



Faculty of Sciences
Department of Chemical and Forensic Sciences

**Botswana herbal tea quality assessment: Antioxidant activity, Proximate and Metal content
analysis of**

***Terminalia Prunioides* pods tea.**

by

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BSc (Forensic Science) (BIUST)

A Dissertation/Thesis Submitted to the Faculty of Science in Partial Fulfilment of the Requirements
for the Award of the Degree of Master of Science in Chemistry of BIUST

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September, 2022

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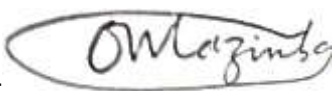
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ACKNOWLEDGEMENTS

I would like to extend my gratitude to my Supervisor Dr. Ofentse Mazimba for his guidance throughout my research. I would also like to thank BIUST chemical and forensic Science and Botswana University of Agriculture and Natural product Food Science and technology laboratory technicians for their continual assistance during my research.

DEDICATION

I dedicate this thesis to my friends and family for their unwavering support throughout my studies.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAAS	Flame Atomic Absorption Spectrometry
GAE	Gallic acid equivalent
ICP-OES	Inductively coupled plasma - optical emission spectrometry
MC	Metal chelation
MeOH	Methanol
PC	Principal component
ROS	Radical Oxygen Species
TPC	Total phenolic content
WHO	World Health Organisation

ABSTRACT

Studies have linked herbal teas to the prevention of many diseases, leading to an increase in their use and demand which has endorsed an increase in their commercial activity and production. Consequently, performing a quality assessment of herbal teas in Botswana to determine their safety, medicinal and economical value has become of greater importance. The quality assessment tools used in this study entail: Antioxidant activity profiling using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Total phenolic content (TPC) and metal chelation activity as well as proximate and metal content analysis. The brewing conditions of 80° C at 60 minutes gave the best antioxidant activity at the lowest DPPH IC₅₀ value of 0.1600 ± 0.0489 µg/mL and the highest TPC of 12319.880 ± 0.622 mg GAE/g. The proximate analysis showed that *Terminalia prunioides* pods contained ash 3.8816 ± 0.1254%, moisture 9.3265 ± 1.5362 %, Crude fiber 7.5908 ± 1.4409 %, and protein 0.00662 ± 0.000717%. The metal content profile showed the presence of the following essential metal elements potassium 10078 ± 325.0 mg/L, calcium 8930 ± 875.1 mg/L, sodium 722.4 ± 250.2 mg/L, and magnesium 2205 ± 65.10 mg/L. The heavy metals present where; Arsenic 1.310 ± 0.08020, chromium 2.380 ± 0.7650 mg/L, copper 16.01 ± 0.3503 mg/L, manganese 28.70 ± 0.6001 mg/L, nickel 0.7600 ± 0.1210 mg/L, lead 4.245 ± 1.575 mg/L, Fe 306.5 ± 1.610, cobalt 1.290 ± 0.1550 mg/L and zinc 1.413 ± 0.6002 mg/L. The elements that were found at permissible values are K, Ca, Mg, Na, Mn, Ni, and Fe, whilst As, Cu, Cr, Co, Zn, and Pb were found at higher values. The tea contains desirable essential minerals such as K, Mg, Ca, Na, Mn, Fe, and Ni and is an excellent source of polyphenolic components that can be explored as a source of dietary antioxidants. The proximate analysis data is also sufficient for herbal tea to be considered as a food product. All three profiles provided preliminary scientific data to ensure the quality and efficacy of *Terminalia prunioides* pods as herbal tea. Therefore, this phytochemistry study shows that *Terminalia prunioides* pods have the potential to be used in herbal tea formulations.

CHAPTER 1

INTRODUCTION

1.1 Importance of herbal products

The field of phytotherapy is rapidly expanding in both developing and developed countries, with an estimated market value of US\$43-60 billion per year, and it is growing gradually (B. Y. Muhammad & Awaisu, 2008). Traditional medicines from developed countries such as China and India have been accepted worldwide; in some countries, they are used alongside modern medicines, while in others, such as the United States, they are sold as over-the-counter medications (Gurib-Fakim, 2006; B. Y. Muhammad & Awaisu, 2008). Because herbal medicine is widely available, it is estimated that 80 percent of the emerging world's population uses it for therapy, and surveys in developed countries such as Germany and Canada show that at least 70 percent of their populations have tried alternative medicines at best once (Chintamunnee & Mahomoodally, 2012; Mahomoodally, 2013). The growing public demand for natural remedies such as herbal teas has led to an increase in their production and commercial activity raising concerns about their quality, safety, and efficacy, as well as demands for patenting rights (B. Y. Muhammad & Awaisu, 2008).

For any natural medicine, quality assurance and correct identification of raw material is an important requirement to ensure repeatable and reproducible quality thus contributing to its safety and efficacy (Geiselhart, 2018). Over 20 years ago, World Health Organisation first issued its quality-control methods for medicinal plant materials guidelines. These guidelines are updated frequently to establish internationally recognized, specific, sensitive, and reliable quality control techniques using both modern and classical instrumental methods for effective assessment of the quality of end products and raw materials (Geiselhart, 2018). These techniques were followed by the publication of monographs of universally significant medicinal plants. The herbal plants that are not listed in these monographs must undergo quality assessment studies to collect scientific data on their quality, safety, and efficacy. Tea is the second most-consumed drink after water throughout the world, with an estimated daily consumption of more than 3 billion cups (Cleverdon et al., 2018).

Tea is said to have been used as a drink and medicine for the first time in China around 2737 BC. Later, it was introduced to Japan in the early 13th century, Europe in the 16th century and then Africa, America, and other parts of the world (Ma et al., 2016). Different types of tea are presently cultivated in over 30

countries around the world (Ma et al., 2016). The interest in the possible health benefits associated with tea consumption, influenced by evidence from epidemiological and animal studies which revealed that tea particularly herbal teas can protect against the development of various chronic diseases, including cardiovascular disease and cancer, has led to the high consumption of this drink hence an increase in its cultivation and production (Cleverdon et al., 2018; Ghuniem et al., 2019; Hajiaghaalipour et al., 2016; Tripoli et al., 2005).

Chronic diseases are believed to be caused by the reactive oxygen species (ROS) produced by human bodies during biochemical reactions. These species of oxygen cause oxidative damage to body organs when they are not quenched effectively (Birben et al., 2012; Pisoschi & Pop, 2015). The endogenous antioxidant defence mechanisms involved in quenching the ROS do not sufficiently counteract their actions, therefore, there is a need for exogenous or diet-derived antioxidants (Chen et al., 2009; Pisoschi & Pop, 2015). Research has demonstrated that herbal teas are an excellent source of diet-derived antioxidants due to the abundance of flavonoids such as catechin. The in-cup antioxidant composition in herbal tea differs depends on the tea location, brewing conditions, and their leaching rates which are influenced by factors, such as water/tea ratio, particle size, time, and temperature (Liu et al., 2018). Therefore, the antioxidant composition of herbal teas can be used to measure their quality.

Herbal teas are aqueous infusions of various plants materials such as leaves fruits, bark, roots, or flowers in hot or cold water to extract the phytochemical constituents which can be correlated to its antioxidants content hence its quality. The quality of herbal teas mostly depends on the abundance of antioxidants and there several factors such as; climate, season, region, geographical variation, horticultural practices, etc. that affect their abundance, therefore it is essential to establish the right conditions under which herbal teas may be cultivated and also for the collection of the best leaves for tea production (Ma et al., 2016). Cultivation of herbal tea plants may also expose the plant to pollutants with adverse health effects on humans.

The accumulation of air pollutants in tea plants is made significantly easier by the large surface area of their leaves, therefore it is not surprising that heavy metals, pesticides, dioxins, and other contaminants have been reported in tea leaves. Tea plants are prone to heavy metal contamination during the growth period, manufacturing process, or both and the absorption of these pollutants can badly affect their safety and efficacy. These heavy metals can be extracted during tea infusions because their solubility ranges from 0- 48 % in water (Brzezicha-Cirocka et al., 2016). Thus, tea can be considered as a significant source of harmful metal ions for humans, therefore there is a need to accurately determine

the percentage of metals leaching into infusions during the evaluation of their safety and efficacy (Ghuniem et al., 2019; Tsushida, 1977). The bioaccumulation of heavy metals such as Lead (Pb) has been linked to numerous disease processes such as immune dysregulation carcinogenesis and insulin resistance (Brzezicha-Cirocka et al., 2016; Chowdhury et al., 2016; Jarup, 2003; Szymczycha-Madeja et al., 2016).

In addition, some researchers have elucidated that excessive levels of both essential and non-essential metals can be toxic and damaging to the organism, therefore it is important to monitor their concentrations, particularly given permissible limits for growth and good health as stipulated by World Health Organisation (WHO) (Duruibe et al., 2007; Ghuniem et al., 2019; Tsushida, 1977). The maximum permitted levels for certain contaminant metals in medicinal plants by WHO guidelines are: Cu 10 mg kg⁻¹, Fe 20 mg kg⁻¹, and Pb 2 mg kg⁻¹ (Nkuba & Mohammed, 2017).

1.2 Problem statement

Botswana has an enormous number of herbal tea plants that have been discovered and used in traditional practices in prehistoric times. These plants have been used for many centuries as a primary source of health care however, few extensive phytochemistry analysis has been performed to establish their purported medicinal and economical value (Motlhanka et al., 2014). This research will focus on the quality assessment of, *Terminalia Prunioides* (Motsiara), of which its fruits and leaves are used to make herbal tea which is linked to the treatment of postnatal abdominal pains, treat coughs, sore throat, and stomach-aches (I. E. Cock, 2015; Pfundstein et al., 2010), even though the plant has been used for medicinal purposes in the Southern part of Africa, Botswana included they have not been extensively studied for both their biological activity and phytochemistry. The study of this plant as a source of healthful quality tea could contribute to the realisation of sustainable development goal number 3 “good health and wellbeing” (UN, 2015).

1.3 Research objectives

1.3.1 Main Objective

To perform quantitative phytochemical and metal content analysis of *Terminalia Prunioides* (Motsiara) herbal tea.

1.3.2 Specific Objective

- Assess the brewing conditions that significantly affect the phenolic and antioxidant activity of herbal tea.
- To determine antioxidant activity and total phenolic content.
- Quantitative determination of essential and non-essential metal content.
- To perform the proximate analysis of the tea samples.

1.4 Research questions

- What are the best brewing conditions needed to extract effective polyphenolic components of herbal *Terminalia Prunioides* tea?
- What could be the quality of herbal teas based on its phenolic content?
- Is the proximate data sufficient for the herbal teas to be considered as food products?

1.5 Justification

An increase in the use and demand for herbal drugs and natural products across the world has led to an increase in their commercial activity and production. The mass production of herbal tea plants, to meet the demand has led to the growing concern pertaining to their quality and safety. World Health Organization has made efforts to address the distress by publishing regularly updated standards on quality control methods for medicinal plant material which can be applied to establish quality parameters. However, this proves to be a challenge to health authorities in developing countries such as Botswana, due to the lack of capacity to monitor many indigenous medicinal plants found in their countries, as a result, there is no or less quality assessment for most of the medicinal plant species found in the country. Therefore, this research is conducted on the local herbal tea plant Motsiara to provide collated and documented scientific data to vouch for their quality, safety, and efficacy as a medicinal plant or herbal tea.

1.6 Significance

The increasing scientific evidence that traditional medication is effective in disease prevention, management, and treatment of non-communicable diseases has led to the growing increase in the global demand for traditional herbal medicine as an alternative to western medication. Studies also show that most Batswana especially those in rural areas prefer herbal medications including daily intake of herbal teas, thus, phytochemistry studies need to be performed on medicinal plants to ensure that both the people and the health sector have adequate access to information on the safety, quality, and effectiveness of the traditional medication. This will also pave the way for the adoption and integration of traditional medicines into the health care system. Phytochemistry studies on local plants will not only help in the determination of the purported medicinal and economical value of the herbal plants of Botswana but will also help in the documentation of indigenous plants.

CHAPTER 2

LITERATURE REVIEW

2.1 *Terminalia* genus

The genus *Terminalia* is one of the largest genus of the family of flowering plants called Combretaceae which is made of 475 species and 20 genera. Approximately 54 species of the Combretaceae family are spread across the tropical and subtropical regions of eastern, western, and southern Africa (I. E. Cock, 2015). The most abundant *Terminalia* species in the western and central Africa part are *Terminalia ivorensis* and *Terminalia superba*, and these species are also grown in the plantations within and outside their natural range, for example in, the Solomon Islands, Fiji, and Hawaii (I. E. Cock, 2015). The most common species that grow as the semi-deciduous to deciduous forests of the southern and eastern part of Africa are *Terminalia prunioides* and *Terminalia brachystema* (Bag et al., 2013; I. Cock & Van Vuuren, 2015)

Terminalia tree's root system is very shallow because their taproots disappear as the tree age (Orwa et al., 2016). *Terminalia ivorensis* tree being the tallest *Terminalia* species is an exception because of its descending roots which start at some distance from the trunk, so as to give it support (I. E. Cock, 2015). The species have obovate and simple leaves which are arranged spirally at the ends of the dwarfed lateral branchlets. In some *Terminalia* trees, such as *Terminalia brassii*, prominent glands can be found at their leaf bases (Akinsulire et al., 2018; I. Cock & Van Vuuren, 2015). The crowns of mature *Terminalia* trees are usually flat or very slightly curved, giving them their distinct shapes. The *Terminalia* trees can also be distinguished by their color. When young, *Terminalia* trees are smooth and pale to grey to dark brown in color, and their branchlets have yellow inner bark (Bag et al., 2013). The bark surface cracks and flakes off in long thin strips or patches as the tree matures, resulting in the formation of deep longitudinal fissures and a blackish color (I. E. Cock, 2015).

Several species of Combretum and *Terminalia* are incorporated into traditional medicinal systems throughout Southern Africa, and several of these uses have already been widely documented (G. Das et al., 2020). A wide variety of Combretaceae species have been used in the treatment of a wide variety of diseases and ailments. Among these are the treatment of various heart problems, abdominal pain, bilharzia, and mental health issues. Both Combretum and *Terminalia* have been used for many medicinal purposes attributed to their antibacterial properties (Chakradhari et al., 2019; G. Das et al., 2020).

2.1.1 *Terminalia prunioides*

Terminalia prunioides are one of the species that are found in Botswana. *Terminalia prunioides* is the scientific name of the tree, its English name is cluster leaf or purple pod *Terminalia*. In Botswana, its local Setswana names are Motshiara, Motororo, Mochara, Mususu, or Motsiyana (Masoko et al., 2005). Whereas in other languages it may be known as sterkbos (Afrikaans), the hareri girachi (Somali), and mwangati-punda (Swahili) (Pfundstein et al., 2010),

T. prunioides has small oval leaves, or egg-shaped, 3 cm broad and 1.3 -7.5 cm long, with the upper surface of the leaf being dark green while the lower one is light green. The leaves are also pubescent when young with the base that is attenuated and the apex either round pointed or notched (Masoko et al., 2005; Pfundstein et al., 2010). Its petiole is obscured by a decurrent leaf base while its leaves are shed irregularly. This species has small oblong-shaped seed pods, which have a red-purple color and are two-winged. The dimensions of the pods are 3.5-6.5 cm long and 2-3 cm wide. The endocarp of the seed pod is hard, woody, and one-seeded. Figure 1 and 2 shows a picture of the *T. prunioides* tree and fruit (seed pod), respectively (Pfundstein et al., 2010).

