



Faculty of Sciences
Department of Chemical and Forensic Sciences

***BOSCIA FOETIDA* SCHINZ. ROOTS AND *XIMENIA AMERICANA* L. FRUITS
EXTRACTS PHYTOCHEMICAL STUDIES, AND *XIMENIA AMERICANA* L. FRUITS
PROXIMATE, MINERAL AND STABILITY ANALYSIS**

by

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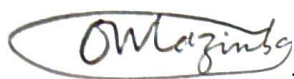


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DEDICATION

I dedicate this work to my younger sister, Charity. She supported and encouraged me so well by sharing the passion for research.

TABLE OF CONTENTS

DECLARATION REGARDING THE WORK AND COPYRIGHT	ii
ACKNOWLEDGEMENTS	iii
DEDICATION	iv
TABLE OF CONTENTS	v
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiii
ABSTRACT	xiv
CHAPTER 1	1
1. INTRODUCTION	1
1.1. Background	1
1.2. Problem Statement	2
1.3. Significance of the study	3
1.4. Aim	3
1.5. Objectives	3
1.6. Research Questions	4
CHAPTER 2	5
2. LITERATURE REVIEW	5
2.1. <i>Boscia</i> Lam genus	5
2.2. <i>Boscia foetida</i> Botanical Description	5
2.2.1. <i>Boscia foetida</i> Distribution and Ecology	7
2.2.2. <i>Boscia foetida</i> Propagation	9
2.2.3. <i>Boscia foetida</i> Ethnomedicinal Uses	9
2.2.4. <i>Boscia foetida</i> other uses	9
2.2.5. Biological Activities of the <i>Boscia</i> species	9
2.2.6. Phytochemistry of <i>Boscia</i> genus	11
2.2.7. <i>Boscia foetida</i> Economic Significance	12
2.3. <i>Ximenia</i> Linn genus	13
2.4. <i>Ximenia americana</i> botanical description	13
2.4.1. Distribution and Ecology	15
2.4.2. <i>Ximenia americana</i> Propagation	16
2.4.3. <i>Ximenia americana</i> ethnomedicinal uses	17
2.4.4. <i>Ximenia americana</i> biological activities	18
2.4.4.1. <i>Ximenia americana</i> antibacterial activity	18

2.4.4.2.	<i>Ximenia americana</i> antifungal activity.....	19
2.4.4.3.	<i>Ximenia americana</i> antiparasitic (Leishmanicidal and Trypanocidal) activity	19
2.4.5.	<i>Ximenia americana</i> phytochemistry	20
2.4.6.	<i>Ximenia americana</i> economic significance	20
CHAPTER 3		22
3.	METHODS.....	22
3.1.	Reagents	22
3.2.	Sample Collection	22
3.3.	Study Design and Experimental site	22
3.4.	Sample Processing.....	23
3.5.	Gas Chromatography Mass Spectrometry Analysis	23
3.5.1.	Extracts Preparation	23
3.6.	Mineral Analysis	24
3.6.1.	Standard Solution Preparation	24
3.6.2.	Microwave Acid-Assisted Digestion	24
3.6.3.	Sample Preparation for Analysis of Calcium.....	24
3.6.4.	Flame Atomic Absorption Spectrophotometry Analysis (FAAS).....	24
3.6.5.	Inductively Coupled Plasma Mass Spectrometry Analysis (ICP-MS)	25
3.6.6.	Health Risk Assessment of Heavy Metals	25
3.6.6.1	Non-carcinogenic risk assessment	25
3.6.6.2	Carcinogenic risk assessment	26
3.6.7.	Mineral Bioavailability Estimation.....	27
3.7.	Nutritional Proximate Analyses	27
3.7.1.	Moisture Content	27
3.7.2.	Ash Content	27
3.7.3.	Crude Fat Content.....	28
3.8.	Anti-Nutritional Factors	28
3.8.1.	Oxalate Content	28
3.8.2.	Alkaloid Content.....	29
3.8.3.	Tannin Content (gallic acid and complex).....	29
3.9.	Statistical Analysis	31
3.10.	Qualitative Preliminary Phytochemical Screening of <i>Boscia foetida</i> Schinz. Leaves and Roots	31
3.10.1.	Alkaloids	31

3.10.1.1. <i>Dragendorff's Reagent Test:</i>	31
3.10.1.2. <i>Wagner's Test:</i>	31
3.10.2. Phenols	32
3.10.2.1. <i>Ferric Chloride Test:</i>	32
3.10.2.2. <i>Lead Acetate Test:</i>	32
3.10.2.3. <i>Ellagic Acid Test:</i>	32
3.10.3. Saponins	32
3.10.4. Terpenoids	32
3.10.5. Coumarins	32
3.10.6. Proteins and Ammino Acids	32
3.10.6.1. <i>Ninhydrin Test:</i>	32
3.10.6.2. <i>Biuret Test:</i>	33
3.10.7. Quinones	33
3.10.7.1. <i>Alcoholic Potassium Hydroxide:</i>	33
3.10.7.2. <i>Concentrated Hydrochloric Acid:</i>	33
3.10.8. Tannins	33
3.10.9. Flavonoids	33
3.10.9.1. <i>Shinoda's Test:</i>	33
3.10.9.2. <i>Lead Acetate Test:</i>	33
3.10.10. Cardiac Glycosides	33
3.10.11. Sterols	34
3.10.11.1. <i>Libermann-Burchard's Test:</i>	34
3.10.11.2. <i>Salkowski's Test:</i>	34
3.10.12. Triterpenoids	34
3.10.13. Reducing Sugars	34
CHAPTER 4	35
4. RESULTS AND DISCUSSION	35
4.1. <i>Boscia foetida</i> preliminary qualitative phytochemical screening of leaves and roots, and mineral element analysis	35
4.1.1. Preliminary qualitative phytochemical screening	35
4.1.2. GC-MS analysis of <i>B. foetida</i>	38
4.1.2.1. Lup-20(29)-en-3-one (Lupenone)	39
4.1.2.2. γ -Sitosterol	39
4.1.2.3. Tetracosane	40
4.1.2.4. 9-Octadecenamide, (Z) (Oleic acid amide)	40

4.1.2.5.	9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	40
4.1.2.6.	Eicosane	41
4.1.2.7.	Phthalic acid, butyl tetradecyl ester.....	41
4.1.2.8.	Octadecane	42
4.1.2.9.	Heptadecane	42
4.1.2.10.	Hexadecane	43
4.1.2.11.	Tetradecane	43
4.1.3.	<i>B. foetida</i> mineral element analysis	44
4.2.	<i>X. Americana</i> (wild plum) mineral content and the effect of long-term frozen storage on mineral content.....	47
4.3.	Health risk assessment of heavy metals in edible parts of <i>B. foetida</i> and <i>X. americana</i>	57
4.4.	<i>X. Americana</i> (wild plum) fruit extracts GC-MS analysis and the effect of storage on the extracts composition.....	59
4.4.1.	GC-MS analysis of wild plum fruit pulp non-polar extracts	59
4.4.2.	GC-MS analysis of wild plum fruit peels non-polar extracts	64
4.4.3.	GC-MS analysis of wild plum fruit coat non-polar extracts.....	68
4.4.4.	GC-MS analysis of wild plum fruit kernels non-polar extracts.....	71
4.5.	Influence of long-term frozen storage on proximate composition of <i>X. americana</i> (wild plum) fruit parts	77
4.6.	Antinutritional factors in <i>Ximenia americana</i> (wild plum) fruit parts	79
4.7.	Pearson correlation between mineral elements and antinutrients	84
CHAPTER 5		90
5.	CONCLUSIONS AND RECOMMENDATIONS	90
REFERENCES		93

