



# *Terminalia Prunioides* pods herbal tea: Antioxidant activity, proximate, and metal content analysis



Laone Thato Juddie Mosii<sup>a,\*</sup>, Tshepo Pheko-Ofithile<sup>a</sup>, Rosemary Kobue-Lekalake<sup>b</sup>, Ofentse Mazimba<sup>a</sup>

<sup>a</sup> Department of Chemical and Forensic Science, Faculty of Science, Botswana International University of Science and Technology, Palapye, Botswana

<sup>b</sup> Department of Food Science & Technology, Botswana University of Agriculture and Natural Resources, P. Bag, Gaborone 0027, Botswana

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## ABSTRACT

Growth in the demand for herbal teas has led to an increase in their research and commercial activities. In this study, the quantitative assessment of Motsiara (*Terminalia prunioides*) pods herbal tea was performed by determining antioxidant activity, total phenolic content, proximate and metal content. The studied extracts had a significant amount of phenolic content and antioxidant activity. The proximate analysis of ash, moisture, fat, crude fiber, protein, and carbohydrates gave results within the permissible limits for plant materials and teas. For metal analysis the following elements were at permissible values: K, Ca, Mg, Na, Mn, Ni, and Fe, while As, Cu, Cr, Co, Zn, and Pb were found at higher values. The preliminary data shows that *T. prunioides* pods are a potential source of natural antioxidants and minerals that can be useful in herbal tea formulations.

## 1. Introduction

Herbal teas are aqueous infusions of various plant materials, such as leaves, fruits, bark, roots, or flowers in hot or cold water. The antioxidant and quality properties of the herbal tea extract are attributed to its phytochemical constituents (Ozkan & Ozcan, 2006; Ravikumar, 2014). The presence of phenolic compounds, which exhibit antioxidant properties has been reported to prevent or slow the advancement of oxidative stress-related disorders (Tan et al., 2018). The fruits (pods) and leaves of *T. prunioides* have been used for making herbal tea in the southern and eastern parts of Africa as a remedy for postnatal abdominal pains, and to treat coughs, sore throat, and stomachaches (Cock, 2015). The *T. prunioides* species are abundant in several districts of Botswana. These plants belong to the Terminalia genus, one of the largest genera of flowering plants called Combretaceae which is made up of 475 species and 20 genera (Bag et al., 2013; Cock, 2015). The local Setswana names for this plant species are Motsiara, Motororo, Mochara, Mususu, or Mot-siyana (Masoko et al., 2005).

*T. Prunioides* species has small, oval leaves which are 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. It also bears small, oblong-shaped, two-winged red-purple seed pods (fruits), which are 3.5–6.5 cm long and 2–3 cm wide (Masoko et al., 2005; Pfundstein et al., 2010). The endocarp of the seed pod is hard, woody, and one-seeded (Pfundstein et al., 2010). Phytochemical studies of *T. prunioides* have not been reported in

the literature, but a review of independent studies of Terminalia species and members of the Combretaceae family indicates that these plants are excellent sources of phenolic compounds, and as a consequence, excellent sources of dietary antioxidants (Bag et al., 2013; Marques et al., 2012; Masoko et al., 2005; Motlhanka et al., 2014; Pfundstein et al., 2010; Pisoschi & Pop, 2015). The study aimed to determine the antioxidant, proximate, and metal content analysis of *T. prunioides* pods herbal tea to establish its quality (World Health Organization, 2011).

## 2. Materials and methods

### 2.1. Sample collection and preparation

*T. prunioides* pods were collected in March 2020 at the Masama farms in Serowe, Central district, Botswana (S 22.41970°, E 26.75500°). The samples were verified by Dr. Andrew Muzila of the University of Botswana Herbarium, voucher number MZ001\_2022. The pods were washed and left to dry in the shade for four weeks. The pods were pulverized to make the herbal tea and the powdered samples were stored in a zip-lock bag.

### 2.2. Tea extract preparation

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

\* Corresponding author.

E-mail address: [thato.mosii@studentmail.biust.ac.bw](mailto:thato.mosii@studentmail.biust.ac.bw) (L.T.J. Mosii).

set at temperatures of 50, 60, 70, 80, and 90 °C at varying times of 15, 30, and 60 min. Re-extraction was also performed bringing the total number of extracts collected to thirty-two. The residues were then filtered using gravitational filtration and then the solvent was evaporated under the fume hood. The percentage yield of the dry extract was determined, and the extracts were stored in a dry place.