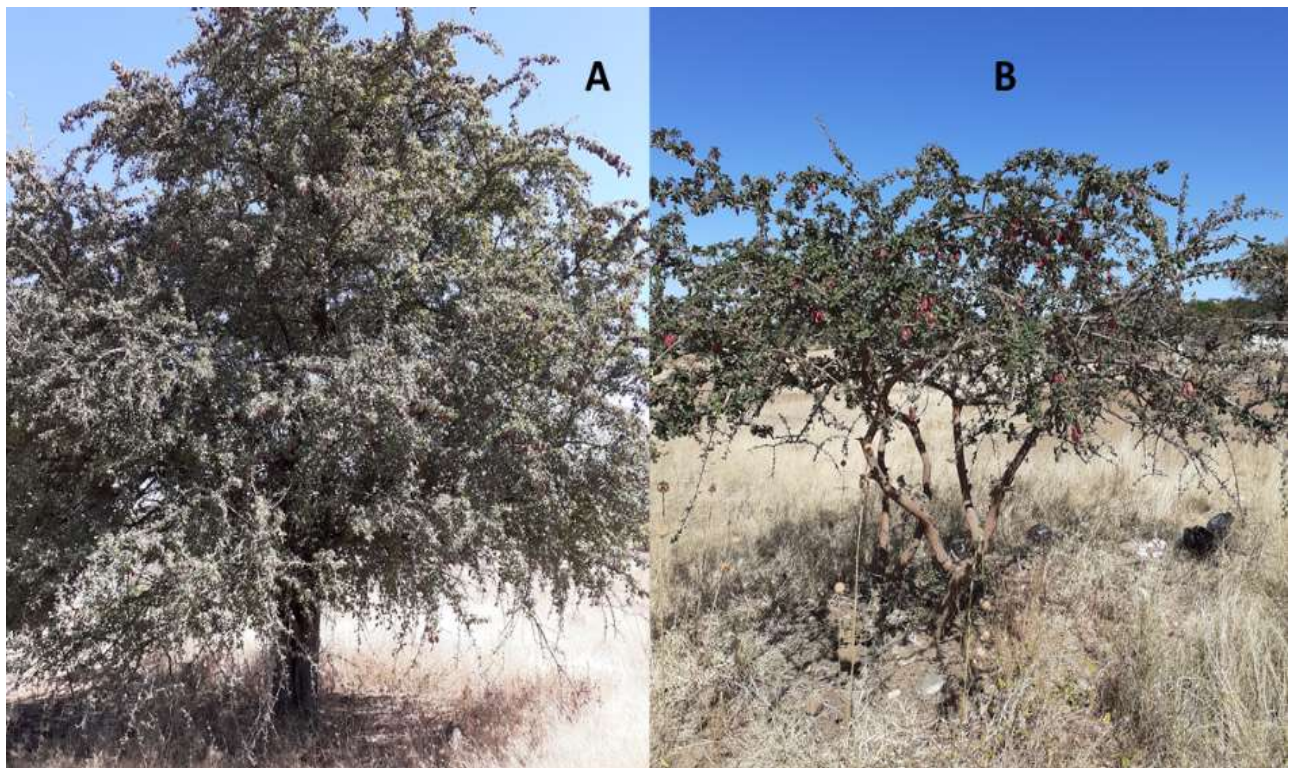


Figure 1: *Terminalia prunioides* tree (A and B)



Figure 2: *Terminalia prunioides* purple seed pods

The *Terminalia prunioides* phytochemical studies have not been reported hence the literature on *Terminalia* species of the Combretaceae family will be reviewed, to identify the biological activity, phytochemicals, antioxidant activities that *T prunioides* may exhibit.

2.1.2 *Terminalia prunioides* bioactivity

The family of Combretaceae has been studied and exhibited plentiful biological activities in various *in-vitro* and *in-vivo* models. Its biological activities include; anti-viral anti-diabetic, anti-hyperlipidemia, antioxidant, hepato-protective anti-bacterial, anti-fungal, cardio-protective activities, anti-inflammatory, anti-cancer, anti-ulcer, anti-parasitic, and antimicrobial (Masoko et al., 2005; Sobeh et al., 2019). Therefore, these species of Combretaceae are used traditionally to cure a variety of infective illnesses such as; diarrhoea, constipation, abdominal pain, coughs and colds, pneumonia, earache, headache, fever, coronary diseases, infertility, inflammation, and swelling (I. E. Cock, 2015; Pfundstein et al., 2010).

Studies have revealed that the therapeutic properties of the Combretaceae may be attributable to their antimicrobial activity (Mbwambo et al. 2011; Mokale Kognou et al. 2011). The potent antimicrobial effects reported against both the Gram-negative and Gram-positive bacteria as well as against dermatophytes (*Epidermophyton spp.*, *Microsporum spp.*, and *Trichophyton spp*) and yeasts (*Candida spp.* and *basidiomycet Cryptococcus neoformans*), can be correlated to the presence of different compounds such as hydrolysable tannins (mostly ellagitannins) and their monomers, ethyl gallate, flavonoids, lignans, stilbenes, saponins, coumarins, and triterpenoid as well as gallic acid and ellagic acid, methylated ellagic acid derivatives (I. E. Cock, 2015; Mokgoatsane, 2011). These antimicrobial

compounds are abundant in all plant parts of the *Terminalia* species (Mbwambo et al., 2011). Numerous reports have indicated that these antimicrobial compounds may inhibit bacteria/fungi through different mechanisms than conventional antibiotics, therefore they are of clinical value in the treatment of resistance (Fyhrquist, 2007; Mokale Kognou et al., 2011; Mokgoatsane, 2011).

A study carried out to establish the anti-microbial activity, using disc diffusion assay, on methanolic and aqueous extracts of different Combretaceae species revealed that these species exhibit excellent inhibitory activity against the Gram-negative and positive bacteria such as *B. subtilis*, *S. aureus*, and *B. cereus* (Fyhrquist, 2007). The research outcomes also indicated that the water extracts of the *Terminalia* species were more effective in inhibiting bacterial growth when compared to other Combretaceae species, this may indicate that *Terminalia* species contain very high concentrations of highly polar tannins which have strong antibacterial activity. Thus, this study confirmed the potential use of the *Terminalia* species for the treatment and control of several bacterial conditions and diseases hence partially validating their application in traditional medicinal systems (Fyhrquist, 2007; Masoko et al., 2005; Mokale Kognou et al., 2011).

Other studies conducted on the *Terminalia* species in Africa have also attested to their compelling antibacterial activity. A very strong antibacterial activity was observed against a wide range of medicinally important bacteria species on the southeast African *Terminalia* species; *T. stenostachya* and *T. spinosa*. Bacteria species under study include several species of *Mycobacterium* such as *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus*, and *Vibrio cholera* (Mbwambo et al., 2011). With the extracts from both, roots and stem bark of *T. spinosa* being highly active against all bacteria except *S. faecalis* and *E. coli* giving MIC values greater than 5 mg/mL. While the stem bark and root extracts of *T. stenostachya* yield MIC values in the range 0.3-2.5 mg/mL making them more active than the leaves (MIC values were ≥ 2.5 mg/mL).

A recent report has also confirmed the antibacterial activity of *T. brownii* against several bacteria's such as *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Escherichia coli*, yielding MIC values ranging between 10-25. Whereas, *Terminalia avicennioides* species has been stated to possess excellent antimicrobial activity towards a methicillin-resistant strain of *Staphylococcus aureus* with 25 mg/ml MIC. (G. Das et al., 2020). There are no reported antimicrobial studies on *T. prunioides*.

2.1.3 *Terminalia prunioides* antioxidant activity

Most *Terminalia* species were reported to possess antioxidant activity. Studies revealed that the Australian, Asian and African Combretaceae possess an exceptionally high antioxidant content (Bag et al., 2013; Masoko et al., 2005; Pfundstein et al., 2010; Pisoschi & Pop, 2015). The antioxidant assay carried out on several extracts of *Terminalia* species indicated that they possessed a potent antioxidant activity as in Table 1.

Table 1: Antioxidant activity of *Terminalia* species

Species	Part and extract	Assay	Activity	Ref
<i>T. chebula</i>	Leaf	2,2-diphenyl-1-picrylhydrazyl (IC ₅₀)	150 mg/mL	(I. E. Cock, 2015)
	Fruit Ethanol	Total Phenolic content	241± 1.57 (mg GAE /kg ext.)	(Saha & Verma, 2016)
		2,2-diphenyl-1-picrylhydrazyl (IC ₅₀)	14 ± 0.05 µg/mL	
<i>T. arjuna</i>	Bark Ethanol	2,2-diphenyl-1-picrylhydrazyl	7.76 µg/mL	(A. Muhammad & Mudi, 2011)
		Total phenolic content	683.174 ± 19.669 (gm GAE /kg ext.)	
<i>T. cattappa</i>	Leaf Ethanol	2,2-diphenyl-1-picrylhydrazyl	80 ± 0.45 %	(Siddiqui et al., 2017)

2.1.4 Terminalia genus reported phytochemicals

Literature survey on phytochemical studies performed on different *Terminalia* species has revealed that this genus has a rich source of triterpenes, and triterpenoid glycosides, gallic acid, and its simple gallate esters, tannins and pseudo tannins, ellagic acid derivatives, and ellagic acid glycosides, flavonoids, chebulic and non-chebulic ellagitannins and phenolic acids (Chafamo et al., 2018; Marston, 2011). These plants' secondary metabolites are responsible for both antioxidant and biological activity in both humans and animals; hence they are accountable for their use as medicinal herbs.

Phytochemical screening of the ethanolic extracts of *T. avicennioides* (Mann et al., 2007) indicated the presence of glycosides, saponins, alkaloids, steroids, tannins, and phenolic secondary metabolites may account for its use as a drug by the Nupe indigenes of Bida and its environs. Analysis of variance shows that graded concentrations of *T. avicennioides* exhibited significant ($P < 0.05$) activity against *S. aureus*, making it an excellent antimicrobial. Another phytochemical study carried out on dichloromethane: methanol (1:1) root extracts of *T. brownii* identified the following classes of secondary metabolites (terpenoids, saponins, tannins, phenols, steroids, flavonoids glycosides, and alkaloids) (Chafamo et al., 2018). A study on *Terminalia Cattappa* (A. Muhammad & Mudi, 2011) also managed to link these phytochemical components to the antimicrobial activity associated with some ethnomedicinal plants. Both studies gave a positive outcome proving that indeed the *Terminalia* species can be of use in the medicinal industry.

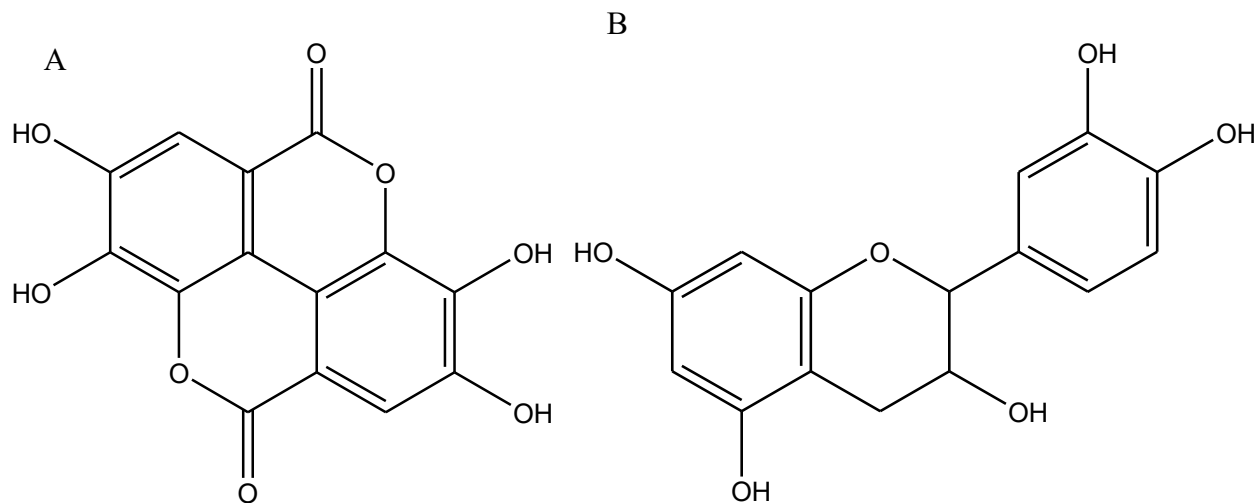


Figure 3: Some of the antioxidant compounds (A) ellagic acid and (B) catechin reported from *Terminalia* species.

2.3 Review of factors used to assess the quality of herbal teas

2.3.1 Total phenolic content and antioxidant activity

A great deal of bioactive compounds can be found in the leaves of herbal teas, namely catechins, which exhibit antioxidant activity. However, recent studies have shown that there are several other phenolic compounds with lower levels of antioxidants (Maisuthisakul et al., 2008). Several polyphenolic compounds have been stated to exhibit antioxidant properties due to their reactivity, which can either donate electrons or hydrogen to attack free radicals. In this regard, plant polyphenols with antioxidant capacity are useful both as scavengers of reactive chemical species and as a protectant against oxidative damage caused by excessive light exposure, making them an important food component for humans and animals alike (Baba & Malik, 2015; Siddiqui et al., 2017).

Numerous studies have revealed a positive correlation between herbal tea consumption and lower risk of oxidative stress-related diseases such as cardiovascular, cancer, and neurodegenerative diseases (Chacko et al., 2010; Pastoriza et al., 2017). Several in vivo and in vitro studies have been conducted on the antioxidant properties of tea infusions and catechins, with the results revealing that tea protects against various types of cancer through its antioxidant properties (Chacko et al., 2010; Pastoriza et al., 2017). A study conducted using animal models has shown that green teas possess the hypocholesterolemic, antibacterial, anti-HIV activity, and anti-inflammatory, furthermore, it has also revealed that green teas have significant protection against Parkinson's disease (Anesini et al., 2008).

Studies conducted on different *Terminalia* medicinal plant species have portrayed that they too are an excellent source of diet-based antioxidants due to the presence of phenolic compounds at high concentrations. An assessment of *T. arjuna* has revealed that the amount of polyphenol in the plant extract is very high in comparison with other common sources of polyphenols such as grapes (Jayaraman et al., 2012). Even though *T. Arjuna* possesses such high polyphenol levels, only three polyphenols have been reported so far. An assessment of the aqueous extracts of *Terminalia Cattappa* pulp showed a higher content of phenolics at 244.33 ± 18.86 mg GAE.g⁻¹ of fruit when compared to the alcoholic extracts, which exhibited 142.84 ± 2.09 mg GAE.g⁻¹ phenolic content (Marques et al., 2012). The other reports on *Terminalia* species showed that the yield of total phenolic of *Terminalia chebula* Retz was 448.7 ± 2.15 mg GAE.g⁻¹, which coincide with the predicted value (447.8 mg GAE.g⁻¹) (Sheng et al., 2018). In Botswana, a study conducted on *Combretum hereroense* a species of the Combretaceae family has shown that it had total phenolic content of 10 680 mg GAE.g⁻¹ which was determined to be ten times more than that of commercial teas (Motlhanka et al., 2014). No

phytochemical studies have been reported on *Terminalia prunioides* but the outcomes of independent research on different species of the *Terminalia* genus and members of the *Combretaceae* family indicate that these plants are an excellent source of total phenolic compounds hence excellent sources of diet-based antioxidants.

2.3.2 Catechin content

Catechin, also called flavan-3-ol, belongs to the flavonoid family and is a naturally occurring phenol, an antioxidant, and a secondary metabolite in plants. The name Catechin derives from the boiled extract of the *Mimosa catechu* plant, which is named Catechu. (Chacko et al., 2010; Xiang et al., 2016). This compound consists of two benzene rings which are usually referred to as ring A, ring B, and ring C. Ring A is analogous to a resorcinol moiety, whereas ring B is akin to a catechol moiety while ring C is a dihydropyran heterocycle with a hydroxyl group attached to carbon-3 (Xiang et al., 2016). Catechin is a constituent of quite a lot of medicinal plants found throughout Africa such as *Ficus cordata*, *Ficus mucoso*, *Ficus-gnaphalocarpa*, *Thalassodendron ciliatum*, *Psidium guajava*, and *Terminalia sericea* (Mbaveng et al., 2014). Catechins are found in many dietary products, fruits (such as apples, blueberries), vegetables, green teas, red wine, cocoa. Tea and red wine are also the most common sources since they are the most consumed beverages in the world. Catechin has been shown to possess antioxidant effects in vitro, in vivo, and through physical methodology (Chacko et al., 2010; Koch et al., n.d.; Vuong et al., 2010; Xiang et al., 2016).

Various research performed across the globe has linked the quality of good herbal tea to its catechins content (Cleverdon et al., 2018; Koch et al., n.d.; Oliveira, 2012; Pastoriza et al., 2017). This compound is more abundant in green teas and thus it is said to contribute mostly to their antioxidant activity, making them of interest in the field of medicinal chemistry. Researchers have linked herbal tea catechins to the prevention of some skin and liver cancers as well as reducing lung, gastric, and breast cancers in vivo and epidemiological studies (Chacko et al., 2010; Xiang et al., 2016).

Spectrometric methods that have been developed to estimate the catechin content includes but are not limited to the following: High Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), Reverse Phase (RP) HPLC, and Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS) (Bronner & Beecher, 1998; Oliveira, 2012). Numerous studies have used High performance liquid chromatographic methods using either diode or fluorescence or mass spectrometry detectors for the detection and quantification of catechin

in tea samples (Bronner & Beecher, 1998; Oliveira, 2012). A study (Saha & Verma, 2016) on the aqueous and methanolic extracts of *Terminalia arjuna* using HPLC and LC-MS analysis, confirmed that the plant species contains flavon-3-ols such as (+)-gallocatechin, (-)-epigallocatechin, and (+)-catechin. Phenolic acids such as ellagic acid, gallic acid, and their derivatives were also detected.