LIST OF TABLES

Table 1: Instrument Operating Conditions and Parameters	25
Table 2: Preliminary phytochemical screening of <i>Boscia foetida</i> leaf and root extracts.	35
Table 3: Phytochemical compounds identified in <i>n</i> -hexane root extract of <i>Boscia foetida</i>	44
Table 4: <i>Boscia foetida</i> roots mineral element profile showing both trace and major elements	45
Table 5: Wild plum (<i>X. americana</i>) fruit peels mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.....	48
Table 6: Wild plum (<i>X. americana</i>) pulp mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.	50
Table 7: Wild plum (<i>X. americana</i>) seed coats mineral element profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.....	52
Table 8: Wild plum (<i>X. americana</i>) kernels mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.	54
Table 9: Nutritional information of the wild plum pulp (essential elements) (mg/100 g per serving).....	56
Table 10: Nutritional information of the wild plum kernels (essential elements) (mg/100 g per serving).....	56
Table 11: Estimated daily intake (EDI) of heavy metals in average adults through the consumption of root preparations of <i>B. foetida</i> , pulp and kernels of wild plum (<i>X. americana</i>)	57
Table 12: Hazard quotient values (HQ) through the consumption of root preparations of <i>B. foetida</i> , pulp and kernels of wild plum (<i>X. americana</i>) from the study areas.....	58
Table 13: Hazard indices (HI) through the consumption of root preparations of <i>B. foetida</i> , pulp and kernels of wild plum (<i>X. americana</i>) from the study areas.....	58
Table 14: Carcinogenic risk assessment values for lead and nickel in the wild plum (<i>X. americana</i>) pulp and kernels and <i>B. foetida</i> roots	58
Table 15: Compounds identified in <i>n</i> -hexane pulp extract after harvest (B1).....	61
Table 16: Compounds identified in <i>n</i> -hexane pulp extract frozen for 3 months (B2).....	62
Table 17: Compounds identified in <i>n</i> -hexane pulp extract frozen for 6 months (B3).....	63
Table 18: Compounds identified in <i>n</i> -hexane peels extract after harvest (B1).....	66
Table 19: Compounds identified in <i>n</i> -hexane peels extract frozen for 3 months (B2).....	67
Table 20: Compounds identified in <i>n</i> -hexane peels extract frozen for 6 months (B3).....	67
Table 21: Compounds identified in <i>n</i> -hexane coats extract after harvest (B1).....	69

Table 22: Compounds identified in <i>n</i> -hexane coats extract frozen for 3 months (B2).....	70
Table 23: Compounds identified in <i>n</i> -hexane coats extract frozen for 6 months (B3).....	71
Table 24: Compounds identified in <i>n</i> -hexane kernels extract after harvest (B1)	73
Table 25: Compounds identified in <i>n</i> -hexane kernels extract frozen for 3 months (B2).....	73
Table 26: Compounds identified in <i>n</i> -hexane kernels extract frozen for 6 months (B3).....	74
Table 27: Fruit pulp proximate analysis	79
Table 28: Fruit peels proximate analysis	79
Table 29: Fruit seed coats proximate analysis	79
Table 30: Fruit kernels proximate analysis	79
Table 31: Oxalate: Mineral molar ratios of <i>Ximenia americana</i> (wild plum) fruit parts.....	80
Table 32: <i>X. americana</i> antinutritional factors	82
Table 33: Pearson correlation coefficients for antinutrients to mineral elements in pulp samples.....	86
Table 34: Pearson correlation coefficients for antinutrients to mineral elements in peel samples.....	87
Table 35: Pearson correlation coefficients for antinutrients to mineral elements in coats samples.....	88
Table 36: Pearson correlation coefficients for antinutrients to mineral elements in kernel samples.....	89

LIST OF FIGURES

Figure 1: <i>B. foetida</i> collected from Serule, Central District, Botswana, where A depicts (leaves and flowers), B (whole plant), C (leaves), D (fruit) and E (roots).....	7
Figure 2: Geographical distribution of <i>Boscia foetida</i> Shinz. (POWO, 2023a)	8
Figure 3: The distribution of the most abundant sub-species of <i>Boscia foetida</i> (Subsp. rehmanniana (Pestal.) Toelken) in Botswana (Setshogo & Venter, 2003).	8
Figure 4: Photos of wild plum (<i>X. americana</i>) collections from Tshimoyapula in Central district and Gasita in Southern district of Botswana where A depicts fruits harvesting, B . <i>Ximenia americana</i> tree in the fruiting stage, C . <i>Ximenia americana</i> whole fruit, D . fruits and leaves.....	14
Figure 5: World geographical distribution of <i>Ximenia americana</i> (POWO, 2023b)	16
Figure 6: Geographical distribution of <i>Ximenia americana</i> in Botswana, Southern Africa (Setshogo & Venter, 2003)	16
Figure 7: GC-MS profile of <i>n</i> -hexane extract of <i>Boscia foetida</i> roots.....	38
Figure 8: Lupenone	39
Figure 9: γ -Sitosterol.....	40
Figure 10: Tetracosane.....	40
Figure 11: 9-Octadecenamide, (Z) (Oleic acid amide)	40
Figure 12: 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	41
Figure 13: Eicosane.....	41
Figure 14: Phthalic acid, butyl tetradecyl ester.....	42
Figure 15: Octadecane	42
Figure 16: Heptadecane	42
Figure 17: Hexadecane.....	43
Figure 18: Tetradecane.....	43
Figure 19: Changes in macro and micro mineral elements content of the wild plum fruit peels after harvest and during storage at -4°C. Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	47
Figure 20: Changes in macro and micro mineral elements content of wild plum fruit pulp after harvest and during storage at -4°C. Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	49

Figure 21: Changes in macro and micro mineral elements content of wild plum fruit seed coats after harvest and during storage at -4°C. Different lower-case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	51
Figure 22: Changes in macro and micro mineral elements content of the wild plum fruit kernels after harvest and during storage at -4°C. Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	53
Figure 23: Influence of long-term frozen storage on <i>n</i> -hexane pulp extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles after 3 months and 6 months of storage respectively.	60
Figure 24: Influence of long-term frozen storage on <i>n</i> -hexane peel extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles for extracts stored for 3 and 6 months respectively.....	65
Figure 25: Influence of long-term frozen storage on <i>n</i> -hexane coats extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles of extracts stored for 3 and 6 months respectively.....	68
Figure 26: Influence of long-term frozen storage on <i>n</i> -hexane kernels extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles frozen stored for 3 months and 6 months respectively.	72
Figure 27: Changes in oxalate content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C. Lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	83
Figure 28: Changes in tannin content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C. Lower case letters indicate significant differences between mean values of elements at ($p \leq 0.05$).	83
Figure 29: Changes in alkaloid content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C. Lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).	84

LIST OF ABBREVIATIONS

FAO– Food and Agriculture Organization of the United Nations

WHO- World Health Organization

GC-MS- Gas Chromatography- Mass Spectrometry

ATSDR- Agency for Toxic Substances and Disease Registry

UV-Vis- Ultraviolet-Visible

ILCR- Incremental Lifetime Cancer Risk

EDI- Estimated Daily Intake

mm- millimetre

cm- centimetre

mL- millilitre

g- gram

ppm- parts per million

ABSTRACT

Boscia foetida Schinz is an indigenous plant with reported medicinal and nutritional importance in the local communities. Likewise, *Ximenia americana* L. is also an indigenous wild plant widely used across Southern African countries with reported phytochemical, nutritional, and bioactivity data. This study was designed to evaluate the preliminary qualitative screening of the roots and leaves of *B. foetida*, phytochemical profile and mineral content of *B. foetida* roots extract, and the effect of long-term frozen storage on the nutritional composition and phytochemical profiles of the wild plum (*Ximenia americana*) fruit parts.

