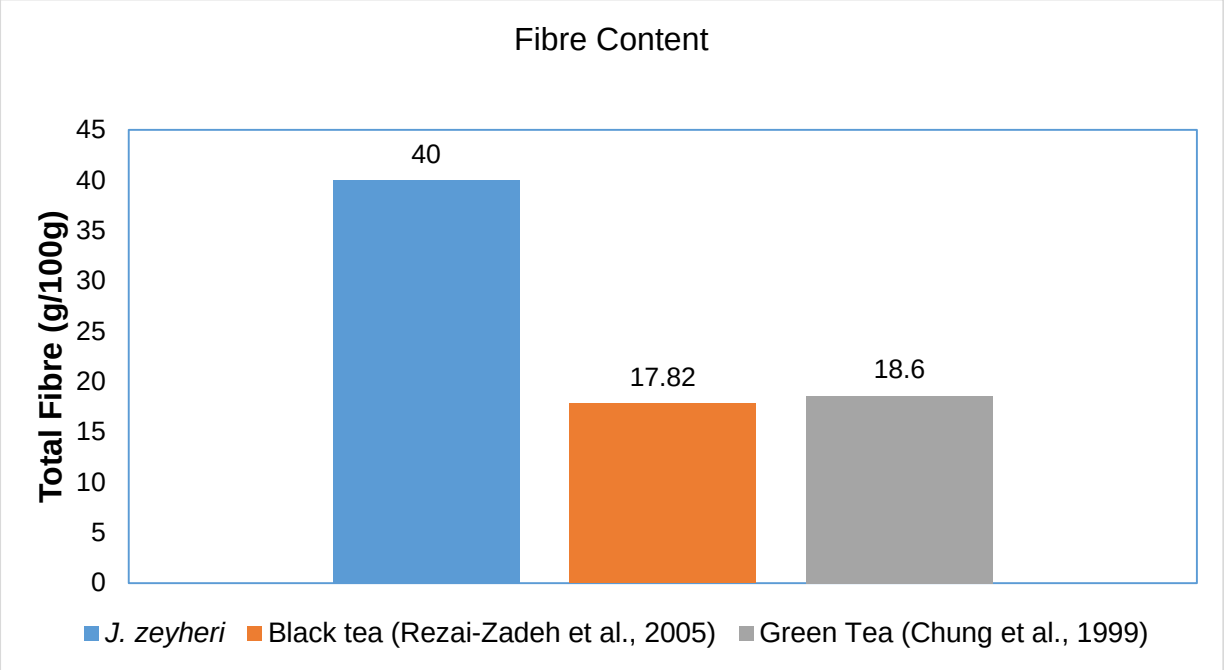

Figure 5.7 Fiber content of *Jatropha zeyheri,* black and green tea.

Drying period at (P<0.05) did not significantly influence energy, protein, carbohydrates, ash and fibre content (Table 5.1). Moisture and fat were significantly increased by drying period (Appendix 5.4 and Appendix 5.6). In this study, it was observed that the energy content of *J. zeyheri* were 786.53 Kj/100g higher than 40.17 Kj/100g and 44.9 Kj/100g values of black and green teas, respectively (Figure 5.3). The protein values of *J. zeyheri* were 18.9 g/100g compared to 17.72 g/100g and 22.36 g/100g protein of black tea (Rezai-Zadeh *et al.*, 2005) and green tea, respectively (Chung *et al.*, 1999) (Figure 5.1). Relative to black and green tea, *J. zeyheri* were 65% and 45% less in carbohydrate content (Figure 5.2).

5.4 Discussion

Moisture content in a tea is known to determine the preserving qualities (Latapi and Barrett, 2006). Higher moisture content deteriorates the desirable tea characteristics by losing briskness with time. The moisture content of *J. zeyheri* were averaged 7.9 g/100g and were 39% and 20% lower to black and green tea moisture content, respectively (Figure 5.5). Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food (Shadung *et al.*, 2012b). Total ash content of *J. zeyheri* had the value of 10.7 g/100g compared to 9.75 g/100g and 9.45 g/100g value of black and green tea, respectively (Figure 5.6). Total fat content of *J. zeyheri* were 3.55 g/100g compared to 19.10 g/100g and 2.74 g/100g total fat contact of black and green tea, respectively (Figure 5.4). *Jatropha zeyheri* has 81% less fat content compared to black tea (Figure 5.4). Total dietary fiber content of *J. zeyheri* were 40 g/100g higher than 17.82 g/100g and 18.6 g/100g values of black and green tea, respectively (Figure 5.7).

In conclusion, drying period does not influence energy, protein, carbohydrates, moisture, fat, ash and fibre content of *J. zeyheri* infusion. *Jatropha zeyheri* infusion has a high energy, ash and fibre content compared to black and green tea, undoubtedly, can be the reason why the Limpopo black community use it as a tea alternative.

CHAPTER 6
SUMMARY, SIGNIFICANCE OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

Jatropha zeyheri Sond*,* is a succulent perennial, monoecious, densely hairy herb producing stems up to 30 cm long that are usually erect but are sometimes prostrate (Arnold *et al.*, 2002). It is widely distributed throughout the eastern parts of South Africa and neighbouring countries, such as Botswana, Zimbabwe, Northern South Africa and Swaziland. It is also utilized to treat sexually transmitted and urinary tract infections by most traditional healers within the black community of South Africa. In Limpopo Province of South Africa, the Pedi speaking communities use it as tea.