2.3.3 Metal content

Several researchers have elucidated that excessive levels of both essential and non-essential metals can be toxic and damaging to the organism, therefore it is significant to monitor the concentrations of metals leaching from the teas leaves, particularly in view of permissible limits for growth and good health as stipulated by World Health Organisation (WHO) (Duruibe et al., 2007; Ghuniem et al., 2019). Therefore, it is of paramount importance to come up with a reliable, accurate, and precise metal detection and quantification analytical method for tea infusions to ensure their quality and safety.

Spectrochemical methods are the most popular and widely used in the analysis of tea infusions. For trace element analysis in tea, plasma emission techniques are often employed due to their low limits of detection, wide concentration ranges, and rapid/simultaneous quantification. While ICP-MS is usually preferred over ICP-OES due to the following advantages: it provides low values of limits of detection (LOD) and quantification (LOQ) (Ammann, 2007; Kar et al., 2008). Even though ICP-MS has proven to be effective, most studies on the metal analysis of herbal teas have used ICP-OES and FAAS (Patrick-Iwuanyanwu & Udowelle, 2017; Szymczycha-Madeja et al., 2016; Tsushida, 1977).

The results of a study on heavy metal analysis of *Terminalia bellirica* fruits using ICP-OES were as follows; The content of Cu was within the normal range of 9.54mg/L, Nickel (Ni) was not detected at all in samples collected from Nepal whereas the concentration of mercury (Hg) was below the detection limit in fruits collected from India and was undetectable in fruits from Nepal (A. Sharma & Shailajan, 2009).

Another study by (Chakradhari et al., 2019) on the mineral elemental composition of the leaves of the following *Terminalia* species showed high content of calcium and potassium, as outlined in Table 2.

Table 2: Metal content of leaves of the *Terminalia* species (*Chakradhari et al., 2019*)

Metal element	Content (mg/kg)			
	<i>Terminalia arjuna</i>	<i>Terminalia billerica</i>	<i>Terminalia cattappa</i>	<i>Terminalia chebula</i>
Phosphorus	51	281	128	772
Potassium	9364	1764	288	6340
Sulphur	426	389	71	606
Magnesium	1868	368	105	121
Calcium	49656	2567	919	207
Chloride	3346	46	54	789
Aluminium	427	21	27	32
Strontium	101	9	2	9
Barium	39	1	2	1
Chromium	1	7	1	2
Manganese	14	21	16	66
Iron	179	229	127	179
Cobalt	6	1	1	1
Zinc	5	6	3	7
Molybdenum	4	1	3	1
Arsenic	1	2	1	1
Lead	2	10	2	2
Copper	15	2	2	1

2.3.4 Proximate analysis

Proximate analysis is the quantitative analysis of macromolecules; moisture, ash, fat, protein, crude fiber, and carbohydrates in food to determine its quality and as well as to provide detailed information on the nutritional value of the tea plant. For instance, the moisture content in food substances indicates that polar vitamins are existing in the sample while the ash content shows the presence of inorganic mineral elements. The moisture content of the tea leaves can also be related to their shelf life that is the higher the moisture content the lower the shelf life. Two independent proximate analysis studies performed on *Terminalia Cattappa* leaves by (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014) showed that the leaves are nutritional, and they gave comparable results as shown in Table 3.

Table 3: Proximate analysis of *Terminalia Cattappa*

Macromolecule	Content	
	(Packirisamy & Krishnamorthi, 2014)	(Offor et al., 2015)
Total protein (mg/g)	86.7±0.75	4.2 ±1.67
Carbohydrates (mg/g)	187.4±0.53	57.9 ± 6.21
Crude fiber (mg/g)	20.47 ± 0.59	12.9±2.94
Ash (%)	20.2±0.48	12.1±2.84
Moisture (%)	7.18±0.18	8.3±2.35
Fat (mg/g)	-	4.6±1.75

CHAPTER 3

METHODS

3.1 Chemicals

The following chemicals were used during this research; AR solvents (Ethanol 99.9% Absolute fully denatured Batch no.20/322 Ethanol SA and Methanol Analytical reagent batch no.220917MT Rochelles Chemicals), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) 97% lot # S27679 ALDRICH, Gallic acid 97.5-102.5% (titration) lot# SLBV4504, 1,10-phenanthroline chemically pure batch no. 050719PE, Sodium carbonate anhydrous AR S2910, EDTA Disodium salt AR E7350 MINEMA, Ferric Chloride AR batch no. FC155 Alpha chemicals, Follin & Ciocalteu's reagent chemically pure Batch no. 270917FL Rochelle chemicals, nitric acid 69-72% batch no. F065GF01 Alpha chemika, HCL 35-36% batch no. HA829 Alpha chemika, H₂O₂ Rochelle Chemicals, Petroleum ether 60-80% Rochelle, NaOH Chemalin, H₂SO₄ 98% Minera, and metal standards (Mn, Cu, Ni, Zn, Co, As, Pb, Fe, Cd, and Cr) Sigma Aldrich.

3.2 Sample collection and preparation

Terminalia prunioides (Motsiara) pods were collected on 12-04-2020 in Serowe, Central district. The plant species was verified by Dr Andrew Muzila of the Herbarium, Department of Biological Sciences, University of Botswana, voucher number MZ001_2022. The pods were washed using tap water and left to dry for four weeks. The dried samples were then ground using Frisch pulverisette 5. They were then placed in sealed zip-lock bags and stored in a dry place.

3.3 Extraction

In a 250 mL round bottom flask, 20 g of the sample was mixed with ethanol. The mixture was then subjected to refluxing using water baths of temperatures 50, 60, 70, 80, and 90°C at varying times of 15, 30, and 60 minutes. Re-extraction was also performed making the total number of extracts collected thirty-two. The residues were then filtered using gravitational filtration and then the solvent was evaporated under the fume hood. Percentage yield of the dry extract determined, and then extracts were stored in a dry place.

3.4 DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution of concentration 720 mg/L was prepared daily by dissolving 0.036 g of DPPH in 50 mL of methanol. A stock solution of concentration 500 mg/L was prepared by dissolving 0.0025 g of the plant extract in 5 mL methanol and then serial dilution was used to prepare five aliquots of concentrations 31.25, 62.5, 125, 250 and 500 µg/mL. Then, 2.5 mL of the extract solution and 3 mL of the DPPH solution were mixed. The mixture was incubated for 0.5, 1, and 3 hours and absorbance measured at 517 nm using the UV-Vis spectrophotometry. Measurements were done in duplicates. The % inhibition was calculated using the formula below and the % inhibitions were used to generate calibration curves which were then used to determine IC₅₀ values.

$$\%Inhibition = \left(\frac{A_o - A}{A_o} \right) \times 100$$

Where A_o is the absorbance of the control and A is the absorbance of the extract.

3.5 Total phenolic content

Gallic acid standards of the following concentrations: 0.1, 0.5, 1.0, 2.5, 5 mg/mL were prepared in 80% methanol. Extract solution of 500 µg/mL was prepared by dissolving 0.0050 g of the extracts in 10 mL 80% methanol. A mixture of 1 mL of the standard/ extract and 1 mL of Follin reagent, was prepared and incubated for 5 minutes in a dark cabinet. Afterward, 2 mL of 20% sodium carbonate was added to the mixture to stop the reaction, then the mixture was incubated for 2 hours. The experiment was performed in triplicates. Absorbance was measured at 760 nm and a calibration curve of gallic standards was used to calculate gallic acid equivalent (GAE/g). Total phenolic content was then determined by multiplication of GAE concentration by volume used /mass of extracts (Blainski et al., 2013).

3.6 Metal chelation

Serial dilution of the extracts was performed to prepare the following concentrations of the extracts: 500, 250, 125, 62.5, and 31.25 µg/mL. Metal chelating activity was measured by adding 2 mL of extracts/EDTA were added to a mixture of 1 mL of o-phenanthroline (0.05% in methanol) and 2 mL of FeCl₂ (0.1% in water). The absorbance of the mixture was recorded at 510 nm after 20 and 40 minutes of incubation. The chelating activity was calculated using the following formula:

$$Metal\ chelating\ activity = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

Where A_{control} is the absorbance of control reaction (without plant extract), and A_{sample} is the absorbance in the presence of a plant extract.

3.7 Proximate analysis

The proximate analysis was done at the Food Science and Technology Laboratory of the Botswana University of Agriculture and Natural Resources (BUAN).

3.7.1 Fat content

Triplicates of about 3 g of grounded and dried sample were placed in the thimble and then the thimble in the Soxhlet extractor. A weighted round-bottom flask was filled with 100 mL of petroleum ether. The whole setting was placed on a heating mantle and the petroleum ether was allowed to boil. The extraction process was run for 6 hours and afterward, the round bottom flasks were moved from the extraction unit and allowed to cool down in a desiccator. The solvent was then evaporated using the rotary evaporator and the round bottom flasks re-weighed. The % fat content was determined using the formula:

$$\% \text{ fat} = \frac{W_{\text{lip}} - W_{\text{em}}}{M_{\text{sample}}} \times 100$$

Where W_{lip} is the weight of the round bottom flask with the lipids and W_{em} is the weight of the empty round bottom flask.

3.7.2 Protein content

One capsule of catalyst (potassium and copper sulphate tablets) and 0.5000g of the sample were combined with 10 mL of concentrated sulfuric acid and the sample was digested overnight in the digestion tube. This resulted in a clear solution of ammonium sulphate. The solution was then diluted with distilled water into a 100mL volumetric flask. Then 20 mL of the diluted solution was mixed with 50 mL of 40% NaOH in the distillation tube and distilled. About 25 mL of the distillate was trapped in 150 mL of 4% boric acid containing two drops of methyl orange indicator then the distillate was titrated with 0.1M HCl, V_{HCl} was recorded. The blank was also prepared following the same method and V_{blank} was recorded. Percentage nitrogen was calculated using the formula below and then % protein was calculated by % nitrogen content $\times 6.25$. The experiment was done in triplicates.

$$\%N = \frac{(V_{HCl} - V_{blank}) \times N_{HCl} \times 0.014 \times 10}{\text{mass of sample (g)}} \times 100$$

Where V_{HCl} is the volume of HCl used to titrate the sample (mL), V_{blank} is the volume of HCl used to titrate blank (mL), N_{HCl} is the molarity of HCl, 0.014 is the atomic mass of nitrogen (mL) and 10 is the dilution factor.

3.7.3 Moisture content

Triplicates of about 3 g of the dry crushed sample were placed in a weighed crucible. The crucibles were then placed in an oven heated to 130°C for 3 hours. They were then removed using the safety tongs and quickly transferred to a desiccator for cooling before weighing. Moisture content was then expressed as % Moisture using the formula below:

$$\% \text{Moisture} = \frac{M_{\text{sample}} - M_{\text{dried}}}{M_{\text{samples}}} \times 100$$

3.7.4 Ash content

Triplicates of about 3 g of the dry crushed sample were placed in a weighed crucible. The crucibles were then placed in a muffle furnace which was ignited at 550 °C overnight. The muffle furnace was turned off and left open until the temperature had dropped significantly. The crucible was then removed using the safety tongs and quickly transferred to a desiccator for cooling before weighing. Ash contents were expressed as % ash using the formula below:

$$\% \text{ Ash (dry basis)} = \frac{M_{\text{ASH}}}{M_{\text{DRY}}} \times 100$$

where M_{ASH} refers to the mass of the ashed sample, and M_{DRY} refers to the original masses of the dried samples.

3.7.5 Crude fiber analysis

About 3 g of ground material was boiled in 200mL of 1.25 % sodium hydroxide solution for 30 minutes the residues were collected by filtering through a cloth. The residues were rinsed off sodium hydroxide using boiling water then they were boiled in 200 mL of 1.25 % H_2SO_4 . The residues were again filtered and rinsed using boiling water. Afterward, the residues were transferred into a crucible for drying using the oven for 2 hours at 130 °C. The crucible was cooled in the desiccator then the cool dry residue was weighed and transferred into a weighed crucible. The sample was then ignited overnight in a furnace

at 550 °C. They were cooled in a desiccator and reweighed. The experiment was performed in triplicates and the % crude fiber was calculated using the following formula:

$$\% \text{crude fibre} = \frac{M_{\text{dry}} - M_{\text{ash}}}{M_{\text{samp}}} \times 100$$

Where M_{dry} is the mass of the sample after moisture was removed, M_{ash} is the mass of the sample after ashing and M_{samp} is the initial mass of the sample.

3.7.6 Carbohydrates content

The carbohydrate content was determined by calculating the percent remaining after all the other components have been measured:

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Crude Fat} + \% \text{ Crude Fiber} + \% \text{ Crude Protein})$$

3.8 Metal analysis

3.8.1 Extraction

Four replicates of 0.5 g of the crushed sample were weighed into the digestion vessels containing a mixture of 2 mL hydrogen peroxide (H_2O_2) and 8 mL of nitric acid (HNO_3) for pre-digestion for 16 hours at room temperature. After 16 hours the digestion vessels were placed in a sample holder and returned to the microwave digester and the plant material method automatically loaded. Two blanks were prepared and run using the same method. A one touch technology microwave digestion system equipped with temperature and pressure regulation was used and the samples were digested using the following heating program: power;1030-1800 minutes, ramp time;20-25 minutes, hold time;10 minutes, cooling;15 minutes. After cooling, the entire digest is transferred into 250 mL volumetric flasks and diluted to the 250 mL mark with distilled water. Reagent blank was prepared similarly to the sample. The sample was filtered to make it is clear for metal analysis.

3.8.2 FAAS analysis of metal elements

Calcium, magnesium, potassium, and sodium standards of concentrations 0.5,1,2,5 and 10 mg/L were prepared and the mixed standard of As, Mn, Zn, Cu, Ni, Co, Pb, Fe, Cr, and Cd of concentrations 0.2,0.4,0.6,0.8 and 1mg/L was prepared as well. A separate standard of Fe was prepared at concentrations 0.5,1,2,4,6 mg/L. The method for running each metal was created using the Analytik

Jena software and the three replicates of the sample and blank were run. The instrument used for analysis was ContrAA. The autosampler used the following injection program; delay time 5s, injection time 10s, load time 10s, and run time 3s. The elements were used to analysed using different parameters and parameters for some meters are outlined below Table 4;

Table 4: Parameters used for running samples with FAAS

Metal element	Parameters		
	Wavelength (nm)	Fuel	Fuel flow rat (l/h)
calcium (Ca)	422	C ₂ H ₂ -Air	215
Magnesium(mg)	285	C ₂ H ₂ -NO ₂	70
Sodium (Na)	588	C ₂ H ₂ -Air	55
Chromium (Cr)	357	C ₂ H ₂ -Air	100
Copper(cu)	324	C ₂ H ₂ -Air	50
Manganese (Mn)	279	C ₂ H ₂ -Air	80
Cobalt (Co)	240	C ₂ H ₂ -Air	50
Lead (Pb)	217	C ₂ H ₂ -Air	65
Zinc (Zn)	213	C ₂ H ₂ -Air	50
Iron (Fe)	248	C ₂ H ₂ -Air	60

3.9 Statistical analysis

Microsoft Excel was used to calculate DPPH radical scavenging activity, total phenolic content metal chelation activity, proximate content, and metal content values. The data was analyzed and represented in tables, charts, and graphs.

XLSTAT by Addinsoft was used for principal component analysis. An analysis of principal components was carried out to determine whether the different methods used to evaluate antioxidant

activity were correlated with one another, and to obtain an overview of the similarities and differences between them.

Chem draw 8.0 by Cambridge was used to draw molecules and reaction mechanisms.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Extracts yields

Phytochemical compounds are extracted from biomass materials primarily through reflux extraction. The goal of extraction is to maximize the number of target compounds and to maximize the antioxidant activity of these compounds (Lezoul et al., 2020). The extraction technique, as well as the solvent used for extraction, are factors that influence both the extract's antioxidant activity and extraction yield. The choice of extraction solvents used on plant materials depends on the type of phytochemicals under study and some of the solvents that have been used include; acetone, ethanol, methanol, and water (Lezoul et al., 2020).