Both *B. foetida* *n*-hexane root extract and wild plum (*X. americana*) *n*-hexane fruit parts extracts were subjected to GC-MS analysis. The root extract revealed the presence of various bioactive compounds such as lupenone, γ -sitosterol, 9-octadecenoic acid (Z)-, methyl ester, and 9-octadecenamide (Z). The preliminary qualitative screening of the roots and leaves showed the presence of phenols, alkaloids, saponins, terpenoids, triterpenoids, coumarins, proteins, reducing sugars, sterols, flavonoids, tannins, glycosides and saponins highlighting the potential of *B. foetida* as a medicinal plant. The roots contained significant amounts of K, Ca, Na and Mg. The following elements were at permissible values: Mn, Cr, Fe, Cu, K, Ca, Mg, and Na, while Pb, Co, Ni, and Zn were found at higher amounts exceeding the permissible values. The preliminary data obtained shows that *B. foetida* roots are a potential source of minerals and phytochemicals that can be useful in herbal products preparations.

Wild fruits play a crucial role in the well-being of people, wild plum (*X. americana*) being one of such fruits. However, one of the main variables influencing the nutritional value of fruits is postharvest treatment, hence it is crucial to protect these nutrients through adequate processing and storage. The effect of long-term storage on the nutrient contents of the wild plum fruit parts at -4°C after 3 and 6 months was investigated. The results showed that mineral elements such as Mg, K, Ca, Cu, Mn, Ni, and Fe were well preserved during the entire frozen storage period with K and Ca being the most retained across the fruit parts. Proximates and antinutrients were also preserved. Although the antinutrients showed a certain degree of retention, their recorded values in the kernels of the wild plum are below the permissible toxic levels, except tannins in the pulp after harvest (post-harvest) relative to the previous limit by FAO/WHO. The phytochemicals showed a significant variation except alkane hydrocarbons. Losses of nutritional qualities mostly occurred in the first month of freezing. Taking into consideration

some essential nutrients losses, to effectively contribute to the nutritional needs and food security, it would be necessary to preserve the wild plum fruits by frozen storage within a period not exceeding 6 months.

Non-carcinogenic and carcinogenic health risk assessments were conducted for the heavy metals detected. The carcinogenic health risk analysis revealed that lead and nickel values in the roots (*B. foetida*) and pulp and kernels of the wild plum (*X. americana*) were above the specified limits set by the United States Environmental Protection Agency (USEPA). Furthermore, there were no non-carcinogenic health risks associated with the studied heavy metals, except lead in the pulp, which showed values of hazard quotient (HQ) and hazard index (HI) above 1. In view of the study result, continuous regulatory control is suggested to ensure the safety of the marketed plant products made from the two plants collected from the study area. The current study result will also contribute to the body of literature on the safety of edible indigenous fruits and plant parts in Botswana.

CHAPTER 1

1. INTRODUCTION

1.1. Background

Indigenous plants refer to plants which are endemic to a particular place, and are well known by local communities as sources of medicine and food. In Africa, these plants have always been of great importance in folkloric traditions of the Sub-Saharan region. Their potential to be used as food and medicine had always been the reason for their widespread popularity, surviving even in civilizations that are gradually losing their close link with nature (Hadjichambis et al., 2008). Although these plants have such benefits, man tend to overexploit them which usually result in them becoming extinct, therefore, it is crucial to obtain as much information as possible about these plants' uses before the locals' knowledge of them is lost. This brings us to the realization of the need to study such knowledge systems (Pieroni et al., 2005). (Van Wyk, 2002) in agreement with the before mentioned idea emphasised the need for "a comprehensive and systematic reference source on indigenous plant use" supported by the Shenzhen Declaration which highlighted the need "to value, document, and protect indigenous, traditional, and local knowledge about plants and nature (Welcome & Van Wyk, 2019)."

Numerous of these plants produce edible fruits that are prized for their therapeutic potential and have dual medicinal and nutritional benefits (Aremu et al., 2019). The medicinal effects are usually associated with phytochemicals, defined as plant-originating chemicals, biosynthesized through secondary metabolism, that are non-nutritive, but essential in defence mechanisms against "pathogenic microorganisms, harmful insects, and adverse environmental changes (Bansal & Priyadarsini, 2022; Sharma & Kaushik, 2021)." At least 4000 of the compounds were archived and classified according to their "protective function, physical characteristics and chemical characteristics (Saxena et al., 2013)." Thousands of phytochemicals have already been identified in fruits and vegetables, nevertheless, a greater percentage is yet to be known (Ferreira et al., 2009; Kumar et al., 2023; Oz & Kafkas, 2017). Quite a number of phytochemicals, especially polyphenols, play a very high significant role in antioxidant activities (protection of body organs from cell damage and/or death induced by free radicals which are mostly reactive oxygen species) (Frag et al., 2020) which could be attested to the greater demand for phytochemicals in many fields, such as medicine and cosmetics amongst others (Aravind et al., 2017). Beyond the phytochemical composition of these plants, it is also imperative to provide information on the composition of micronutrients and macronutrients to possibly meet the consumer's needs. Macronutrients are those required "for energy and to maintain the body's structure and systems (Alexander, 2020)" for the entire life, and are normally needed by the body in relatively large amounts;

carbohydrates, proteins and fats are typical examples of macronutrients (Youdim, 2021), whereas micronutrients are required in quite small amounts to engage in various physiological functions for normal growth and development, and to maintain a healthy life (World Health Organization, 2006)." The body cannot produce these nutrients on its own (Youdim, 2021).

These indigenous fruit plants are mostly found in wild environments, however, some are cultivated (Rural Radio Resource Pack, 2007). *Adansonia digitata*, *Sclerocarya birrea*, *Kigelia africana*, and *Ximenia caffra* are some examples of valued indigenous fruit trees in sub-Saharan Africa (Aremu et al., 2019). The fact that these plants are naturally well adapted to their local conditions and/or climates is the best of all their benefits (Cemansky, 2015). They are less prone to chronic damage from pests and diseases making them ecologically more useful than exotic species, in particular "for the conservation of native flora and fauna as well as for the conservation of water (Gemechu et al., 2021)."