### 2.3. In-vitro antioxidant activity

#### 2.3.1. DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity of *T. prunioides* pod extracts following the modified method (Baliyan et al., 2022). Percentage (%) inhibition was calculated using the formula below where  $A_0$  is the absorbance of the control and  $A$  is the absorbance of the extract. The % inhibitions were used to generate calibration curves which were then used to determine  $IC_{50}$  values.

$$\% \text{ Inhibition} = \left( \frac{A_0 - A}{A_0} \right) \times 100 \quad (1)$$

#### 2.3.2. Metal ( $Fe^{2+}$ ions) chelation activity

The ability of *T. prunioides* pod extract to chelate  $Fe^{2+}$  ions was measured using a slightly modified method (Benzie & Strain, 1996). The chelating activity was calculated using the following formula, where  $A_{control}$  is the absorbance of the control reaction (without plant extract), and  $A_{sample}$  is the absorbance in the presence of a plant extract.

$$\text{Metal chelating activity} = \frac{((A_{control} - A_{sample}))}{A_{control}} \times 100 \quad (2)$$

### 2.4. Total phenolic content

The Folin-Ciocalteu reagent method (Blainski et al., 2013) was used to determine the Total phenolic content (TPC) and it was expressed in terms of gallic acid equivalent.

### 2.5. Proximate analysis

Association of Official Analytical Chemists (AOAC) methods (Baur & Ensminger, 1977) were used to determine the proximate analysis of *T. prunioides* pods herbal tea samples (carbohydrates, fats, proteins, moisture, and ash) while carbohydrate content was calculated using the following formula:

$$\text{Carbohydrate} = [100 - (\text{Protein} + \text{Fats} + \text{moisture} + \text{ash})]. \quad (3)$$

All proximate analysis values were presented as percentages (%).

### 2.6. Metal analysis

Metal elements were extracted from the pod samples using a microwave digestion method (Xing & Veneman, 1998). The contents of heavy metals (Cd, Cu, Ni, Pb, Zn) were determined directly in their respective solutions by the ContrAA Flame Atomic Absorption Spectrum with the air-acetylene flame and hollow cathode lamps. Calcium, magnesium, potassium, and sodium standards of concentrations 0.5, 1, 2, 5 and 10 ppm were prepared, respectively. 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 one ppm were prepared as well. A separate standard of Fe was prepared at concentrations of 0.5, 1, 2, 4, and 6 ppm. The method for running each metal was created using the Analytik Jena software and the three replicates of the standards, samples, and blanks was run. The calibration curve of standards was used to calculate the concentration of metal elements in the samples.

**Table 1**

*T. prunioides* ethanol extracts yields at different brewing conditions.

Extract	Brewing conditions		1st extraction % Yield	2nd extraction
	Temperature (°C)	Time (minutes)		
1	50	15	4.6	2.2
2		30	5.4	3.3
3		60	5.6	4.2
4	60	15	7.2	2.5
5		30	8.0	3.5
6		60	9.8	3.6
7	70	15	6.9	3.4
8		30	7.2	3.6
9		60	11.8	5.1
10	80	15	6.9	4.4
11		30	9.6	4.7
12		60	9.9	7.2
13	90	15	5.8	3.3
14		30	8.8	4.0
15		60	9.2	4.1

### 2.7. Statistical analysis

Microsoft Excel was used to calculate DPPH radical scavenging activity, total phenolic content metal chelation activity, proximate content, and metal content values.

## 3. Results and discussion

### 3.1. Extraction yield

The extraction yields under varying brewing conditions of the first and second extracts are shown in Table 1. Ethanol has been widely used to extract polar antioxidant compounds from plants and plant-based foods (Lezoul et al., 2020) and the hot solvent reflux extraction method has shown to be an effective extraction technique for phytochemicals, resulting in higher yields regardless of the solvent used or plant material (Sultana et al., 2009). Therefore, the hot solvent reflux extraction method of *T. prunioides* pods herbal tea has led to more efficient retrieval of antioxidant components resulting in higher extract yields ranging from 2.2 to 11.8%.

The percentage yield of extracts was dependent on the extraction conditions. Longer extraction times (60 min) resulted in higher percentage yields because they provided sufficient time for the plant material to be in contact with the solvent (Lezoul et al., 2020). Variations in extraction temperatures also affected extraction yields. In this study, an increase in extraction temperature led to an increase in extract yields. However, the extraction rates dropped at 90 °C; this was probably due to heat-sensitive phytochemical degradations (Tambunan et al., 2017). Heat-sensitive phytochemicals in the extract can be degraded at higher temperatures (Tambunan et al., 2017), and the ethanolic plant extracts typically contain polar molecules such as hydrolysable tannins, which are composed of gallic and ellagic acids with a sugar moiety in their structure and may cleave when exposed to higher temperatures (Das et al., 2020).