Ethanol was used for extraction, and it extracted high extract yield ranging from 2.1870 to 11.8080% in both the first and second extraction as shown in Table 5. According to (Lezoul et al., 2020), methanol and ethanol are both widely used to obtain antioxidant compounds from plants and plant-based foods because the extraction efficiency of phytochemicals tends to favor these highly polar solvents. Reflux extraction was proven to be quite effective by (Sultana et al., 2009), who showed that the yields of the extract were superior when utilizing this extraction approach, regardless of the solvent utilized or plant material. Therefore, hot solvent reflux extraction has led to more efficient retrieval of antioxidant components resulting in higher extract yields.

Table 5: % yield of ethanol extracts of *Terminalia prunioides* at varying brewing conditions

	Brewing conditions		1st extraction	2nd extraction
Extract	Temperature (°C)	Time (min)	% Yield	
1	50	15	4.6425	2.1875
2		30	5.4070	3.2695
3		60	5.5595	4.2450
4	60	15	7.1790	2.5015
5		30	8.0290	3.519
6		60	9.7945	3.6100
7	70	15	6.9255	3.4090
8		30	7.1790	3.6255
9		60	11.8080	5.1415
10	80	15	6.8975	4.3790
11		30	9.5515	4.6580
12		60	9.8955	7.1565
13	90	15	5.7720	3.2670
14		30	8.7710	3.9970
15		60	9.2100	4.0625

The results for both extractions agree with the hypothesis that a longer extraction time results in a higher percentage yield as shown by Figure 4. The prolonged extraction time values provide more time for the solute to be in contact with the solvent (Lezoul et al., 2020; Nguyen & Chuyen, 2020). Figure 4 also confirmed that temperature parameters varied in this study affected percentage yield. The results indicated that increasing the temperature during the extraction process has led to percentage yield lowering after 70°C at all extraction times. A higher heating process can degrade the heat sensitive phytochemicals in the extract which may adversely affect the extraction yield and efficacy of medicinal plants (Tambunan et al., 2017). The ethanolic plant extract usually contains polar molecules such as hydrolysable tannins which are comprised of gallic and ellagic acids with a sugar core mainly glucose in their structure which when exposed to higher temperature may cleave leading to lower extraction yields (A. K. Das et al., 2020).

Based on these results, it suggests the extraction technique for 60 minutes at 70°C using ethanol as a solvent is the most efficient method to obtain the highest total yield extract in *Terminalia prunioides* pods.

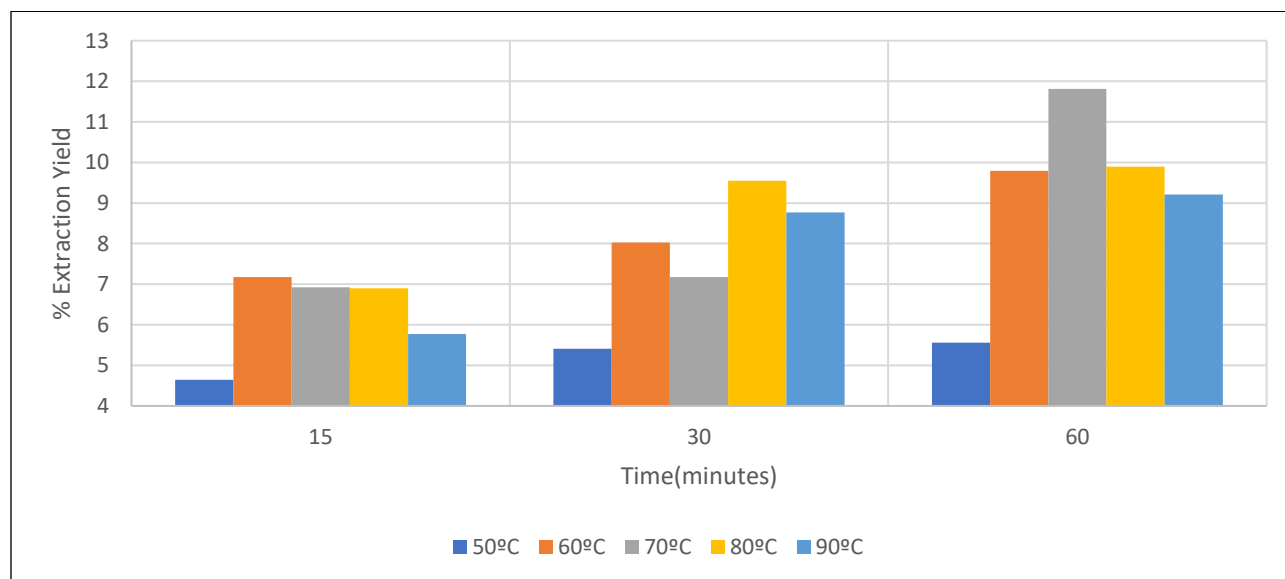


Figure 4: The effects of time and temperature on ethanolic extraction of *Terminalia prunioides* pods.

Antioxidants are substances that inhibit and stabilize the damage caused by unstable free radicals in the human body by supplying them with electrons, thus, turning these disease causing free radicals into waste by-products that are eliminated from the body (Baba & Malik, 2015; Land, 1990). This assay enables the quantification of the ability of antioxidants to quench 2,2-diphenyl-1-picrylhydrazyl

(DPPH) radical which is unstable due to the delocalization of a spare electron over the molecule Figure 5 (Molyneux, 2004; A. Sharma & Shailajan, 2009). The unstable radical produces a dark purple colour in methanol which will fade to yellow color if it is reduced by an electron provided by antioxidants to form its non-radical stable form.

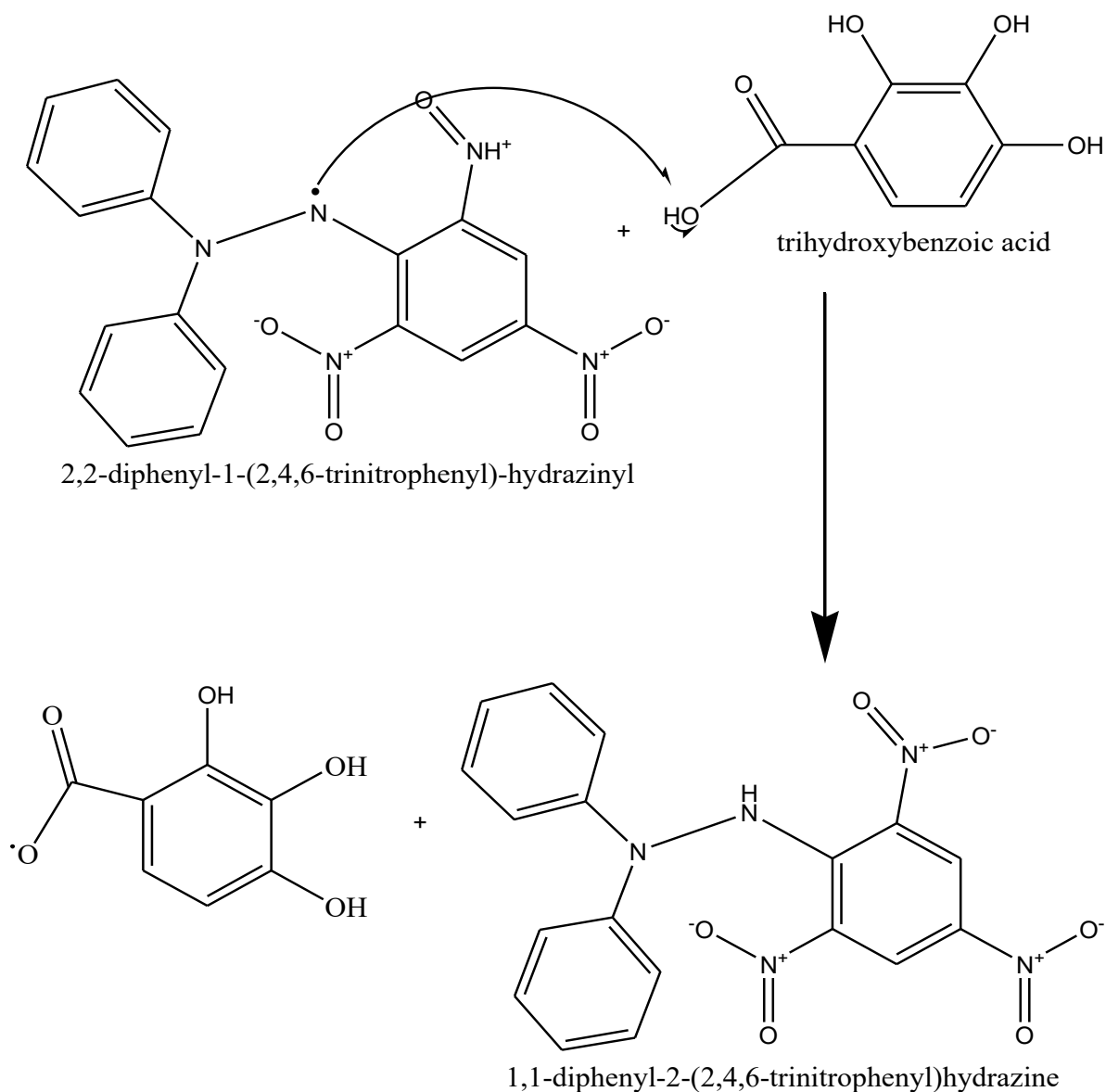


Figure 5: Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.

All the assessed *Terminalia prunioides* extracts revealed a reduction in the purple-coloured DPPH radical into the yellow-coloured DPPH[•]-H complex and this reduction can be correlated to antioxidant activity, and it was expressed as percentage inhibition. Percentage Inhibition of the extracts varied with concentration as shown by Figure 6-8. The graphs indicate that percentage inhibition increased with an

increase in concentration, which shows that sample extracts with high concentrations contain higher amounts of antioxidants as compared to those of lower concentrations. The higher antioxidant concentration means that there are more available phenolic compounds to quench the DPPH radicals (O. P. Sharma & Bhat, 2009).

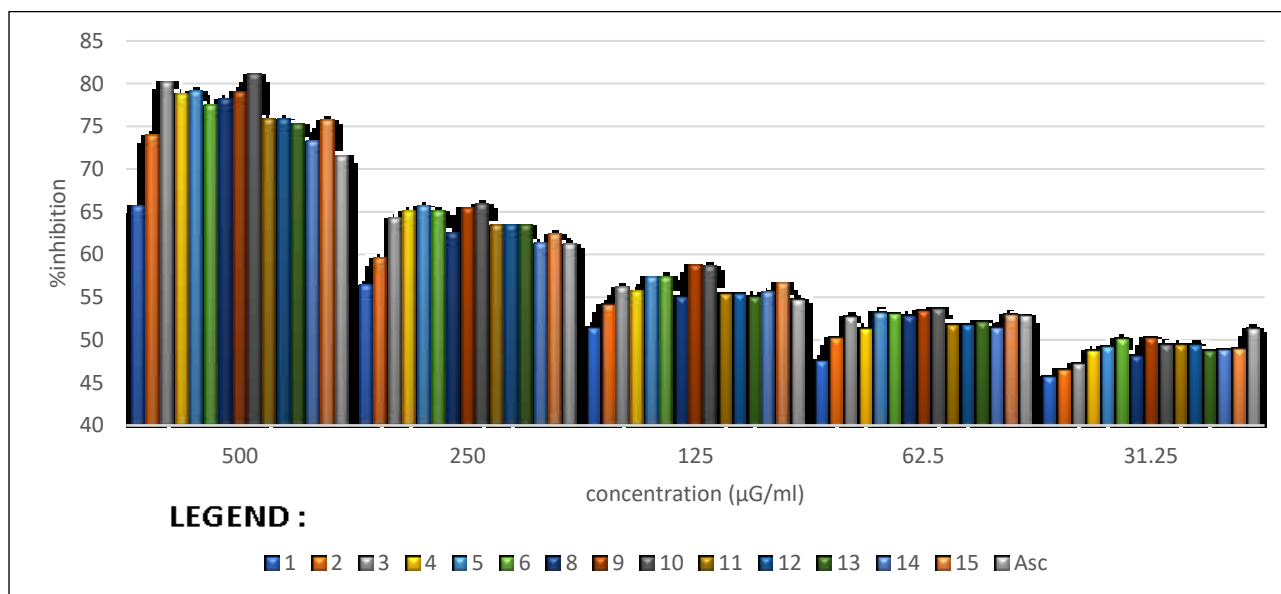


Figure 6: 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition at various concentrations (µg/mL) of *Terminalia prunioides* pods extracts at 30 minutes incubation.

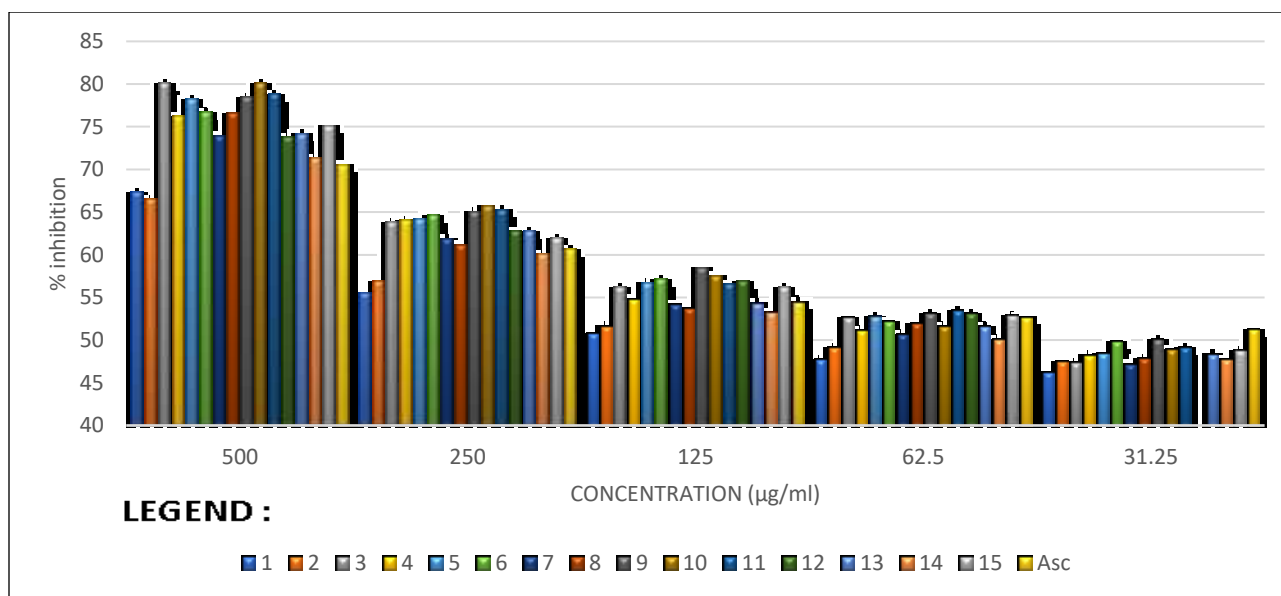


Figure 7: 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition at various concentrations (µg/mL) of *Terminalia prunioides* pods extracts at 60 minutes incubation.

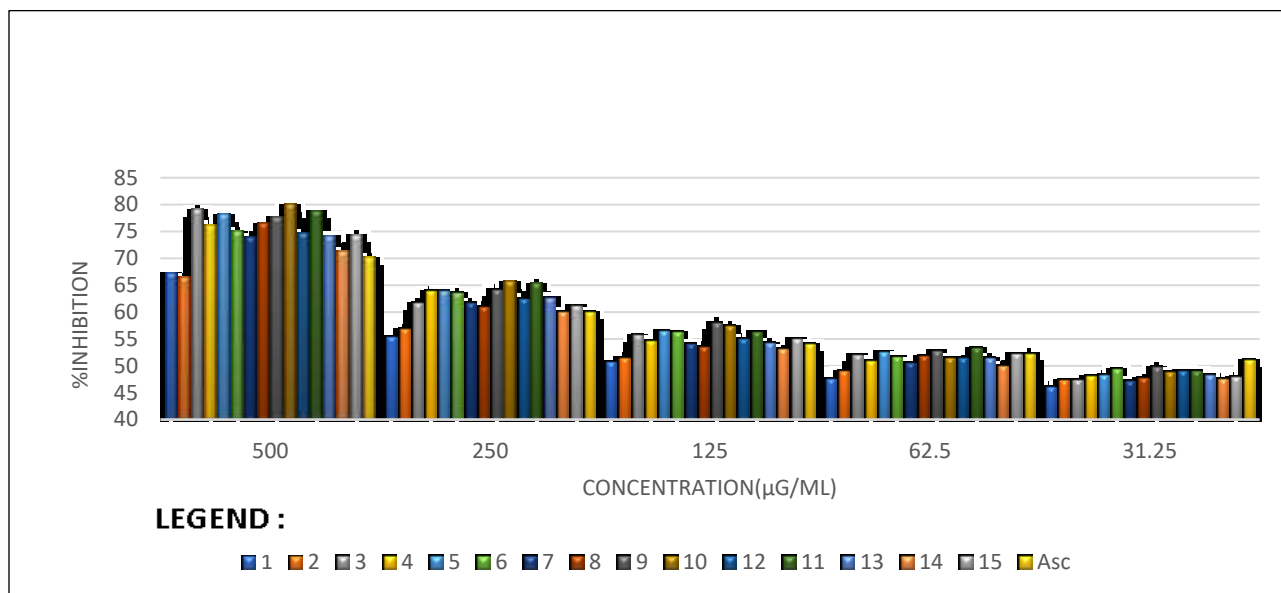


Figure 8: 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition against various concentrations ($\mu\text{g/mL}$) of *Terminalia prunioides* pods extracts at 3 hours incubation.