1.2. Problem Statement

One of the nations that uses native flora as a source of food and medicine is Botswana. The efforts to scientifically corroborate the reported potential of African indigenous plants as source of nutrition are ongoing. Various studies have been conducted in several countries on *Boscia* Lam. species regarding their phytochemical and nutritional composition and biological activities of different plant parts. However, it has come to light that data is lacking on the phytochemical and nutritional investigations of *Boscia foetida* Schinz plant species. The nutritional value of the plant's leaves and twigs in grazing animals, particularly browsers, was the subject of reported experiments on the species (Aganga et al., 2005; Aganga & Adogla-Bessa, 1999; Havenga et al., 2004). Likewise, *Ximenia americana* L. is one of the widely consumed indigenous fruit plant in most localities of Southern Africa. *X. americana* fruit, was found to possess good nutritional qualities which include, high total phenolic and flavonoid content, high levels of citric acid, ascorbic acid (vitamin C) and potassium (Goosen et al., 2018; Maroyi, 2016), these are important nutritive values in human diet. Reported studies generally focused much on *Ximenia americana* L. fruit as a source of oil, and other plant parts for remediation of different ailments, but there are no reports regarding the composition of non-polar extracts of the fruit pulp, peels, and seed coats taking into consideration the extensive use of the plant fruit. With the kernels, only a few comparative studies have been reported. Although there appears to be broad agreement regarding the biologically active substances found in *Ximenia americana* fruits (wild plum), little is known about changes that occur in their phytochemical and nutritional composition over the course of storage. One of the main elements affecting the stability of phyto-compounds and nutritional components in fruits after harvest is storage time which is one of the factors that, in the long run could impact the fruit's

resilience and marketability after harvest. Storage stability is an important factor for seasonal fruits such as wild plum. Generally, low-temperature or controlled environment storage is frequently used to increase the shelf life of fruits. This study aims at investigating the stability of *X. americana* fruit during storage.

1.3. Significance of the study

The study will generate new knowledge to augment the existing one on the phytochemical and nutritional properties of *Boscia* Lam. and *Ximenia* Linn. genera plant phytochemistry. With the persisting health issue of malnutrition in most African countries, nutritional data could assist in submitting recommendations in terms of edible indigenous plants nutritive value to regulatory agencies such as World Health Organisation (WHO). The demand for traditional nutritious fruits has shown to be in surge, however, just like any other fruit, wild plum (*X. americana*) is highly perishable after harvest (Emongor & Ramagonono, 2019; Galani et al., 2017) due to many factors of which several of them have been investigated, and reported. In this study we sought to investigate the effect of prolonged frozen storage of wild plum (*X. americana*) fruit parts of which the outcomes might increase the chances of the fruit to reach the local market, to impart knowledge on the local consumers regarding the changes that take place in bioactive compounds and the nutritional quality of their consumables, particularly fruits after storage. This knowledge might also be extended to the "health and food industries" (Galani et al., 2017). This project aligns with the United Nations Sustainable Development Goal number 2 (SDG 2) (End hunger, achieve food security and improved nutrition and promote sustainable agriculture) (Grosso et al., 2020) by combating food shortages in rural areas during drought seasons and exploring storage treatments to sustain fruit shelf life. *X. americana* could be used to make nutritional products such as milk, fortified fruit juice, soap oils, and hair conditioners. On the other hand, *B. foetida* is resourceful in making animal feeds, herbal medicine, and coffee.

1.4. Aim

To evaluate the phytochemical and nutritional composition of *Boscia foetida* Schinz and *Ximenia americana* Linn. plants and the storage stability of the wild plum (*X. americana*) fruit.

1.5. Objectives

- To establish a GC-MS phytochemical profile of the non-polar extracts of *Ximenia americana* fruit and the roots of *B. foetida*.
- To perform a preliminary qualitative phytochemical screening of *B. foetida* leaves.
- To determine the nutritional composition of the wild plum (*X. americana*) fruit parts.

- To determine the effect of long-term frozen storage on the nutritional and phytochemical composition of *X. americana* fruit.

1.6. Research Questions

- What is the phytochemical composition of *Boscia foetida* Schinz roots and *Ximenia americana* L. fruit parts extracts?
- What is the nutritional composition of the wild plum (*X. americana*) fruit parts?
- Are there variations in phytochemical and nutritional composition of the wild plum (*X. americana*) fruit when frozen for a prolonged time?

CHAPTER 2

2. LITERATURE REVIEW

2.1. *Boscia* Lam genus

Boscia Lam. is a genus of small trees and shrubs which belong to the subfamily "Capparoideae" of the Capparaceae family (Kers, 2003). Capparaceae is an angiosperm plant family with a pantropical distribution comprising of approximately "40 to 45 genera and 700 to 900 species" throughout the tropics of the world. Botanical records confirmed eight of the genera to be endemic to southern Africa (SANBI, 2022a) with five identified and located in Botswana (Hyde et al., 2022a). A new species belonging to the genus *Thilachium* was identified from the collection material recovered during an expedition in Madagascar in 1938, which originally was identified as *Capparis* sp (Fici, 2021). This significantly brought about an increase in the number of species both within the genus and the family. Capparaceae is a morphologically diverse plant group with low number of collections as a limiting factor to better understand the interrelations among closely related species in terms of morphology. This family comprises of species exhibiting varying growth habits ranging from perennial herbs, shrubs and subshrubs, trees, climbers, scramblers and lianas and occurs primarily at low altitudes (Hall et al., 2002; Kers, 2003; Luber et al., 2022). Flowers can either be actinomorphic or radially symmetric, zygomorphic or bilaterally symmetric (Joly & Schoen, 2021; Luber et al., 2022), on rare occasions, unisexual and bisexual (Kers, 2003). Some animal species such as hummingbirds, bees, hawkmoths, and bats necessitates the process of pollination with bat pollination the most recurrent in the family (Hall et al., 2002; Kers, 2003).

2.2. *Boscia foetida* Botanical Description

Boscia foetida Shinz. is a shrub or small tree that can reach a height of 10 meters (Maroyi, 2019b). It resembles the closely related *Boscia albitrunca*, which can be distinguished from most subspecies by its smooth fruit and discoloured, noticeably larger leaves. Additionally, it could be mistaken for *Maerua parvifolia* (iNaturalist, 2023). It has spreading branches that typically culminate with spines (WFO (The World Flora Online), 2023). The stems have an unpleasant aroma when cut. On short terminal lateral stems, the blooms grow in dense clusters and have a disagreeable aroma and yellowish green colour (Maroyi, 2019b).

The leaves are linear to oblanceolate in shape, leathery, and both sides range in colour from dark green to grey-green (Maroyi, 2019b) and on tiny knobs are grouped in compact clusters (iNaturalist, 2023). The petiole is about 1-3 mm long. Mesophyll sclereids differed into a foot, stalk, and occasionally a

branching apex that reached into the center of the leaf, were solitary, similar on both sides, or were significantly shorter or non-existent on the abaxial side of the leaves. The plant has an axillary, racemose, sessile inflorescence with a pedicel that is 0.3-2 cm long and hairy. It has four sepals, 2-3 mm long, elliptic, hairy, or at least glabrous within. The flower also comprises of about 6-15 stamens, 2-3 mm long filaments, hairy gynophore, approximately 0.5 mm long style, and a capitate stigma with an ovary capable of producing 8-16 ovules (WFO (The World Flora Online), 2023). Additionally, they give off a foul smell, after which the tree is named (*iNaturalist*, 2023). With darker bark showing in the crevices, the smooth bark is light grey to grey in color. Young branchlets exhibit plum-colored underbark above grey bark (*iNaturalist*, 2023).

The fruit has a spherical shape, about 1 cm in diameter, and typically have one brown seed (*iNaturalist*, 2023; WFO (The World Flora Online), 2023). It is coated in many hairs, and is a velvety, yellowish in colour (Maroyi, 2019b).