### 3.2. Total phenolic content and antioxidant activity

The results of the total phenolic content of *T. prunioides* pod herbal tea extracts are outlined in Table 2. Total phenolic content is the quantification of the concentration of different phenolic compounds present in the extract, and it is vital to measure these compounds as they are attributed to high antioxidant activity (Blainski et al., 2013). Phenolic components with high antioxidant activity can scavenge reactive chemical species such as reactive oxygen species (ROS). The reactive chemical species are involved in the oxidative damage of cells and organs in the body and have been associated with diseases such as cardiovascular, cancer, and neurodegenerative diseases

**Table 2***T. prunioides* pod extracts DPPH radical scavenger activity, Metal chelation activity, and Total phenolic content.

Extract	Total phenolic content (mg/g)	IC <sub>50</sub> values (DPPH)	IC <sub>50</sub> values (Fe <sup>2+</sup> Chelation)
1	8184.304 ± 0.622	112.09 ± 0.05	262.9597 ± 0.0065
2	10,336.925 ± 0.622	69.71 ± 0.05	260.0292 ± 0.0065
3	11,012.383 ± 0.622	42.53 ± 0.05	353.6983 ± 0.0065
4	10,502.573 ± 0.622	38.2 ± 0.05	222.3290 ± 0.0065
5	10,743.808 ± 0.622	16.9 ± 0.05	240.4541 ± 0.0065
6	11,559.183 ± 0.622	7.83 ± 0.05	294.3414 ± 0.0065
7	10,150.370 ± 0.622	42.87 ± 0.05	265.4979 ± 0.0065
8	10,361.049 ± 0.622	39.98 ± 0.05	277.2912 ± 0.0065
9	10,738.984 ± 0.622	2.08 ± 0.05	352.1971 ± 0.0065
10	10,663.397 ± 0.622	11.87 ± 0.05	334.2042 ± 0.0065
11	10,972.178 ± 0.622	9.14 ± 0.05	343.8336 ± 0.0065
12	12,319.878 ± 0.622	0.16 ± 0.05	352.6494 ± 0.0065
13	10,668.221 ± 0.622	31.72 ± 0.05	299.2039 ± 0.0065
14	10,701.994 ± 0.622	8.74 ± 0.05	310.9210 ± 0.0065
15	12,186.394 ± 0.622	21.86 ± 0.05	328.5971 ± 0.0065
Ascorbic acid	–	0.8776 ± 0.0011	–
EDTA	–	–	152.7740 ± 0.0067

(Chacko et al., 2010). The ethanolic extracts of *T. prunioides* pod herbal tea showed higher concentrations of phenolic compounds, ranging from 8184.304 ± 0.622 to 12,319.880 ± 0.622 mg GAE/g. Aqueous infusions of Rooibos tea (*Aspalathus linearis*) were reported to have a lower TPC of 860 mg GAE/L while white tea exhibited a comparable TPC of 1293 mg GAE/L (Santos et al., 2018). Other Terminalia species exhibited lower TPC values, *Terminalia Cattappa* water extract (244.33 ± 18.86 mg GAE.g<sup>-1</sup>) and alcoholic extract (142.84 ± 2.09 mg GAE.g<sup>-1</sup>) and *Terminalia chebula* Retz aqueous extract (448.7 ± 2.15 mg GAE.g<sup>-1</sup>) (Marques et al., 2012). While comparable TPC values were reported for *Combretum hereroense* (10 680 mg GAE.g<sup>-1</sup>) (Motlhanka et al., 2014).

The *T. prunioides* pods herbal tea extracts were also assessed for antioxidant activity using DPPH radical scavenger assay and the metal (Fe<sup>2+</sup>) chelation activity methods. All assessed extracts exhibited DPPH scavenger activity with extract number 12 (Table 1) having the highest activity of IC<sub>50</sub> value 0.1600 ± 0.0489 µg/mL. Extract 12 had an IC<sub>50</sub> value lower than the value of the standard ascorbic acid, 0.8776 ± 0.0011 µg/mL. The herbal tea extract also contained higher radical scavenging activity when compared to the prior studies of the methanolic leaf extract of *Terminalia chebula* which exhibited an IC<sub>50</sub> value of 0.1500 × 10<sup>6</sup> µg/mL (150 mg/mL) (Cock, 2015). The extracts also exhibited a significant metal chelation activity as shown in Table 2. The extract's IC<sub>50</sub> values were higher than that of standard chelator EDTA, 152.7740 ± 0.0067 µg/mL, implying that all of the extracts tested have a lower capacity to chelate ferrous ions than EDTA.