Figure 9 shows that % inhibition was not significantly affected by incubation times, for instance, the % inhibition of the ascorbic acid standard at concentration 500 $\mu\text{g/mL}$ varied from 70.5, 71.5, and 70.2 % at 0.5, 1, and 3 hours respectively.

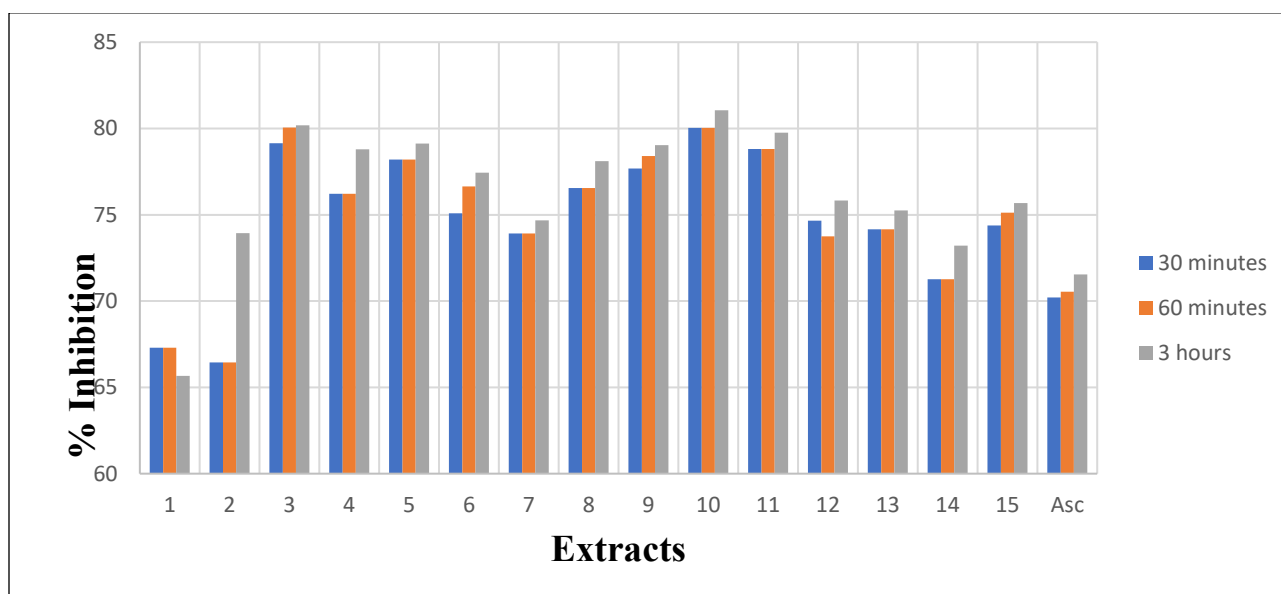


Figure 9: 2,2-diphenyl-1-picrylhydrazyl (DPPH) % inhibition at 500 $\mu\text{g/mL}$ concentrations of *Terminalia prunioides* pods extracts at 30 minutes, 60 minutes, and 3 hours incubation.

Percentage inhibition of the sample extract is better expressed as IC_{50} (O. P. Sharma & Bhat, 2009). The IC_{50} value represents the amount of sample extract required to reduce DPPH activity by 50%. Lower IC_{50} values indicate stronger DPPH radical scavenging activity of the sample. IC_{50} values can be correlated to radical scavenging activity of the extracts, that is extracts with IC_{50} values less than 10 $\mu\text{g/mL}$ have an excellent activity, those with IC_{50} values ranging from 10-50 $\mu\text{g/mL}$ have good activity while those ranging from 50 -100 $\mu\text{g/mL}$ have significant activity (Brighente et al., 2007). The percentage inhibitions were used to determine the IC_{50} values by plotting them against concentrations as shown by the graph Figure 10. The correlation factors (R^2) for all the sample extracts were 0.99, suggesting that the percentage inhibition and concentration are positively correlated as shown by Appendix A. The IC_{50} values were then determined using the equation of the line, $Y=mX +C$ where X was the unknown concentration and Y was the 50 % inhibition concentration (Brighente et al., 2007; Xiao et al., 2020).

The extracts under study demonstrated a difference in their radical scavenging activity as shown in Figure 10. The graph also shows that the IC_{50} values decrease from left to right which indicates that radical scavenging activity increases with increasing extraction time and temperature. This means that extracts that were brewed for shorter times and lower temperatures had poor radical scavenger activity when compared to those brewed for a longer time at higher temperatures. Therefore, it can be concluded that both brewing time and temperature play a significant role in the extraction of antioxidants from tea pods. Figure 11 also shows lower IC_{50} values for the extracts extracted at a temperature of 80°C, thus it can be depicted that the radical scavenger activity was higher for 80°C extracts. From the graph, it can also be observed that there is no significant change in IC_{50} values with increasing incubation time.

The sample extract that displayed the highest DPPH scavenger activity gave an IC_{50} value of $(0.1600 \pm 0.0489) \mu\text{g/mL}$ which is lower than the IC_{50} value of ascorbic acid standard $(0.8776 \pm 0.0011) \mu\text{g/mL}$. The type of phenolic compounds present in an extract and their chemical structure determine the antioxidant activity of the extract (Mathew et al., 2015). It has been determined that phenolic compounds and their ester's antioxidant activity depend on the number and position of electron donating hydroxyl groups within the molecule. Therefore, the high antioxidant activity of the 80°C (at 60 minutes) extract could be related to the presence of compounds containing a high number of the hydroxyl groups situated on either the ortho and/or para positions (Mathew et al., 2015). *Terminalia prunioides* pods extract contained higher radical scavenging activity when compared to the prior studies of the methanolic leaf extract of *Terminalia chebula* which exhibited an IC_{50} value of 0.1500

$\times 10^6 \mu\text{g/mL}$ (150 mg/mL) (I. E. Cock, 2015). Therefore, *Terminalia prunioides* pods can make good herbal tea due to their high radical scavenger activity.

A previous report determined that the best extraction conditions range between 5-10 minutes and 80-100 °C for plant extracts preparations and these will result in infusions with greater antioxidant capacity than teas made using a shorter brewing time at lower temperatures (Banerjee & Chatterjee, 2014). From this study, the extract that exhibited higher antioxidant activity was rather extracted at a longer extraction time which exceeds the stipulated extraction time range for tea brewing. Hence, the best brewing conditions for *Terminalia prunioides* herbal tea at 80 °C for 60 minutes is ideal for tea that would be extracted to be packaged and not for a quick (less than 10 minutes) cup of tea.

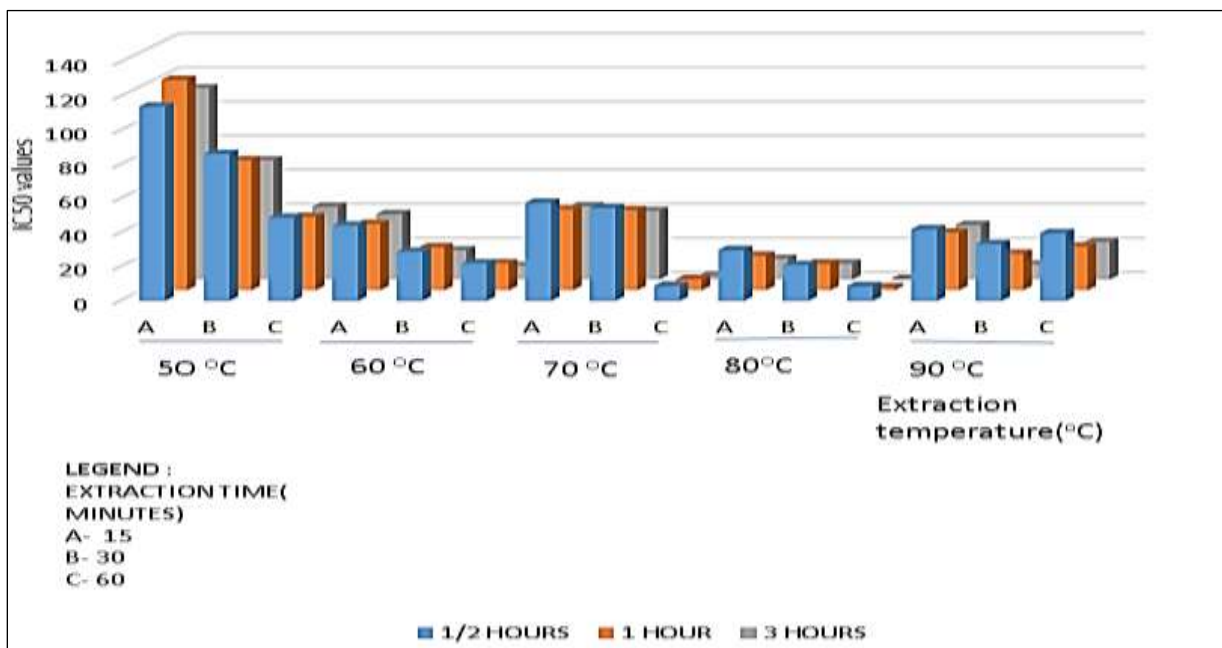


Figure 10: Changes in IC₅₀ values with incubation time, extraction time, and temperature.

4.2 Total phenolic content

Total phenolic content is the quantification of the concentration of phenolic compounds present in the extract and it's vital to measure these compounds as they are attributed to high antioxidant activity (Blainski et al., 2013; Siddiqi et al., 2011). Phenolic compounds display their antioxidant activity through several mechanisms such as donating electrons or hydrogen atoms to free radicals, scavenging other reactive species such as OH[•], N₂O₃, NO₂[•], HOCl, and ONOOH (Maisuthisakul et al., 2008). The gallic acid equivalent method was used in the estimation of the concentration of phenolic content in the pod's extracts of *Terminalia prunioides* (Blainski et al., 2013). The absorbance of known concentrations

of gallic acid standards was measured and a calibration curve of standards obeyed Beer's law as shown in Appendix B.

An assessment of the methanolic extracts of *Terminalia prunioides* pods showed that they have a higher concentration of phenolic compounds ranging from (8184.304 ± 0.622) to (12319.880 ± 0.622) mg GAE/g. Figure 11 indicates that phenolic compounds concentrations in the extracts increased with increasing extraction time. For instance, the phenolic content of extract 1 brewed at 50°C for 15 minutes was (8184.304 ± 0.622) mg GAE/g while that of extract 3 brewed at the same temperature for 60 minutes was found to be (11012.380 ± 0.622) mg GAE/g. Therefore, there was a 35% increase in total phenolic content. An increase in extraction temperature also led to an increase in phenolic content and as exhibited in Figure 11 which indicates a skewing of the graph to the right side. For instance, extract 1 and 4 which were extracted at the same extraction time, but differing extraction temperatures demonstrated a huge difference in their phenolic content with (8184.304 ± 0.622) and (10502.570 ± 0.622) mg GAE/g, respectively. A 28% increase in total phenolic content was observed upon changing extraction temperature. Thus, both extraction time and temperature show an important role in the extraction of phenolic compounds from the extracts.

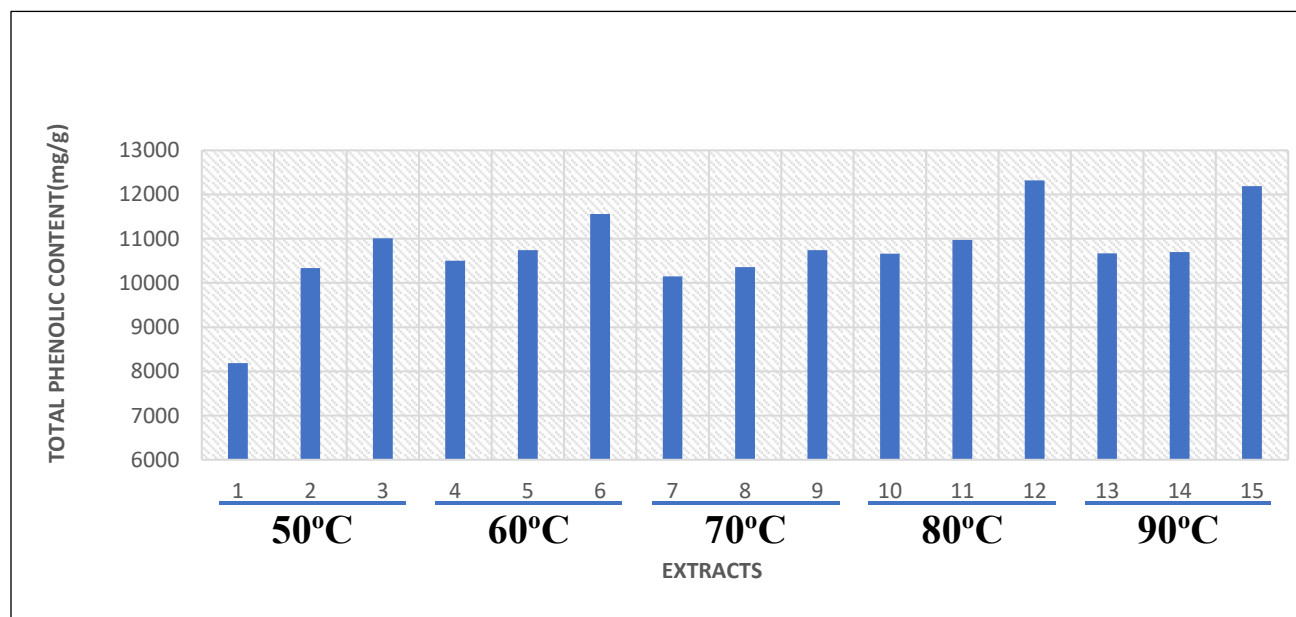


Figure 11: Total phenolic content (GAE mg/g) of *Terminalia prunioides* extracts at varying brewing conditions.

The first two principal components, PC1, and PC2 explained 89.82% and 10.18% of the total variance of the data set, respectively, and entirely accounted for 100 % of the total variance of the data matrix

from DPPH and TPC. The biplot and correlation circle Figure 12 indicate that there was a strong negative correlation among DPPH and TPC. According to (Karadag et al., 2020), when two variables are far from the center and on opposite sides in the correlation circle they are significantly negatively correlated that is there R^2 is close to -1. Therefore, both the bio-plot and correlation circle show that extracts with the highest strength in scavenging DPPH radical (lower IC_{50} values) possessed high amount of phenolic compounds. From the study extract 12 brewed at 80°C for 60 minutes exhibited the highest total phenolic content and DPPH scavenging activity of (12319.880 ± 0.622) mg GAE/g and (0.1600 ± 0.0489) $\mu\text{g/mL}$, respectively when compared to other extracts. The high phenolic content of this extract could be related to high antioxidant activity therefore these are the best brewing conditions of *Terminalia prunioides* pods according to this study.