Boscia foetida is split into five sub-species based on the number of stamens in the flower and their location. These include *Boscia foetida* subspecies "*Filipes* (Gilg) M.C. Lötter", "*Foetida* Schinz", "*longipedicellata* (Gilg) Toelken", "*minima* Toelken", and "*rehmanniana* (Pestal.) Toelken" (Maroyi, 2019b; SANBI, 2022c). The *filipes* (Gilg) M.C. Lötter subspecies is generally found in northern Kwazulu-Natal and Limpopo (South Africa), eastern Eswatini, and southern Mozambique. It grows up to 4 m long with 6-8 stamens within the inflorescence. The *Foetida* is characterized by 11-15 stamens within its flowers, and it is found in the vicinity of Namibia stretching to the Northern Cape (South Africa). Subspecies *longipedicellata* (Gilg) Toelken also has 11-15 stamens but with long and slender flower stalks, and single-stemmed reaching up to 1 m long in growth (*iNaturalist*, 2023). While, *minima* Toelken/ (Gilg) M.C.Lötter is characterized by small shrublets and is found in the North West province, Western Limpopo, Northern Gauteng, and the vicinities of Kruger National Park in South Africa. The Subspecies *rehmanniana* (Pestal.) Toelken comprises of 5-7 stamens within its flowers, and is distributed in the Transvaal bushveld, northern Mpumalanga stretching towards central Botswana (*iNaturalist*, 2023; SANBI, 2022c).

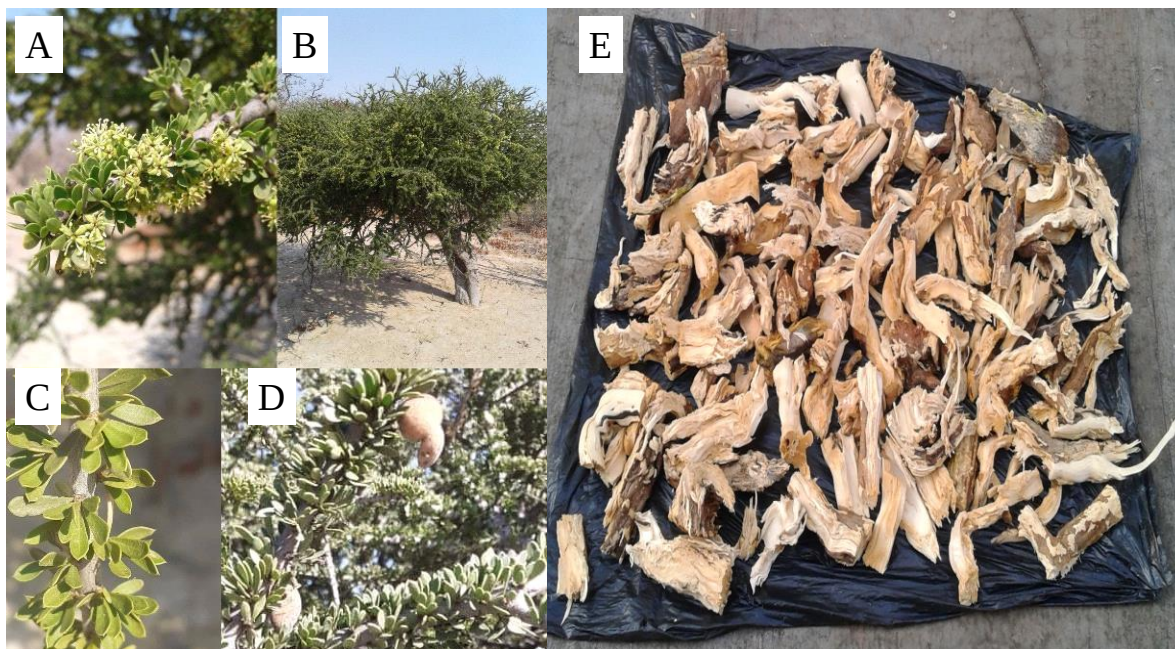


Figure 1: *B. foetida* collected from Serule, Central District, Botswana, where **A** depicts (leaves and flowers), **B** (whole plant), **C** (leaves), **D** (fruit) and **E** (roots).

2.2.1. *Boscia foetida* Distribution and Ecology

Boscia foetida covers the Cape provinces, Kwazulu-Natal, northern provinces of the republic of South Africa, Botswana (mostly in the central region), Namibia, Zimbabwe (mostly in the southern), Mozambique and Swaziland, present day Eswatini (*iNaturalist*, 2023; POWO, 2023a)

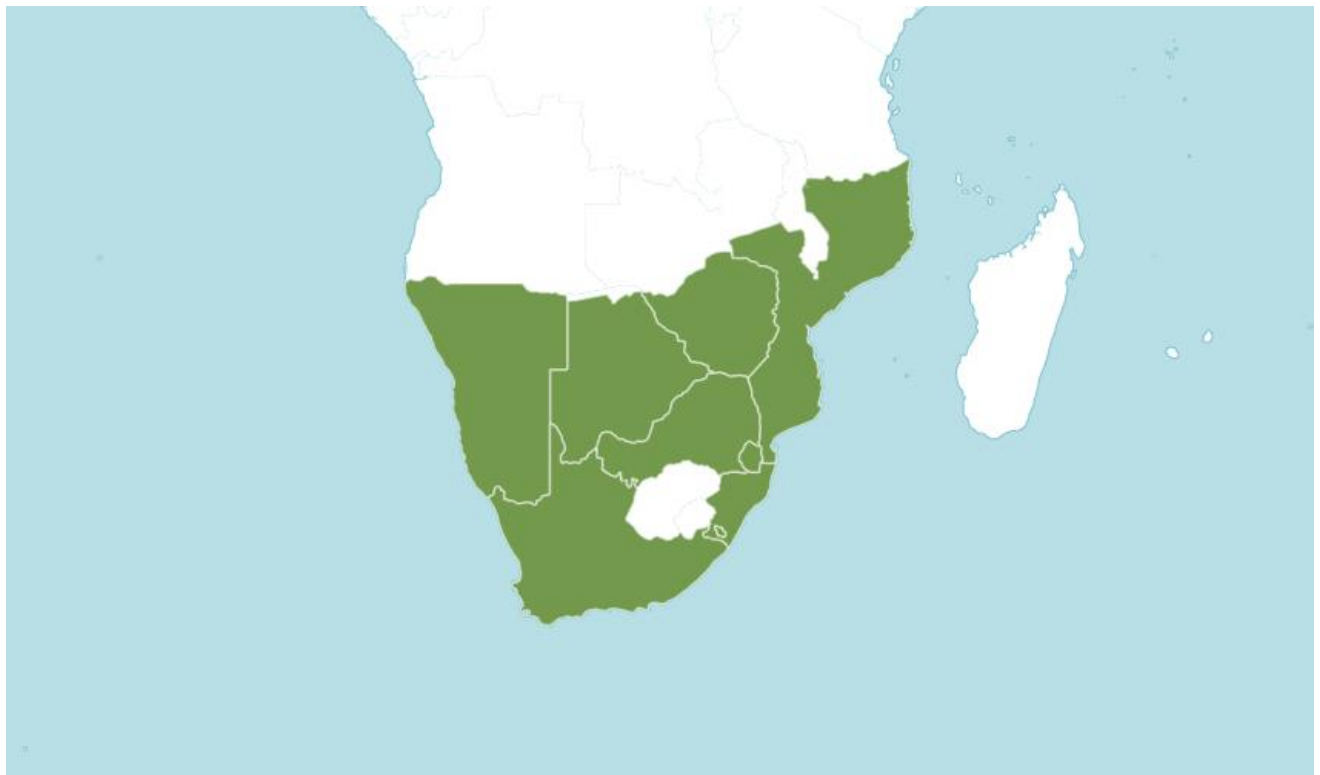


Figure 2: Geographical distribution of *Boscia foetida* Shinz. (POWO, 2023a)