Independent studies were conducted to investigate effect of drying period on natural antioxidant phenolic compounds, proximate and mineral elements composition of *J. zeyheri* tea. The four drying periods that were used as treatments were 0, 24, 48 and 72 hours and were replicated five times in a completely randomised design. Drying period significantly increased total phenolic content, antioxidant capacity and tannin content. The studied found that *J. zeyheri* is high in energy, ash and fibre content compared to black and green tea. Drying period increased Mg, K, P, S, AL, Co, Mn, Si and Zn content and inversely decreased Na, Ca, and Ni quantities.

The current study has provided information on chemical composition (tea phenolic compounds, mineral elements and proximate composition) of *J. zeyheri* leaves. The results have made available to tea product development experts in the country. The findings of these research would contribute to economic growth of the rural parts of South Africa, especially where the indigenous plant are grown.

The results of the study demonstrated that drying period improves the bioavailability of some essential minerals elements. It also demonstrated that increasing drying period increases total phenolic content, antioxidant capacity and tannin in *J. zeyheri* infusion. The data provided by the study added valuable information that would aid the tea industry in the development of *J. zeyheri* processed tea products.

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APPENDICES

APPENDIX 3.1 Analysis of variance for total polyphenols under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	11.7896	3.92985	115.86	0.0000
Error	16	0.5427	0.03392		
Total	19	12.3323			

APPENDIX 3.2 Analysis of variance for antioxidant capacity under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	0.71414	0.23805	13.80	0.0001
Error	16	0.27604	0.01725		
Total	19	0.99018			

APPENDIX 3.3 Analysis of variance for tannin under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	162.767	54.2555	75.62	0.0000
Error	16	11.480	0.7175		
Total	19	174.246			

APPENDIX 4.1 Analysis of variance for magnesium under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	153.014	51.0047	192.27	0.0000
Error	16	4.245	0.2653		
Total	19	157.258			

APPENDIX 4.2 Analysis of variance for sodium under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	4.18968	1.39656	79.16	0.0000
Error	16	0.28229	0.01764		
Total	19	4.47198			

APPENDIX 4.3 Analysis of variance for potassium under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	192.456	64.1520	9.88	0.0006
Error	16	103.851	6.4907		
Total	19	296.307			

APPENDIX 4.4 Analysis of variance for phosphorus under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	154.363	51.4544	13.11	0.0001
Error	16	62.809	3.9255		
Total	19	217.172			

APPENDIX 4.5 Analysis of variance for calcium under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	1001.41	333.803	37.20	0.0000
Error	16	143.56	8.973		
Total	19	1144.97			

APPENDIX 4.6 Analysis of variance for sulphur under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	81.960	27.3201	17.94	0.0000
Error	16	24.362	1.5226		
Total	19	106.322			

APPENDIX 4.7 Analysis of variance for aluminium under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	9.0059	3.00198	19.55	0.0000
Error	16	2.4563	0.15352		
Total	19	11.4622			

APPENDIX 4.8 Analysis of variance for cobalt under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	1.83080	0.61027	15.65	0.0001
Error	16	0.62392	0.03899		
Total	19	2.45472			

APPENDIX 4.9 Analysis of variance for manganese under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	10.9632	3.65441	18.24	0.0000
Error	16	3.2058	0.20036		
Total	19	14.1690			

APPENDIX 4.10 Analysis of variance for nickel under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	0.05095	0.01698	6.55	0.0042
Error	16	0.04146	0.00259		
Total	19	0.09241			

APPENDIX 4.11 Analysis of variance for silicon under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	1.56473	0.52158	20.67	0.0000
Error	16	0.40380	0.02524		
Total	19	1.96852			

APPENDIX 4.12 Analysis of variance for zinc under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	0.11701	0.03900	24.61	0.0000
Error	16	0.02535	0.00158		
Total	19	0.14237			

APPENDIX 5.1 Analysis of variance for energy content under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	1332.3	444.11	0.24	0.8697
Error	16	30059.8	1878.74		
Total	19	31392.1			

APPENDIX 5.2 Analysis of variance for protein under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	0.5828	0.19427	0.19	0.9032
Error	16	16.5516	1.03448		
Total	19	17.1344			

APPENDIX 5.3 Analysis of variance for carbohydrates under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	41.450	13.8167	2.57	0.0908
Error	16	86.121	5.3826		
Total	19	127.571			

APPENDIX 5.4 Analysis of variance for moisture content under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	9.1511	3.05036	4.06	0.0253
Error	16	12.0149	0.75093		
Total	19	21.1660			

APPENDIX 5.5 Analysis of variance for ash contant under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	2.4884	0.82947	1.57	0.2352
Error	16	8.4451	0.52782		
Total	19	10.9335			

APPENDIX 5.6 Analysis of variance for total fat under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	4.56268	1.52089	6.16	0.0055
Error	16	3.95064	0.24692		
Total	19	8.51332			

APPENDIX 5.7 Analysis of variance for total dietary fibre content under time-based hot air drying method of *Jatropha zeyheri* tea.

Source of variation	DF	SS	MS	F	P
Treatment	3	3.4356	1.14522	0.28	0.8417
Error	16	66.3313	4.14570		
Total	19	69.7669			