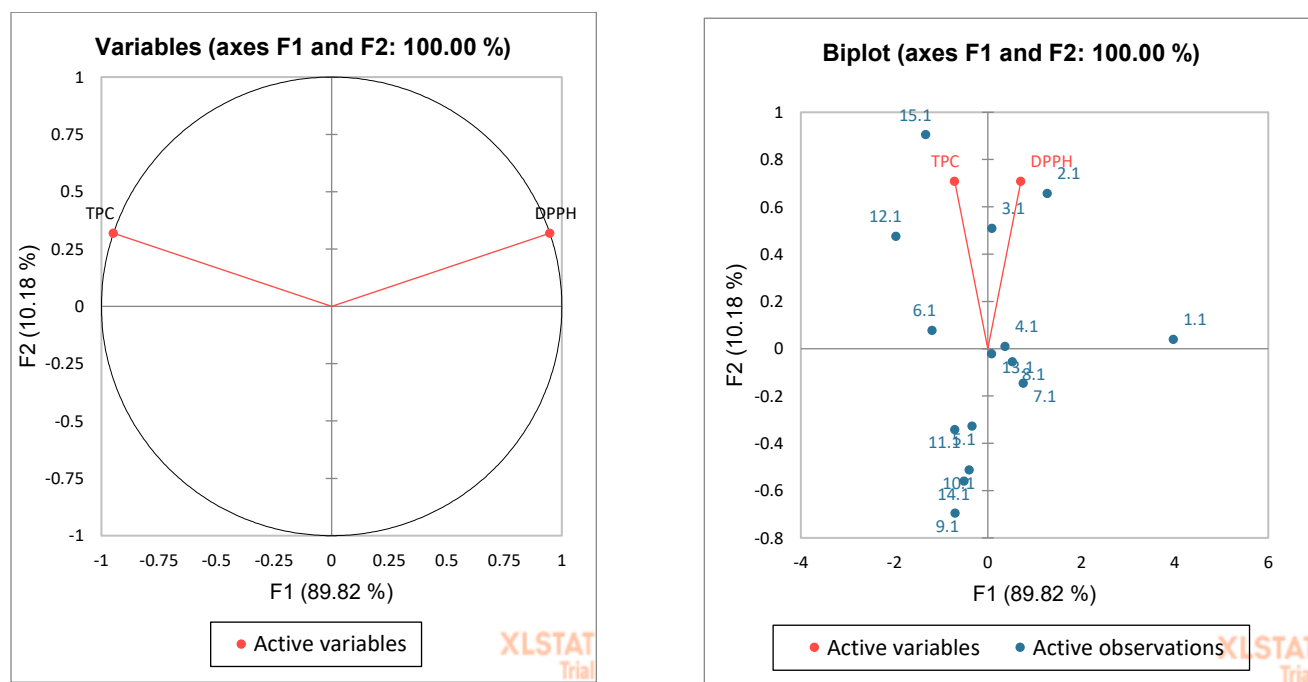


Figure 12: Biplot and Correlation circle showing the relationship of 2,2-diphenyl-1-picrylhydrazyl and Total phenolic content

Extract 12 also exhibited higher total phenolic content when compared to other *Terminalia* species. A study on *Terminalia cattappa* pulp showed the concentration of phenolics at 244.33 ± 18.86 mg GAE.g⁻¹ (Marques et al., 2012), while another study showed that the yield of total phenolic of *Terminalia chebula* retz was 448.70 ± 2.15 mg GAE/g (Sheng et al., 2018).

4.3 Metal chelation

Trace amounts of a transition metal such as iron, copper, and lead can be useful in the human body as they play role in major biochemical reactions but when in excess these metals can be detrimental to the body as they possess the ability to form reactive oxygen species that damage DNA, proteins, and lipid (Duruibe et al., 2007; Jarup, 2003). To relieve itself of the excess transition metal ions the body utilizes both the endogenous and exogenous antioxidants (Flora & Pachauri, 2010). Herbal teas have proven to be the best sources of polyphenols that possess high antioxidant activity. Literature shows that polyphenols are organic metal chelators, that is they have a high binding affinity for toxic metal ions forming a complex structure that can be easily excreted from the body. (Flora & Pachauri, 2010; Sellal et al., 2019; Shahidi & Zhong, 2015). A good metal chelator is a chemical compound that can react with metal ions to form a stable water-soluble complex and these compounds should be able to form several bonds with the metal ion to form chelates. Fe^{2+} metal chelation assay was carried out to assess the chelation capacity of the crude extracts of *Terminalia prunioides* pods. This assay enables the quantification of the ability of polyphenols to reduce Fe^{2+} metal ions to form a chelate as shown by Figure 13 (Shahidi & Zhong, 2015).

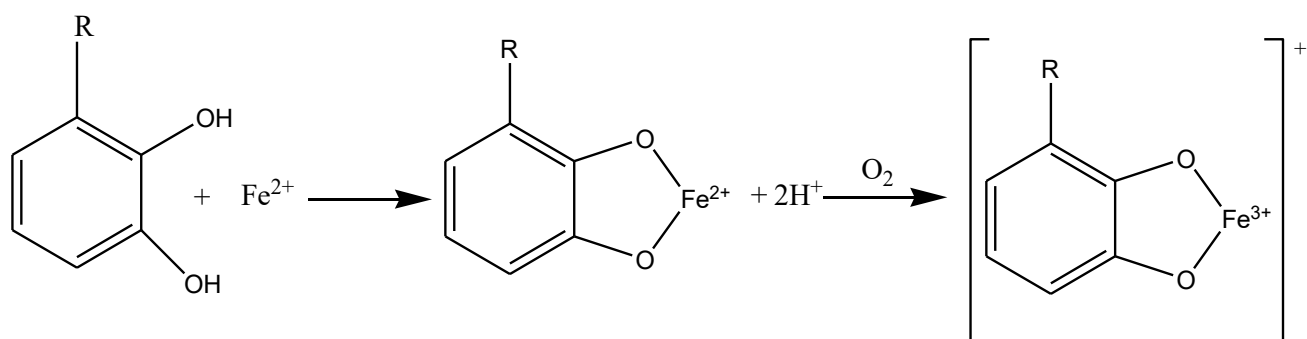


Figure 13: Reduction of Fe^{2+} by polyphenols

All the assessed *Terminalia prunioides* extracts exhibited a metal chelation activity which was then expressed as percentage metal chelation. Percentage metal chelation can be correlated to the antioxidant activity of extracts (Flora & Pachauri, 2010). The metal chelation of the extracts was concentration dependent as shown by Figure 14 and Figure 15, that is % metal chelation activity increased with an increase in concentration, which shows that sample extracts with high concentrations contain higher amounts of metal chelators when compared to those of lower concentrations.

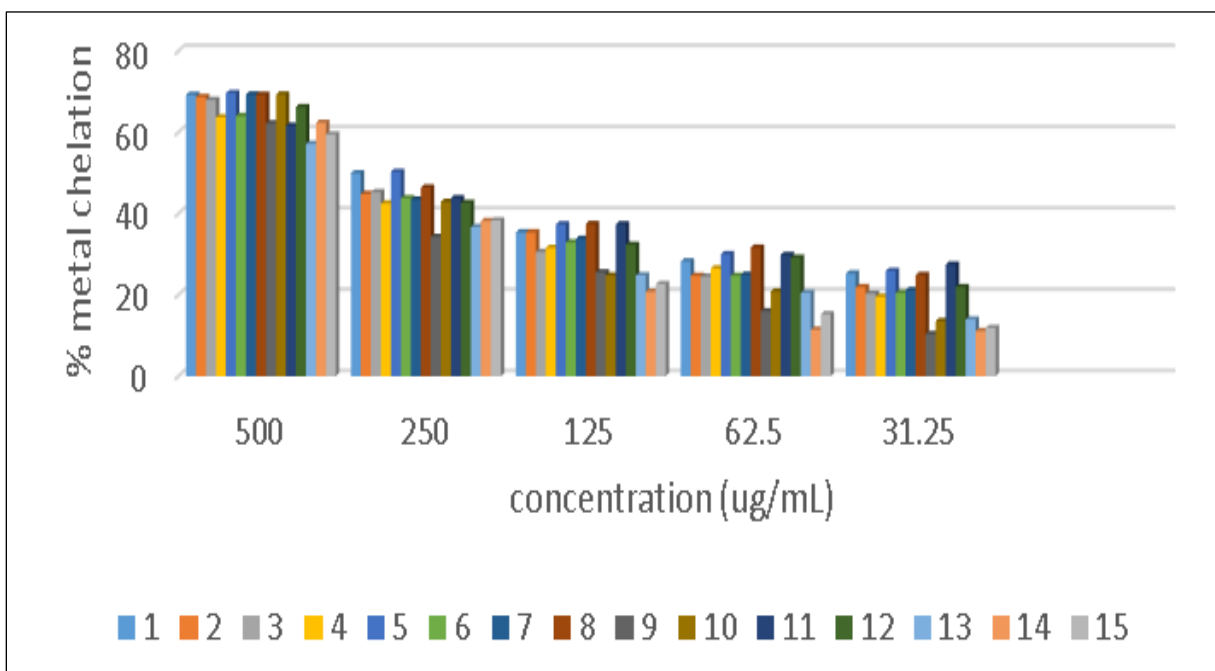


Figure 14: Metal chelation activity of *Terminalia prunioides* at 20 minutes incubation.

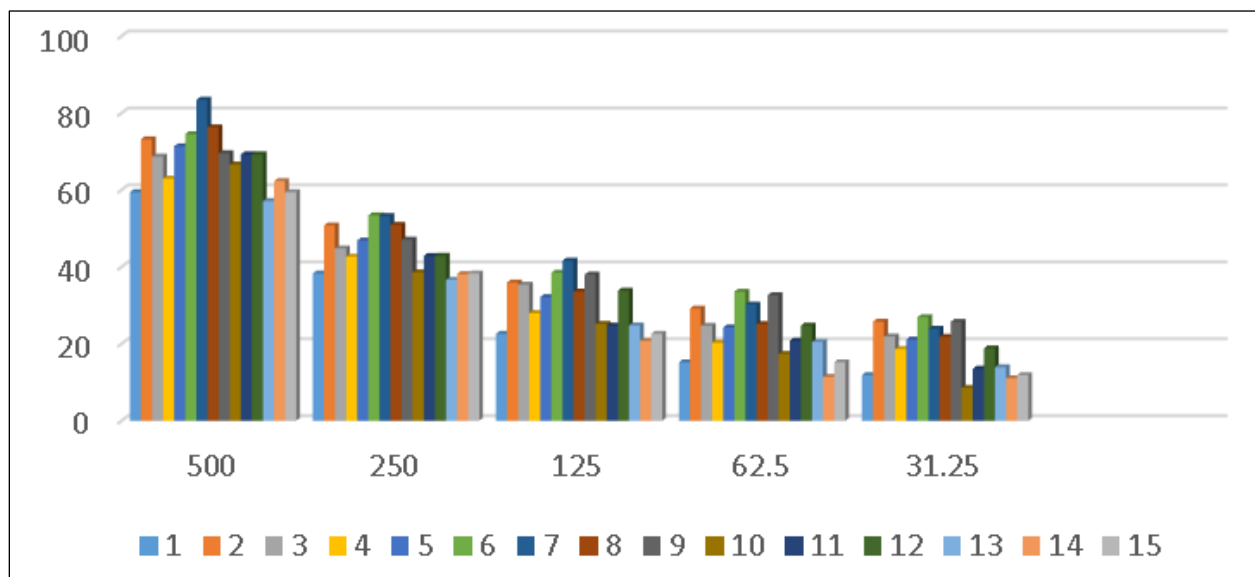


Figure 15: Metal chelation activity of *Terminalia prunioides* at 40 minutes incubation.

The IC₅₀ values for the extracts metal chelation activity were calculated from the equation of the line of calibration plot of % metal chelation against the concentration of extracts. The correlation coefficient of the graphs was maintained at 0.99 and the line fitted Beer's law. The IC₅₀ values were determined and used to explain the metal chelation activity of the extracts as shown in Appendix C.

The sample extracts under study demonstrated a difference in their metal chelation activity as shown by Figure 16. Metal chelation activity was higher in extracts brewed for a shorter time than those extracted at longer times. For instance, extract 1 brewed for 15 minutes had higher metal chelation activity $262.9600 \pm 0.0065 \mu\text{g/mL}$ compared to $353.6983 \pm 0.0065 \mu\text{g/mL}$ of extract 3 brewed for 60 minutes resulting in a 35% decrease in activity. The metal chelation activity was also significantly affected by brewing temperatures. Higher metal chelation activity was observed for Extracts 4 to 8 brewed at temperatures 60 and 70°C compared to 50,80 and 90°C. Figure 16 also indicates that IC_{50} values decreased with increasing incubation time, therefore it can be concluded that longer incubation periods significantly increased the metal chelation activity. Extract 6 at 20 minutes incubation had an IC_{50} value of $341.2236 \pm 0.0065 \mu\text{g/mL}$ which then lower to $294.3413 \pm 0.0065 \mu\text{g/mL}$ at 40 minutes incubation. Thus, from this study, both brewing conditions and incubation time played a significant role in the metal chelation activity of the polyphenolic compounds of the *Terminalia prunioides* pods.

Extracts of the *Terminalia prunioides* pods samples under study exhibited lowered metal chelation activity when compared to positive control EDTA. Literature suggests that the smaller the IC_{50} value, the higher the metal chelating activity of the extract (Md Yusof et al., 2015). The IC_{50} values of extracts were higher than $152.7740 \pm 0.0067 \mu\text{g/mL}$ of the EDTA, which infers that all evaluated extracts have a lesser capacity to chelate ferrous ions compared to the EDTA (standard chelator).

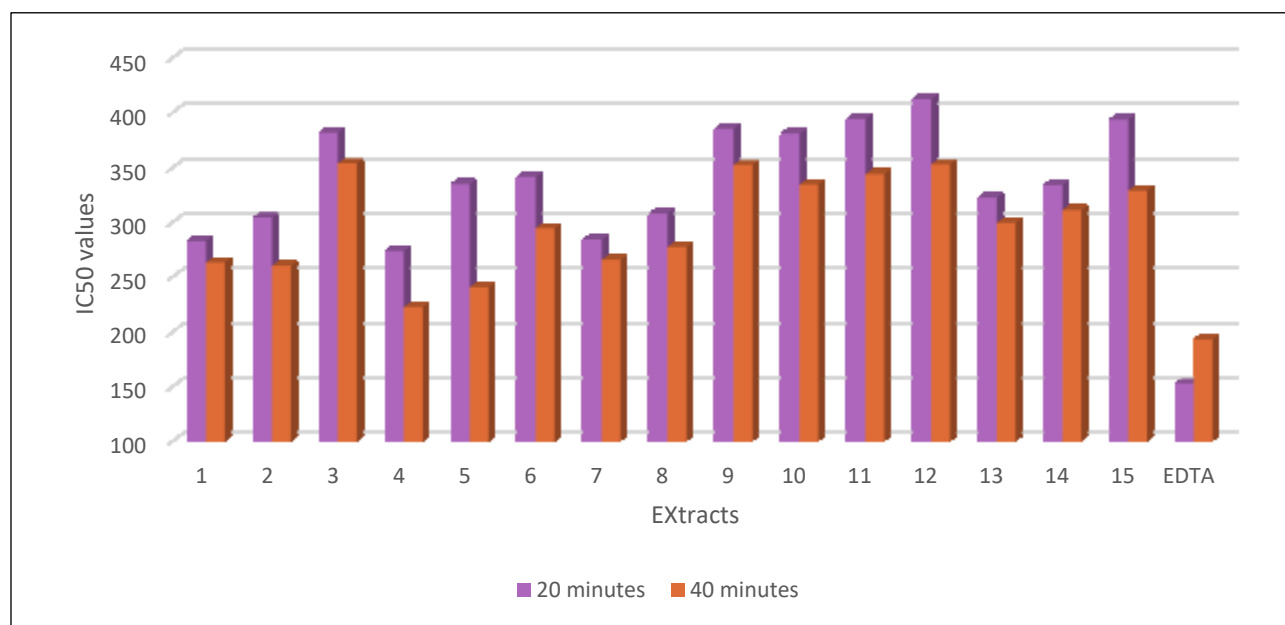


Figure 16: Illustrate the IC_{50} of different *Terminalia prunioides* extracts and Ethylenediamine tetraacetic acid (EDTA) standards at different incubation times.

A combined principal component analysis of the three variables DPPH, TPC, and metal chelation gave the PC1 and PC2, which accounted for 81.60 % and 11.77 % of the total variance of the data set and entirely accounted for 93.40% of the total variance of the data matrix. Through the analysis of PC1, it was possible to observe a strong negative correlation for both total phenolic content and metal chelation activity with DPPH, indicated by Figure 17. This indicates that extracts with the highest strength in scavenging DPPH radical (lower IC₅₀ values) also had higher total phenolic contents and were moderate metal chelation agents (higher IC₅₀ values) as confirmed by extracts 9,10, 11,12 and 15. The phenolic compound's chelation activity is reliant on its unique phenolic structure as well as the number and location of the hydroxyl groups (Hider et al., 2001). Therefore, moderate metal chelation of extracts can depict that the phenolic compounds present in the *Terminalia prunioides* pods possess fewer hydroxyl groups when compared to the EDTA standard molecule.