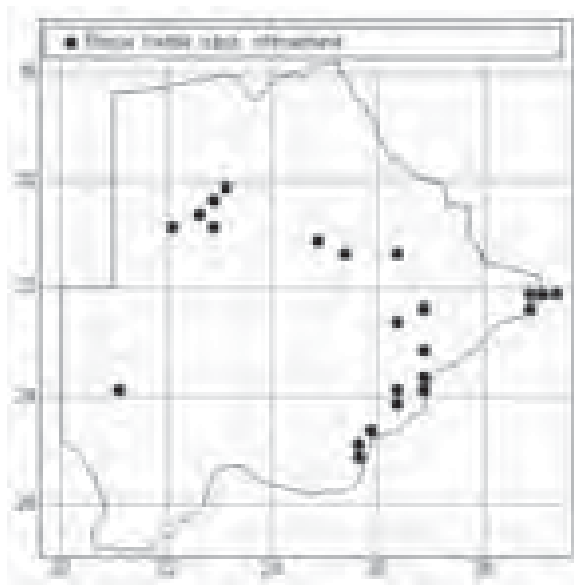


Figure 3: The distribution of the most abundant sub-species of *Boscia foetida* (Subsp. *rehmanniana* (Pestal.) Toelken) in Botswana (Setshogo & Venter, 2003).

Boscia foetida has been found at altitudes between 50 m and 1400 m above sea level on stony or rocky slopes in karroid, semi-desert regions, dry river beds, dry bushveld, and on termite mounds (*iNaturalist*,

2023; Maroyi, 2019b). It maintains its foliage all year long and has a deep root system. A significant increase of the species have been noted in the north-western part of Ghanzi, Botswana because of the availability of ground water (Auret, 2018). *Boscia foetida* was reported to be growing in clusters (Käller, 2003).

2.2.2. *Boscia foetida* Propagation

Germination studies reported that 50% of the plant seeds were viable and produced young healthy plants clearly indicating the plant's means of propagation (Galpin & Galpin, 1935).

2.2.3. *Boscia foetida* Ethnomedicinal Uses

The use of different plant parts of *Boscia foetida* across the regions of the world for medicinal purposes is diverse. In Namibia, the leaves and twigs are used to treat back pains, colds, ear and eye problems, and stomach conditions which are also treated by a combination of twigs and roots (Maroyi, 2019b; Van Damme et al., 1922). Furthermore, in South Africa, leaves are used as a remedy to treat womb problems, roots in treatment of menstrual problems, and flowers are used in a superstition of going against lightning. Roots were reported to treat impotence in Kenya and Nigeria. In Kenya, roots are mixed with the roots of *Uvaria leptopoda* (King) R.E. Fr. and *Combretum hereroense* Schinz. Mozambican natives uses the roots as a remedy for kidney problems, and the leaves and twigs are used for stomach issues (Kaingu et al., 2013; Koopman, 2011; Maroyi, 2019b; Philander, 2011; Ribeiro et al., 2010). In Botswana, leaves are used as an ethnoveterinary medicine to treat eye problems in livestock in a form of crushed dried leaves mixed with either salt or sugar (Gabalebatse et al., 2013).

2.2.4. *Boscia foetida* other uses

The uses of *Boscia foetida* Schinz plant also include the use of dried, roasted and pounded roots to make coffee, and the pounded meal of roots can also be used to make soft porridge, fruits are used to make a sweet drink, mixed with either milk or water or as a flavouring agent in food (Maroyi, 2019b; Moteetee et al., 2019). Leaves, and twigs are utilized as fodder for livestock since ancient times and shown to be nutritious, contributing to the diet of browsers such as goats (Aganga et al., 2005; Aganga & Adogla-Bessa, 1999; Havenga et al., 2004), and the plant's wood is capable of making furniture as indicated by (Neelo et al., 2015).

2.2.5. Biological Activities of *Boscia* species

There aren't any accounts of the *Boscia foetida* plant's bioactivity, but scientists have looked into the bioactivities of other species in the same genus. An *in-vitro* study was conducted (Pendota et al., 2015) to assess the antimicrobial (bacterial and fungal) activities of the leaves of *Boscia albitrunca*. The *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol leaf extracts were tested against four bacteria:

Bacillus subtilis, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*, and one fungal strain, *Candida albicans* using micro-dilution assay in 96-well microplates. All the extracts exhibited a wide range of activities against bacteria but the *n*-butanol fraction showed the best with MIC value ranging from 390.0-1560.0 µg/ml. The *n*-butanol fraction further afforded a compound called martynoside which also showed good antibacterial activity against *Bacillus subtilis* and *Klebsiella pneumoniae* strains with lowest MIC values of 7.81 and 31.2 µg/ml respectively. Therefore, the study suggested that the leaf extracts might be useful for treating ocular infections.

Boscia angustifolia hydro-alcoholic (methanol/water) leaf extract was tested against the extremely contagious avian influenza A virus (H5N1) found in Egypt which infected chickens in the year 2013 using plaque reduction assay with confluent 24hr old monolayer of MDCK cells in six-well plate (Salem, Hussein, El-Sharawy, El-Khateeb, et al., 2016). The crude extract further afforded a pure compound, "Ombuin," and they were all subjected to antiviral activity assessment. The isolated Ombuin exhibited outstanding inhibition of 68.5% at a concentration of 80 µg/µl against H5N1 virus whereas the crude extract inhibited at a rate of 63% at the same concentration (Salem, Hussein, El-Sharawy, El-Khateeb, et al., 2016).

The anti-inflammatory activity of the hydro-methanolic leaf extract of *Boscia coriacea* was evaluated using carrageenan-induced pore edema model. The extract exhibited a significant inhibition rate of 75% after six hours of carrageenan injection. The study showed that there are anti-inflammatory agents in the leaf extract of *Boscia coriacea* (Tseha et al., 2022).

In Cameroon, *Boscia senegalensis* was evaluated for antibacterial activity of its *n*-hexane, chloroform, methanol and water extracts against gram-positive *Staphylococcus aureus*, and gram-negative *Escherichia coli* and *Salmonella typhi* bacterial strains using agar disc diffusion and broth dilution techniques. The results showed a significant inhibition of *Staphylococcus aureus* by the hexane extract covering 16-, 14.75- and 13.25-mm zones of inhibition at concentrations of 20, 10 and 5 mg/ml respectively. On the other strains were no inhibitions (Vougat Ngom & Foyet, 2022).

Boscia salicifolia aqueous and methanolic stem bark extracts were tested *in-vitro* and *in-vivo* against chloroquine-sensitive and resistant *Plasmodium falciparum* using semi-automated micro-dilution method as well as against chloroquine-sensitive *Plasmodium berghei* strain ANKA based on a four-day suppressive test respectively. For *in-vitro*, the extracts showed IC₅₀ values of 1.0 µg/ml and 3.7 µg/ml for chloroquine-sensitive, and 8.9 to 10.1 µg/ml activity against the resistant strain. For *in-vivo*,

the extracts showed activity in inter-peritoneal injection therapy with 86.5% and 43.8% chemo-suppression, which was less than the 99.0% chemo-suppression of malaria pathogens shown by chloroquine as a positive control (Maroyi, 2019c).