The ability of the phenolic compounds in *T. prunioides* pod herbal tea extract to chelate Fe<sup>2+</sup> ions and scavenge the DPPH radical shows that the pods can be used as a source of dietary antioxidants. Studies have shown that dietary antioxidants are responsible for regulating different cellular pathways making them essential in promoting human health (Kurutas, 2015). The antioxidant activity of *T. prunioides* pods herbal tea explains why this plant has been used for centuries to treat a variety of ailments (Chakradhari et al., 2019; Das et al., 2020). Herbal tea extract number four had the highest metal chelation (83%). Literature has shown that extracts capable of chelating transition metals and showing moderate antioxidant activities tend to have phenolic compounds with *ortho*-hydroxyl groups such as chlorogenic acid, catechins, and flavanols (Santos et al., 2018).

*Origanum Sipyleum* which is used in herbal teas was reported to show 203.57 mg GAE/g TPC, 102.75 µg/mL DPPH IC<sub>50</sub> and 20.68% metal chelation on its ethanol extract (Kaska, 2018). The values were lower than for the reported *T. prunioides*. The activities of *O. sipyleum* were ascribed to phenolic components such as rutin, gallic acid, and chlorogenic acid (Kaska, 2018).

### 3.3. Proximate analysis

Ash is the inorganic residue left after water and organic materials have been removed from a food sample by heating it in the presence of oxidizing agents, and it serves as a measure of the overall amount of minerals in that food (Park, 1996). In this study, the *T. prunioides* herbal tea samples were found to contain 3.8816 ± 0.1254% of ash content which was within the recommended ash content in herbal teas of 4 to 8% (Kc et al., 2020). The moisture content was found to be 9.3265 ± 1.5362% which was comparable to the moisture content of *Terminalia Cattappa* leaves; 7.18 ± 0.18% and 8.30 ± 2.35%, respectively (Offor et al., 2015; Packirisamy & Krishnamorthi, 2014). Moisture content should be controlled within the range of 2.5 and 6.5% to overcome a decomposition reaction, which enables the safe storage of samples over a prolonged period and better stability (Jayawardhane et al., 2016). As a result, the samples under study should be given more time to dry or an alternative method, such as using dryers at specific temperatures, to reduce their moisture content (Müller & Heindl, 2006).

The *T. prunioides* pods herbal tea sample contained no fat which was expected given that brewed tea should have no or fewer calories (Wierzejska, 2014). The sample also contained 7.5908 ± 1.4409% crude fiber which was comparable to less than 16% of the standard set by the WHO (Jayawardhane et al., 2016). Therefore, this herbal tea will make an excellent source of dietary crude fiber. The tea also had a protein content of 0.00662 ± 0.000717% which was significantly closer to 0% as found in most teas (Poswal et al., 2019) and thus cannot be used as a protein supplement. The *T. prunioides* pods herbal tea contained 79.8 ± 1.91% carbohydrates which were higher than the 18.44 ± 0.053% and 5.79 ± 0.62% in the leaves of *Terminalia cattappa* reported by Offor et al. (2015), Packirisamy and Krishnamorthi (2014). Therefore, this herbal tea can provide carbohydrates. The proximate analysis data is summarized on Table 3.

**Table 3***T. prunioides* proximate analysis.

Parameter	Mean ± SD
Ash content (%)	3.88 ± 0.13
Moisture content (%)	9.33 ± 1.54
Fat content (%)	0 ± 0
Protein content (%)	0.00662 ± 0.000717
Crude fiber (%)	7.59 ± 1.44
Carbohydrates content (%)	79.8 ± 1.9

**Table 4**  
T. prunioides metal elements and permissible concentrations.

Metal element	Concentration (ppm) Mean $\pm$ SE	Permissible concentrations (ppm)*	Metal element	Concentration (ppm) Mean $\pm$ SE	Permissible concentrations (ppm)*
Potassium(K)	10,078 $\pm$ 325	–	Chromium (Cr)	2.380 $\pm$ 0.765	1.3
Calcium (Ca)	8930 $\pm$ 875	–	Copper (Cu)	16.01 $\pm$ 0.35	10
Magnesium (Mg)	2205 $\pm$ 65	2000	Cobalt (Co)	1.290 $\pm$ 0.155	0.48
Sodium (Na)	722 $\pm$ 250	–	Lead (Pb)	4.245 $\pm$ 1.575	2
Nickel (Ni)	0.76 $\pm$ 0.12	10	Zinc (Zn)	1.41 $\pm$ 0.60	0.6
Manganese (Mn)	28.7 $\pm$ 0.6	200	Arsenic (As)	1.31 $\pm$ 0.08	1
Iron (Fe)	306.50 $\pm$ 1.61	20			

\* Permissible minimum content by WHO for medicinal plants and herbs (Ghuniem, 2019).