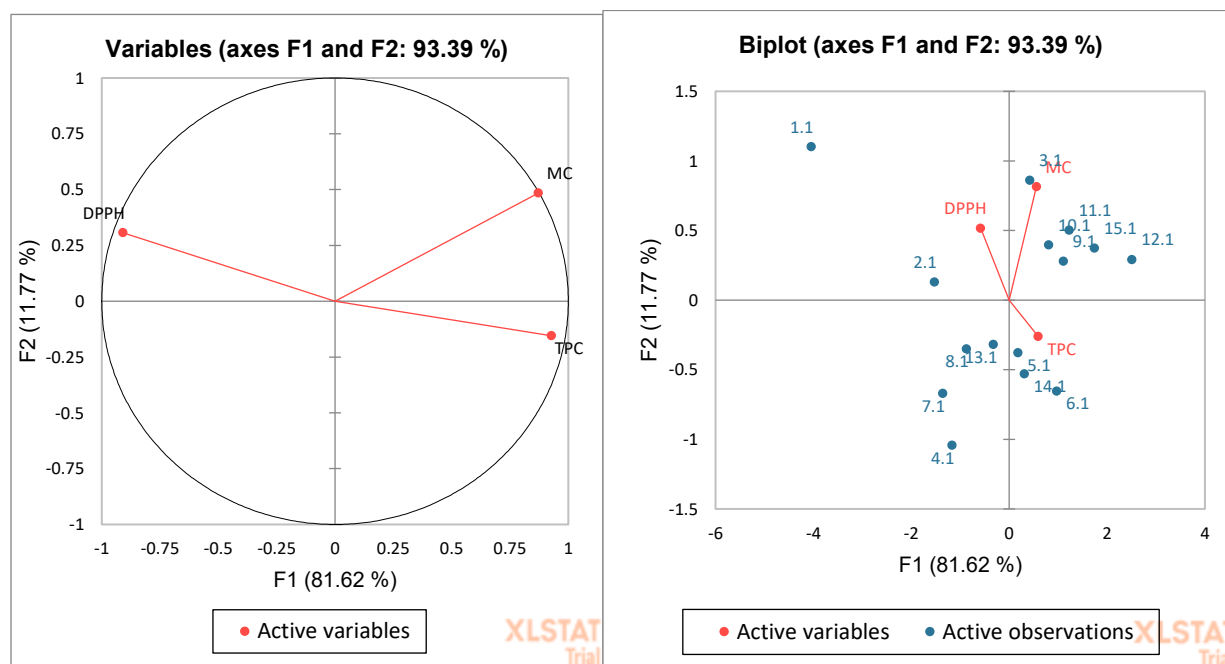


Figure 17: Bio-plot and Correlation circle showing the relationship of Total phenolic content, 2,2-diphenyl-1-picrylhydrazyl and Metal chelation activity of *Terminalia prunioides* pods extract.

4.5 Proximate analysis

4.5.1 Ash content

The *Terminalia prunioides* pods sample was found to have an ash content of $(3.8816 \pm 0.1254 \%)$ as shown in Table 6. Ash is the inorganic residue left after water and organic materials have been removed from a food by heating in the presence of oxidizing agents, and it serves as a measure of the overall

amount of minerals in that food (Park, 1996). Herbal teas are known to be excellent sources of minerals; therefore, they form a major portion of the diets as mineral supplements. Minerals are essential for humans because they are accountable for building strong bones and teeth as well as controlling body fluids inside and outside the cell and they also play a major role in respiration. A deficiency in any type of mineral can cause a variety of diseases such as iron deficiency anaemia and goitre due to iodine deficiency (Nkuba & Mohammed, 2017). Nepal standards for herbal teas indicate that the total ash content in teas should range between 4 and 8 % (KC et al., 2020), which the *Terminalia prunioides* tea under study was close (3.88 %) as shown in Table 6 to this range, thus these tea pods can be used as a good mineral supplement. The Ash content of *Terminalia prunioides* was lower when compared to the ash content of *Terminalia Cattappa* leaves 20.20 ± 0.48 % and 12.10 ± 2.84 % by (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014), respectively.

4.5.2 Moisture content

The moisture content of *Terminalia prunioides* was found to be 9.3265 ± 1.5362 %. The moisture content of the tea leaves can be linked to their shelf life that is the higher the moisture content the lower the shelf life due to an increased rate of food decomposition (Okamura & Zhang, 2000). Therefore moisture content should be controlled between 2.5 and 6.5% to overcome a decomposition reaction, which enables safe storage over a prolonged period and better stability (Jayawardhane et al., 2016). The moisture content of the dried *Terminalia prunioides* pods was found to be above the stipulated range Table 6 shows that the air-drying method was not effective in the removal of water. More time or an alternative method such as the use of dryers at specific temperatures could be trialed to achieve a lower moisture content of the sample (Müller & Heindl, 2006). Its moisture content was comparable to the moisture content of *Terminalia Cattappa* leaves at 7.18 ± 0.18 % and 8.3 ± 2.35 % (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014).

4.5.3 Fat content

The *Terminalia prunioides* sample contained no fat as shown in Table 6. Food sources with very high fat content can contribute to excess adiposity which is responsible for the development of coronary heart diseases, diabetes, cancers, and other disorder therefore it is advisable to reduce or avoid them. Plainly brewed tea should contain no or fewer calories (Wierzejska, 2014). This quality makes *Terminalia* herbal tea a potential weight loss drink. The fat content of *Terminalia prunioides* was comparable to that of *Terminalia Cattappa* leaves 0% and 0.46 ± 0.18 % found by (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014).

4.5.4 Crude fiber content

The dietary fiber is a complex blend of several components such as cellulose, hemicellulose, and pectin. This complex mixture is thought to be responsible for decreasing the blood level of low-density lipoprotein cholesterol by binding it to bile acids. Its binding ability also enables it to play a major role in the elimination of waste from the gastrointestinal tract by binding it to water to soften the stool to allow its easy movement, therefore, preventing constipation (Kartika et al., 2011). Dietary fiber consumption has been demonstrated in scientific research to provide a variety of health benefits, including a lower risk of cardiovascular disease and cancer (Aroyeun, 2013; Kartika et al., 2011; Poswal et al., 2019). Crude fiber can also be used to determine the quality of tea and studies have shown that when the fiber content in teas increases most of the quality characteristics components of tea decrease (Poswal et al., 2019).

The recommendation for fiber content in good quality tea according to WHO standards are less than 16 % and this fiber content is related to maintaining the high quality of tea during storage (Jayawardhane et al., 2016). The *Terminalia prunioides* sample contained 7.5908 ± 1.4409 % crude fiber which was comparable to WHO standard. According to other reports (Aroyeun, 2013), the fiber content in tea products usually varies and ranges between 7 to 20% of which the crude fiber in *Terminalia prunioides* pods sample falls within the given range. Therefore, this herbal tea can be used as an excellent source of dietary crude fiber.

4.5.5 Protein content

Protein is essential for human health and well-being because it provides energy, aids in the movement of biochemicals across cellular membranes, and facilitates enzyme function. In addition, studies have connected adequate protein intake in the diet to the prevention of disorders like sarcopenia in an aging global population (Hayes, 2020; Kartika et al., 2011).

Many herbal teas do not contain any carbohydrates, fat, or protein, and there are no calories in unsweetened tea. The percentage protein content in most teas is 0 % therefore they cannot be taken as protein supplements (Poswal et al., 2019). *Terminalia prunioides* tea contained a protein content of $0.00662 \pm 0.000717\%$ which was significantly closer to 0 %, thus *T. prunioides* tea cannot be used as a protein source. The *Terminalia prunioides* pods contained less protein content when compared to the leaves of *Terminalia cattappa* with 8.67 ± 0.0750 % (or 86.7 ± 0.75 mg/g) (Packirisamy & Krishnamorthi, 2014) and $0.4200 \pm 0.167\%$ (or $(4.2 \pm 1.67$ mg/g) (Offor et al., 2015).

4.5.6 Carbohydrates content

Carbohydrates are carbon, hydrogen, and oxygen-containing molecules produced by plants and animals, and include starches, sugars, and plant and animal fibers. These molecules are important for health because they provide energy and help to diminish the risk of contracting some type of cancer, coronary heart disease, diabetes, and constipation (Guo et al., 2021). Tea leaves and flowers contain water soluble polysaccharides in their cell walls, which provide a variety of health benefits including antioxidant, hypoglycemic, anti-cancer, anti-blood coagulant, anti-radiation, and hepatoprotective properties. (Du et al., 2016).

The *Terminalia prunioides* sample contained 79.8 ± 1.91 % carbohydrates , which is higher than 18.44 ± 0.053 % and 5.79 ± 0.62 % in leaves of *Terminalia cattappa* (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014). Therefore, this herbal tea can be used as a good source of carbohydrates.

Table 6: Proximate analysis results of *Terminalia prunioides*

Parameter	Mean $\pm$ SD
Ash content (%)	3.88 ± 0.13
Moisture content (%)	9.33 ± 1.54
Fat content (%)	0
Protein content (%)	0.00662 ± 0.000717
Crude fiber (%)	7.59 ± 1.44
Carbohydrates content (%)	79.80 ± 1.90

4.6 Metal content

Minerals are found in all tissues of the body and have a role in establishing the proper course of numerous biological processes. Excessive amounts of these minerals in the diet can cause a variety of problems and raise the risk of diseases like cancer, osteoporosis, hypertension, coronary heart disease, and diabetes. The minerals K, Mg, Ca, Na, Mn, As, Cu, Cr, Co, Ni, Fe, Pb, and Zn were found in the *Terminalia prunioides* sample extract in the concentrations given in Table 7.

Copper is regarded as an essential element for all living organisms because it is a component of numerous enzyme systems, including lysis oxidase, cytochrome oxidase, and ceruloplasmin. (Flemming & Trevors, 1989). Copper deficiency and toxicity in living things can both cause anaemia and a congenital inability to excrete copper, which results in Wilson's disease. Even though copper is required for the natural development of living things, it can be toxic depending on the dose and duration of exposure (Flemming & Trevors, 1989; Tsushida, 1977). The concentration of copper in the *Terminalia prunioides* tea sample in this study was found to be 16.01 ± 0.3503 mg/L, which is above the acceptable maximum value set by WHO in both medicinal and edible plants at 10 mg/L (Flemming & Trevors, 1989). Therefore, the concentration of copper in the *Terminalia prunioides* tea sample is a concern from the toxicity perspective. The results of this analysis were compared with the copper concentrations of *Terminalia* species by (Chakradhari et al., 2019) ranging at 1-15 mg/L.

Chromium is an essential element in the human body as it is involved in the normal metabolism of carbohydrates and lipids, as well as the biosynthesis of protein and cholesterol. The function of chromium in the body can be directly linked to the function of insulin (Brzezicha-Cirocka et al., 2016; Duruibe et al., 2007; Tchounwou et al., 2012). Chromium deficiency and toxicity can both be harmful to the human body. Its deficiencies include disruptions in protein metabolism, lipids, and glucose, whereas its toxicity includes nose irritations, skin rashes, bleeds, kidney, stomach upset, and liver damage. The chromium value in all tea samples studied was 2.380 ± 0.7650 mg/L, which is above the 1.3 mg/L permissible limit for chromium in plants (Brzezicha-Cirocka et al., 2016). A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 1-7 mg/L which is comparable to the *Terminalia prunioides* tea sample.

Cobalt (Co) is a trace element that is required for the metabolic processes of humans, animals, and plants. It contains vitamin B12, which is necessary for the formation of red blood cells and the prevention of anaemia. Intake of larger amounts of cobalt can be harmful to the human body, causing complications such as blood pressure, slowed respiration, nausea, diarrhoea, dermatitis, and pulmonary disorders vomiting, whereas deficiency causes weight loss, retarded growth, and anaemia (Brzezicha-Cirocka et al., 2016; Ezeofor et al., 2017). In this study, the concentration of Co was found to be 1.290 ± 0.1550 mg/L which is higher than the WHO allowed limit for Co in medicinal plants of 0.48 mg/L (Brzezicha-Cirocka et al., 2016). A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 1-6 mg/L which is comparable to the *Terminalia prunioides* tea sample.

Manganese is a vital micronutrient in the human body and is commonly found in a variety of foods. It is involved in amino acid, glucose, cholesterol and carbohydrate metabolism, reactive oxygen species scavenging, bone formation, reproduction, and immune response as a cofactor for many enzymes, including manganese arginase, superoxide dismutase, and pyruvate carboxylase (Zidenberg-Cherr et al., 1985). Tea drinkers may consume more manganese than the general population because tea is a good source of manganese. This element's overexposure and deficiency can both have negative consequences. Excessive manganese consumption can have neurotoxic side effects (Basta, 2006; Jarup, 2003). The value of manganese in the tea sample under study was 28.70 ± 0.6001 mg/L which is very low when compared to 200 mg/L of the permissible limit of manganese in medicinal plants (Brzezicha-Cirocka et al., 2016). A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 14-66 mg/L which is comparable to the *Terminalia prunioides* tea sample.

Nickel is a trace element with numerous roles in human bodies, and it is required in small amounts in the pancreas, where it plays a significant role in insulin synthesis. As a result, a lack of it can lead to liver problems (Lockwood et al., 2015). Nickel is not a cumulative toxin in the body, but in excess it can cause toxicity and carcinogenicity, earning it the names such as; genotoxic, nephrotoxic, reproductive toxic, neurotoxic, pulmonary toxic, hepatotoxic, immunotoxin, and carcinogenic agent. (Jarup, 2003; Lockwood et al., 2015). The content of nickel in the tea pod sample under study was determined to be 0.7600 ± 0.1210 mg/L which was below the permissible limit of 10 mg/L, thus Nickel toxicity is not a concern for this plant sample (Ghuniem, 2019).

Lead is a highly toxic trace element with no known function in plants or human bodies. This metal has the potential to cause irreversible adverse health effects on the renal, blood, skeleton, muscular, nervous, cardiovascular and reproductive systems in humans, resulting in poor muscular coordination; thus, its ingestion can result in both chronic and acute toxicity (Chowdhury et al., 2016; Tsushida, 1977; Tuzen, 2003). The amount of lead in the sample under study was 4.245 ± 1.575 mg/L which was above the permissible limit of 2 mg/L according to the WHO (Brzezicha-Cirocka et al., 2016). The study shows that lead (Pb) toxicity from *Terminalia prunioides* tea is a concern with respect to the permissible amount in medicinal plants. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 2-10 mg/L which is comparable to the *Terminalia prunioides* tea sample.

Zinc is a trace element that is required by all plants, animals, and humans. This metal is necessary for normal growth, thyroid function, blood clotting, and as well as protein and DNA synthesis in the human body. Its deficiency, as well as toxicity, can have negative consequences in the body (Jarup, 2003; Roux & Sahut, 1997; Tsushida, 1977). The deficiency of zinc has a negative impact on organ systems in humans, including the immune, gastrointestinal, integumentary, reproductive, and central nervous system. A high level of zinc intake, on the other hand, can cause epigastric pain, fatigue, vomiting, lethargy, and nausea. The WHO allows 0.60 mg/L of zinc in plants (Brzezicha-Cirocka et al., 2016), but the tea under study had a higher zinc content of 1.410 ± 0.6002 mg/L. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 3-7 mg/L which is higher than that of *Terminalia prunioides* tea sample.

Arsenic is a common metalloid found in nature, and in man-made products, including some pesticides and it is classified as heavy metal. It can exist in two chemical forms; inorganic and organic, with the inorganic arsenic compounds being more toxic than ones (Brzezicha-Cirocka et al., 2016; Duruibe et al., 2007; Tchounwou et al., 2012). Long-term subjection to high levels of inorganic arsenic has been linked to an adverse effect on the following organs and systems, lung, kidney, bladder, cardiovascular systems, central nervous system, and even death. The concentration of arsenic was found to be 1.310 ± 0.08020 mg/L in the *Terminalia prunioides* tea leaves sample which is greater than the maximum allowed content of arsenic in medicinal plants of 1 mg/L by WHO (Ghuniem, 2019). A possible cause of this high dose of arsenic could be environmental factors, as the plant was harvested outside ploughing fields and farms. The plant may have absorbed the arsenic from both underground and outflowing water from the fields. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 1-2 mg/L which is comparable to the *Terminalia prunioides* tea sample.