Boscia variabilis Collet and Hemsl ethyl acetate, 70% ethanol, and water flower extracts were tested against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Staphylococcus aureas* and *Candida albicans*. All the extracts exhibited significant zone of inhibition within a range of 11-20 mm, and among them, ethyl acetate extract showed remarkable activity (activity range) against all tested microbes. The minimum inhibitory concentration of the three extracts were determined against *E.coli* and *S.aureas* in particular which have been reported to be the most common causes of diarrhoea, and the ethyl acetate extract exhibited the lowest MIC value of 12.5 mg/ml. The study suggested the possible use of *Boscia variabilis* thamone flowers in treatment of diarrhoea (Aye et al., 2018).

2.2.6. Phytochemistry of *Boscia* genus

The qualitative phytochemical screening of water, acetone and acetone/water leaf extracts of *Boscia albitrunca* revealed the presence of alkaloids, steroids, saponins, terpenoids, tannins, flavonoids, anthraquinones and coumarins (Shatri & Mumbengegwi, 2020). The phenolic compound, martynoside was isolated from the *n*-butanol fraction of 20% aqueous methanolic leaf extract of *Boscia albitrunca*, and subjected to antimicrobial activity assessment which showed low MIC values of 7.81 and 31.2 µg/ml against *B.subtilis* and *K. pneumoniae* indicating substantial activity (Pendota et al., 2015). *Boscia mossambicensis* leaves and twigs reportedly contain 13.9 and 29.4 µg/g dry matter of α -tocopherol, and 0.4 and 1.5 µg/g of γ -tocopherol respectively (Maroyi, 2019b). Furthermore, sulfuric acid lignin (9.5-18.5%) was also measured from the leaves and twigs (Maroyi, 2019b). *Boscia foetida* leaves, stems and twigs were reported to contain 37.1-45.5 % of acidic detergent fibre, 136.0-222.0 g/kg of acidic detergent lignin, 0.3 and 0.4 % condensed tannins and total tannin respectively (Aganga & Adogla-Bessa, 1999; Maroyi, 2019b).

GC-MS profiling of the silylated (derivatized) aqueous methanolic leaf extract of *Boscia angustifolia* revealed a mixture of 42 compounds. The extract afforded "Ombuin" (7,4'-dimethoxy quercetin) which showed a remarkable antiviral activity at a rate of 68.5% at a concentration of 80 µg/µl against avian influenza A virus (H5N1) (Salem, Hussein, El-Sharawy, El-Khateeb, et al., 2016). Furthermore, (Salem, Hussein, El-Sharawy, Ragab, et al., 2016) a total of 10 compounds were reported, namely; tamarixetin-7-O-methylether, tamarixetin-3-O- β -sophoroside-7-O-methylether, rhamnocitrin-4'-O-

methylether, rhamnocitrin-3-O- β -glucopyranoside, rhamnocitrin-3-O- β -(6'-O-E-p-coumaroyl)-glucopyranoside, rhamnocitrin-3-O- β -sophoroside, esculetin, esculetin 6,7-dimethylether, methyl-4-methoxy benzoate and phthalic acid decyl octyl ester. The 80% methanol leaf extract of *Boscia coriacea* reportedly contain alkaloids, flavonoids, tannins, cardiac glycosides, phenols, quinones, and saponins (Maroyi, 2019b; Tseha et al., 2022). Fruit pulp and seeds of *Boscia senegalensis* were subjected to qualitative phytochemical screening. The screening let out the presence of sterols and triterpenes, alkaloids, flavonoids and polyphenols in all the screened parts (Awa et al., 2020). Alkaloids, phytosteroids, and triterpenoids were abundant in the root alcohol extract, while saponins, anthraquinones, and tannins were detected in only modest amounts. Flavonoids were not detectable (Osuala et al., 2022). A aqueous methanolic extract fraction afforded a total of eight compounds upon isolation, and these were identified as 4'-hydroxy-3,3',4,5,5'-pentamethoxy-7,9':7'9'-diepoxy lignane, pinoresinol-4'-O- β -D-glucopyranoside, liriodendrin, alangilignoside D, (+)-lariciresinol-9-O- β -D-glucopyranoside, (+)-lyoniresinol-3 α -O- β -D-glucopyranoside, (-)-lyoniresinol-3 α -O- β -D-glucopyranoside, dehydrodiconiferyl alcohol-4-O- β -D-glucopyranoside, rhamnocitrin-3-O- β -D-glucopyranoside, rhamnocitrin-3-O- β -D-(6''-O-E-p-coumaroyl)-glucopyranoside, rhamnocitrin-3-O- β -D-(6''-O-E-feruloyl)-glucopyranoside, syringin, methyl isothiocyanate, methyl-2-propyl, lasianthionoside A, and isopropyl isothiocyanate (Maroyi, 2019d; Morgan et al., 2015, 2017).

Qualitative phytochemical screening of 80% methanol leaf extract of *Boscia salicifolia* revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and terpenoids (Elon et al., 2022). A total of four flavonoids were isolated from the leaves, and identified as rhamnocitrin-3-O- β -neohesperidoside, rhamnetin-3- β -O-neohesperidoside, rhamnocitrin-3-O- β -glucopyranoside and rhamnetin-3-O- β -glucopyranoside (Walter & Sequin*, 1990). Moreover, boscialin and boscialin 4'-O-glucoside were isolated from the leaves, and a GC-MS profile revealed a complex mixture of 1,2,3-propantriol, 9-octadecenal, 9-octadecanoic acid, 2-(2-hydroxy-2-phenylethoxy) phenol, 3,5-di-tert-butylphenol, 13-octadecanoate, methyl-12-methyltetradecanoate, n-hexadecanoic acid and n-octadecanoic acid (Maroyi, 2019c). Some reported pharmacological activities could directly be linked to these phytochemicals. *Boscia variabilis* edible parts were reported to contain a significant amount of alkaloids (14.61), phenols (31.75), tannins (24.94), saponins (26.41) and flavonoids (26.72) mg/g dry weight (Mon, 2018).

2.2.7. *Boscia foetida* Economic Significance

The roots of the plant are sold in Mozambique and South Africa's black market for herbal remedies. (Krog et al., 2006; Williams et al., 2001).

2.3. *Ximenia* Linn genus.

The genus *Ximenia* Linn. belongs to the plant family "Olacaceae," and is characterized by shrubs or small trees typically with axillary spines (Hyde et al., 2022b; Nair et al., 2013). Olacaceae is a flowering plant family of shrubs or small trees known to possess parasitic activity. The family comprises of 41 genera with pantropical distribution throughout African, American and Asian tropics (Kuijt & Hansen, 2015), (Albuquerque et al., 2018) with 200 species and an estimate of eight species of *Ximenia* genus. Furthermore, two genera with a total of five species are native to Southern Africa (SANBI, 2022b), however, (The Angiosperm Phylogeny Group, 2016) there exist insufficiency of corroborating evidence to address the existing ambiguity on taxonomical classification of order Santalales, and Olacaceae family.. Molecular phylogeny studies have shown Olacaceae to be polyphyletic, therefore could possibly be split into numerous families (Malécot & Nickrent, 2008). Flowers are usually bisexual with a varying inflorescence (Kuijt & Hansen, 2015). (Djonwangwe et al., 2011) and the pollination pathway of *Ximenia Americana* flowers was induced by bees (*apis mellifera adansonii* species) and this study suggested that the findings classified "Olacaceae as a very highly nectariferous and highly polliniferous bee plant."