### 3.4. Metal content analysis

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, Table 4. The essential elements that were found at permissible values are K, Ca, Mg, Na, Mn, Ni, Zn and Fe, whilst As, Cu, Cr, Co, and Pb were found at higher levels. In comparison to the mineral content of mountain tea, the *T. prunioides* pods tea has been found to contain a higher concentration of potassium, copper, and magnesium, and lower concentrations of other elements (Özcan, 2004). Furthermore, when compared to the commonly consumed green teas, *Camellia sinensis* it was found that *T. prunioides* pods tea contains higher levels of essential dietary elements such as calcium, iron, sodium, and magnesium, making it a great source of these nutrients (Özcan et al., 2008). Nonetheless, it's important to highlight that this tea also contains higher levels of toxic elements, such as chromium, copper, and cobalt, when compared to *C. sinensis*. The contamination of the tea sample by heavy metals (As and Pb) was attributed to environmental pollution since the plant material were collected on the periphery of (S 22.41970°, E 26.75500°) Serowe village farms.

Comparatively, the mountain tea and *C. sinensis* exhibited increased levels of toxic elements such as Zn, in contrast to the pod tea. This highlights that the mineral content of herbal plants can differ due to multiple factors, such as growth conditions, genetic variations, soil composition discrepancies based on location, environmental factors, and the analytical methods utilized to quantify the mineral content (Hamurcu et al., 2010; Özcan, 2004; Özcan & Akbulut, 2008). Additionally, a recent review article by Hlihor et al. (2022) have reported that heavy metal concentrations in medicinal plants, such as *Echinacea pallida*, *Matricaria chamomilla*, *Camellia Sinensis*, and *Calendula officinalis*, vary by species and metal element. Therefore, it is crucial to monitor heavy metal contaminations as one of the quality control parameters when collecting *T. prunioides* pods intended for human consumption.

### 4. Conclusion and recommendations

The extracts of *Terminalia prunioides* pods herbal tea exhibited high total phenolic content. These extracts also exhibited high antioxidant activity as they were able to scavenge the DPPH radical and chelate the Fe<sup>2+</sup> metal ion, with the best antioxidant activity exhibited by extract number 12. This extract exhibited excellent% DPPH radical scavenger activity (IC<sub>50</sub> = 0.1600  $\pm$  0.0489  $\mu$ g/mL), the highest TPC (12,319.880  $\pm$  0.622 mg GAE/g), and moderate metal chelation activity (IC<sub>50</sub> = 352.1971  $\pm$  0.0065  $\mu$ g/mL). The proximate analysis gave results within the permissible limits for plant materials and teas.

The metal content analysis of *T. prunioides* showed the presence of K, Ca, Mg, Na, Mn, Ni, Zn and Fe in permissible amounts while As, Cu, Cr, Co, and Pb were found at higher concentrations. The contamination of the tea sample was caused by heavy metals such as As, and Pb; therefore, more studies need to be conducted to establish the source of heavy metal

contamination of plant samples and apply measures to reduce harmful heavy metals especially As and Pb, from the collection site.

Results indicate that *T. prunioides* can be one of the sources of K, Ca, Mg, dietary fiber, carbohydrates, and antioxidant tea extracts. However, further studies on the polar extracts could be done to identify the compounds accountable for antioxidant and metal chelation activities. The content of catechin, flavanols, tannins, and chlorogenic acids in herbal tea can also be quantified to further determine the quality of this herbal tea. Lastly, requires more studies to be conducted to establish the safety of herbal teas collected within the periphery of farms.

### Declaration of Competing Interest

The authors declare that there is no conflict of interest.

### CRediT authorship contribution statement

**Laone Thato Juddie Mosii:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Tshepo Pheko-Ofithile:** Conceptualization, Supervision, Writing – review & editing. **Rosemary Kobue-Lekalake:** Conceptualization, Supervision, Formal analysis, Writing – review & editing. **Ofentse Mazimba:** Conceptualization, Supervision, Project administration, Writing – review & editing.

### Data availability

Data will be made available on request.

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