Iron is a trace element that is necessary for human growth, organ function, haemoglobin production, and cell development regulation. Low iron intake leads to nose bleeding, gastrointestinal infection, myocardial infection, and anaemia, whereas excessive iron intake can result in iron toxicity (Abbaspour et al., 2014; Kartika et al., 2011). Iron toxicity can be detected by the following signs; vomiting, nausea, diarrhoea, and stomach pain and its bioaccumulation in the body tissues can cause lethal damage to both the brain and liver. The iron level in the *Terminalia prunioides* tea sample under study was found to be 306.5 ± 1.610 mg/L, which is higher than the WHO permissible limit of 20 mg/L in both medicinal and edible plants (Ghuniem, 2019). *Terminalia prunioides* green tea is, therefore, a good source of iron.

As a result, using it as an iron supplement in a diet could aid in the treatment of iron deficiency anaemia, which affects many people worldwide. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study ranged from 127-227 mg/L which was lower than *Terminalia prunioides* tea sample.

Potassium is a necessary nutrient required for the maintenance of the following: regulate body acids, cell function as well as electrolyte balance. It is also important for water photosynthesis protein, enzyme activation, starch synthesis, and prevention of osteoporosis. Potassium is commonly found in a variety of unrefined foods, especially fruits and vegetables. Its deficiency has been associated with hypertension and cardiovascular diseases (Brzezicha-Cirocka et al., 2016). Whereas its acute toxicity is usually from enormously potassium intake in supplement form but so far none from any food sources (Ezeofor et al., 2017). The concentration of K was 10078 ± 325.0 mg/L in the *Terminalia prunioides* tea pods sample which shows that this tea can be used as a source of dietary potassium. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 288-9364 mg/L which indicates that *Terminalia prunioides* tea sample is a better source of Potassium when compared to other *Terminalia* species.

Magnesium is a vital nutrient that the body requires to stay healthy. This element facilitates many enzymes catalyzed reactions, particularly those involving nucleotides and the reactive species being the magnesium salt, such as MgATP2 (Ezeofor et al., 2017). Mg deficiency is to conditions such as convulsions and abnormal irritability of muscle whereas its surplus has been linked to the central nervous system depression. Green tea infusions have a higher magnesium content because this element is important in the structure of chlorophyll, which changes quite easily due to tea leaf processing (Brzezicha-Cirocka et al., 2016). The magnesium content in the *Terminalia prunioides* tea sample was 2205 ± 65.01 mg/L which is higher than the 2000 mg/L permissible limit. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 105-186 mg/L which indicate that the *Terminalia prunioides* tea sample is a better source of magnesium when compared to other *Terminalia* species

The body needs calcium to maintain the hardness and structure of bones as well as to carry out many imperative functions such as regulation of endo- and exo-enzymes and regulation of blood pressure. Calcium is also required by the body for muscles to move and nerves to transmit messages between the brain and body parts (Cormick & Belizán, 2019; Ezeofor et al., 2017; Kartika et al., 2011). Both calcium

deficiency (hypocalcemia) and excess (hypercalcemia) have adverse effects on the body. Hypocalcemia leads to cataracts, dental changes, osteoporosis, and alterations in the brain, whereas hypercalcemia leads to kidney stones, weakened bones as well as impaired heart and brain function (Cormick & Belizán, 2019; Pravina et al., 2013). The calcium content in *Terminalia prunioides* tea sample was 8930 ± 875.1 mg/L, which shows that this tea can be used as a source of dietary calcium. A study by (Chakradhari et al., 2019) determined that the concentration of this element in *Terminalia* species under study range from 919-965 mg/L which indicates that *Terminalia prunioides* tea sample is an excellent source of calcium when compared to other *Terminalia* species.

Sodium is responsible for maintaining blood pressure, nerve and muscle function, and fluid balance in the body. The total amount of sodium in the body is affected when sodium consumption and loss are out of balance. Food substances contain salt naturally, or salt can be added as an ingredient during cooking as table salt or sodium bicarbonate (Ezeofor et al., 2017; Kartika et al., 2011; Szymczycha-Madeja et al., 2016). Both low(hyponatremia) and high levels of sodium have detrimental effects on the human body. Signs of hyponatremia occur when in the body includes confusion, lethargy, and fatigue. Hyponatremia can also affect body organs, for instance, it can cause kidney failure. Consumption of high levels of sodium can result in the following conditions: heart diseases, high blood pressure, and stroke. It can also result in calcium losses, some of which may be drawn from bone (Grillo et al., 2019). The sodium content in the *Terminalia prunioides* tea sample was 722.4 ± 250.2 mg/L, thus *Terminalia prunioides* tea can be a dietary source of sodium.

Table 7: Metal elements content in the *Terminalia prunioides* pods.

Metal element	Concentration (mg/L)	Permissible concentrations (mg/L)*
Potassium(K)	10078 $\pm$ 325.0	
calcium (Ca)	8930 $\pm$ 875.1	
Magnesium(mg)	2205 $\pm$ 65.10	2000
Sodium (Na)	722.4 $\pm$ 250.2	
Iron (Fe)	306.5 $\pm$ 1.610	20
Chromium (Cr)	2.380 $\pm$ 0.7650	1.3
Copper(cu)	16.01 $\pm$ 0.3503	10
Manganese (Mn)	28.70 $\pm$ 0.6001	200
Cobalt (Co)	1.290 $\pm$ 0.1550	0.48
Lead (Pb)	4.245 $\pm$ 1.575	2
Zinc (Zn)	1.410 $\pm$ 0.6002	0.6
Arsenic (As)	1.310 $\pm$ 0.08020	1
Nickel	0.7600 $\pm$ 0.1210	10

*Permissible minimum content by World Health Organisation (WHO) (Flemming & Trevors, 1989; Ghuniem, 2019)

The contamination of the tea sample by heavy metals such as, Cu, Cr, Co, Zn, and Pb points to environmental pollution issues at the plant collection site, therefore going forward checking for these metal contaminations should be one of the quality control measures before consuming *T prunioides* tea from around the villages and farms. But the tea has desirable essential minerals such as K, Mg, Ca, Na, Mn, Fe, and Ni.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Terminalia prunioides pods tea qualities were assessed using total phenolic content, antioxidant activity, proximate analysis, and metal content determinations. The phytochemical compounds were extracted from the sample using methanol at different temperatures and times. All the antioxidant assays under study; 2,2-diphenyl-1-picrylhydrazyl, total phenolic content, and metal chelation assays provided sufficient scientific evidence that the pod's samples possessed substantial antioxidant activity. It was also demonstrated that both extraction temperature and time affect the antioxidant activity. The best brewing conditions needed to extract effective polyphenolic components for *Terminalia prunioides* herbal tea were determined to be 80° C at 60 minutes as it exhibited the lowest DPPH IC₅₀ value ($0.1600 \pm 0.0489 \mu\text{g/mL}$) and the highest TPC of (12319.880 ± 0.622) mg GAE/g. From the correlations of the antioxidant assays, it was shown that extracts with the highest strength in scavenging DPPH radical contained more phenolic components but were poor metal chelating agents.

The proximate profile of the *Terminalia prunioides* tea was determined to be ash 3.8816 ± 0.1254 %, moisture 9.3265 ± 1.5362 %, Fat 0 %, Crude fiber 7.5908 ± 1.4409 %, and protein at 0.00662 ± 0.000717 %.

The quantification of metal elements showed the presence of the following essential and heavy metals; potassium, calcium, magnesium, sodium, chromium, copper, cobalt, manganese, nickel, arsenic, lead, iron, and zinc at concentrations of 10078 ± 325.0 mg/L, 8930 ± 875.1 mg/L, 2205 ± 65.10 mg/L, 722.4 ± 250.2 mg/L, 2.380 ± 0.7650 mg/L, 16.01 ± 0.3503 mg/L, 1.290 ± 0.1550 mg/L, 28.70 ± 0.6001 mg/L, 0.7600 ± 0.1210 , 1.310 ± 0.08002 , 4.245 ± 1.575 mg/L, 306.50 ± 1.610 mg/L and 1.413 ± 0.6002 mg/L, respectively. The essential elements that were found at permissible values are K, Ca, Mg, Na, Mn, Ni, and Fe, whilst As, Cu, Cr, Co, Zn, and Pb were found at higher levels. The contamination of the tea sample by heavy metals (As, Zn Pb) was attributed to environmental pollution since the plant material was collected on the periphery of Serowe village and next to farms. And this should be one of the quality controls measures in choosing collection points for *T. prunioides* tea.

The tea contains desirable essential minerals such as K, Mg, Ca, Na, Mn, Ni, and Fe and is a potential source of polyphenolic components that can be explored as a source of dietary antioxidants. The proximate analysis data is also sufficient for herbal tea to be considered a food product. All three

profiles provided preliminary scientific data for the quality and efficacy of *Terminalia prunioides* pods as a medicinal plant or herbal tea. Therefore, this phytochemistry study shows that *Terminalia prunioides* pods have the potential to be used in herbal tea formulations.

5.2 Recommendations

The *Terminalia prunioides* phytochemical profile data is not available in the literature, isolations of polar extracts could be done to possibly identify the polyphenolic compounds accountable for antioxidant activity. The catechin and other polyphenol content of the tea sample can also be quantified by HPLC and LC-MS to further determine the quality of herbal tea. The quality assessment can be conducted on samples collected at different seasons and regions to investigate how changes in seasons and environmental pollution impact the plant's antioxidant activity, proximate, and metal content. The above recommendations are meant to address some of the limitations of the study such as small sample size, non-quantification, and identification of the plant polyphenols by HPLC and LC-MS. More antioxidant assays could be used to determine the antioxidant potentials of the plant. The plant could also be assessed for heavy metal binding affinities for environmental remediations. More studies need to be conducted to establish the safety of herbal teas collected in the Masama farms.

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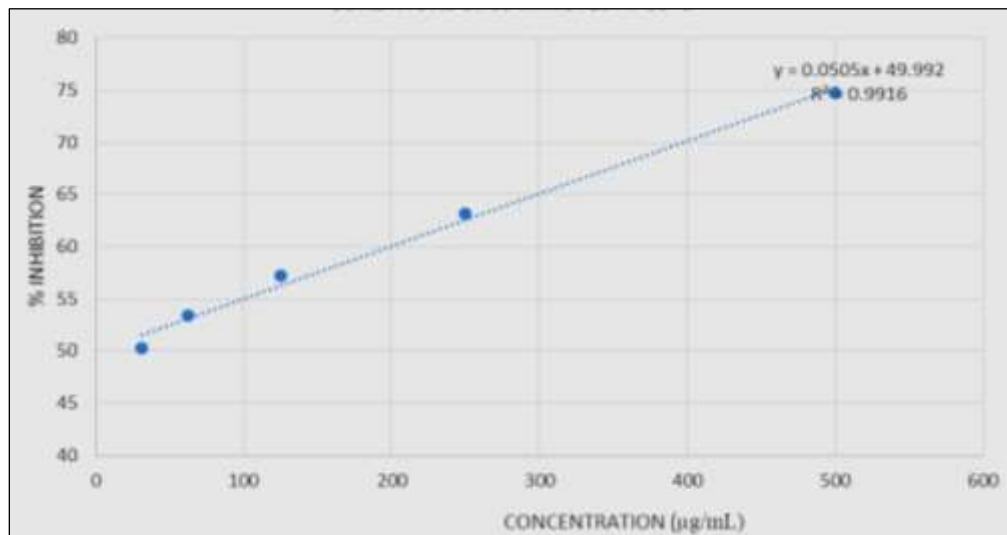
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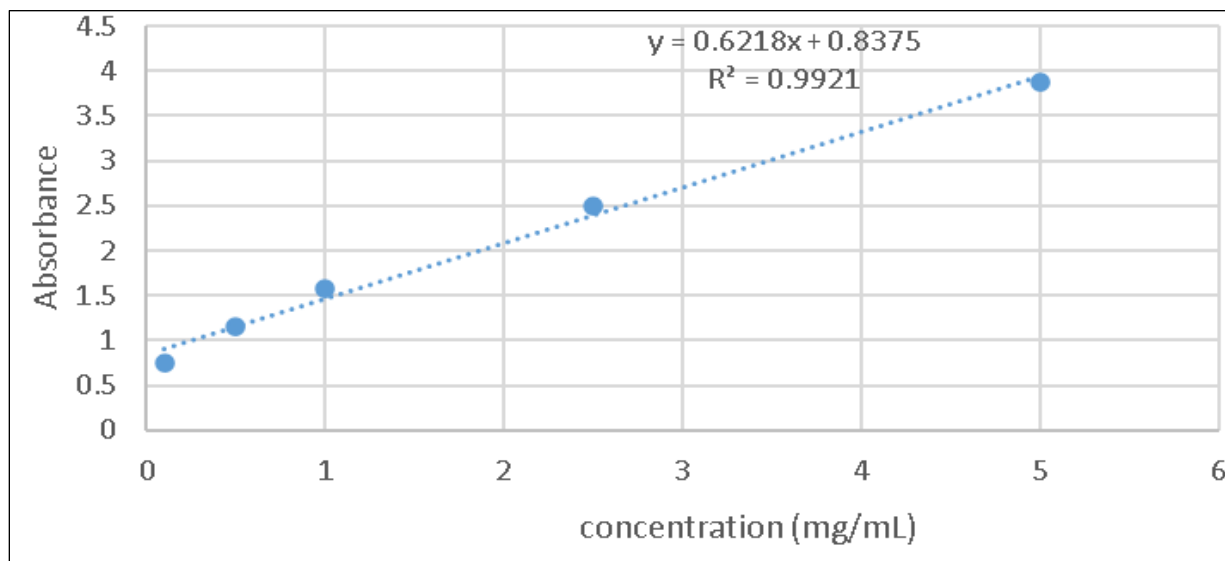
APPENDICES

Appendix A: Shows the calibration curve of standards used to determine the IC₅₀ of the DPPH radical scavenger activity.



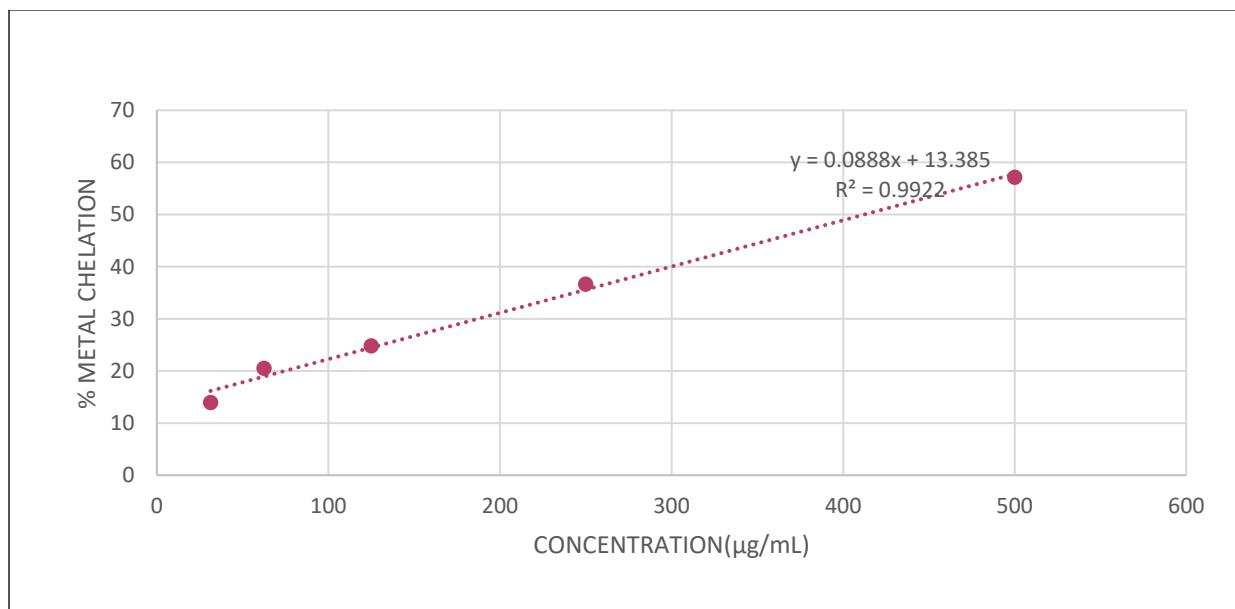
% Inhibition against the concentration of *Terminalia prunioides* extract at brewing conditions 60 minutes and 80°C.

Appendix B: Shows the gallic acid standards curve which was used to calculate the gallic acid equivalents (GAE) concentration in different extracts under study.



Graph of absorbance against the concentration of gallic acids standards.

Appendix C: Shows the calibration curve of standards used to determine the IC₅₀ of the metal chelation activity.



A graph of % metal chelation of *Terminalia prunoides* extracts versus concentration