2.4. *Ximenia americana* botanical description

Ximenia americana is described as a semi-scandent bush-forming shrub or small tree with a height ranging between two and seven metres (Kefelegn & Desta, 2021), and a range of four to eight metres at maturity (Lompo et al., 2021). It's trunk has a maximum diameter of 10 cm, and dark brown to light grey and smooth to scaly bark. The branches create a downward arch, and the branchlets have 1 cm long, straight, thin spines that protrude out, and are coloured purple-red with a waxy bloom (Albuquerque et al., 2018; Kefelegn & Desta, 2021). The plant occasionally expresses semiparasitic nature with haustoria on the roots (Kefelegn & Desta, 2021). The fruit is an oval, approximately 20 to 30 mm long drupe that oddly resembles a little yellowish plum. A distinct almond-like aroma is present in immature fruits and is brought on by cyanogenetic chemicals, the most significant of which is sambunigrine (Urso et al., 2013). It has a thin, shiny peel and a tart pulp, but eating it is incredibly reviving. It has one substantial endospermic seed, up to 60% of which is oil, (Albuquerque et al., 2018; Ruffo et al., 2002). Leaves are simple and alternating or clustered on spur shoots and are generally hairless as compared to the leaves of *Ximenia caffra*, its closely related specie. The morphology of seedlings varies; when they are young, the leaves are densely hairy, but as they mature, they become smooth and lustrous (Feyssa et al., 2012). Leaf-lamina is 2-8 to 1-4 cm, oblong-elliptic, retuse at the tip, coriaceous, 3-6 mm long petiole, canaliculate, puberulous or pubescent above, three to six pairs of

lateral nerves that are subinconspicuous on both surfaces (Albuquerque et al., 2018; Ruffo et al., 2002). Young leaves produce a smell like bitter almonds when crushed (Kefelegn & Desta, 2021). Flowers are tiny, greenish-white, fragrant, and five to ten millimetres long, borne in "pedunculate racemose or umbelliform cymes" on branched inflorescences (clusters are branched in a single stock) (Albuquerque et al., 2018; Ruffo et al., 2002).

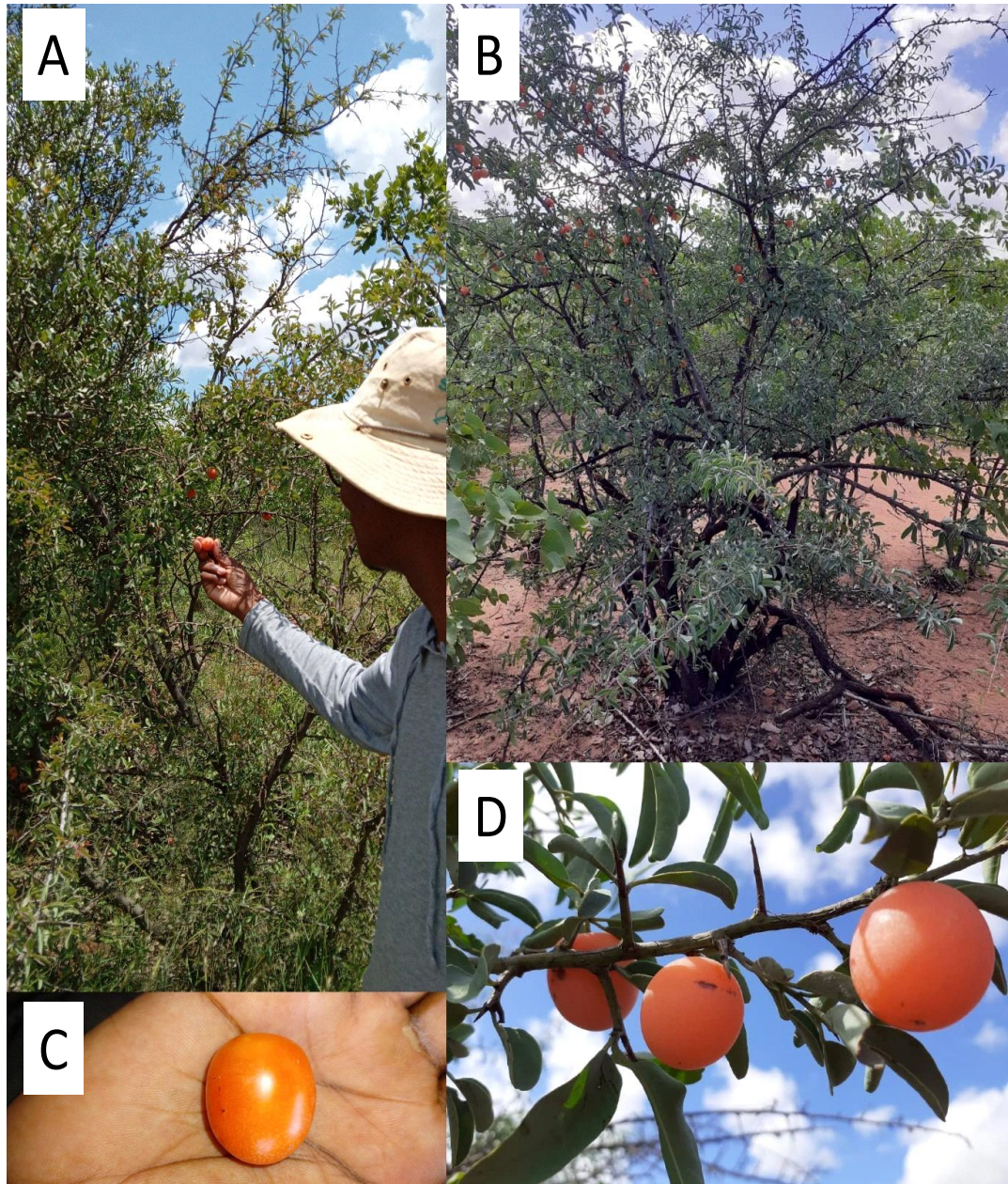


Figure 4: Photos of wild plum (*X. americana*) collections from Tshimoyapula in Central district and Gasita in Southern district of Botswana where **A** depicts fruits harvesting, **B**. *Ximenia americana* tree in the fruiting stage, **C**. *Ximenia americana* whole fruit, **D**. fruits and leaves

2.4.1. Distribution and Ecology

Ximenia americana L. has several common names based on native languages and the location. For instance, in English it is termed sour plum, wild plum, hog plum, false sandalwood, small sour plum, seaside plum, wild olive, and tallow wood, French (cerise de mer, macaby, citron de mer, croc), Swahili (mpingi, mtundakula, timbui timbui, mtumbui tumbui), Tswana (moretologa wa pudi), and Arabic (kelto, abu khamira, medica, humeid abiad) (Orwa et al., 2009; Setshogo, 1998). Apparently, the plant taxonomy community discourages the use of these names due the variations in their use from one region to another (Setshogo, 1998). *Ximenia americana* is widely distributed across the tropics. It covers Africa, India, South East Asia to Australia, New Zealand, Pacific Islands, West Indies, Central and South America, and particularly prevalent in Africa and Brazil (Albuquerque et al., 2018; Queiroz Monte et al., 2012). These locations experience moderate to warm summer temperatures and little to no frost during the winter. These have annual average summer rainfall ranging from 750 to 1 250 mm, depending on the location (Mathalauga, 2020). This plant can be found in a variety of habitats, although it is most commonly found in semi-arid bushland, various types of dry woodland, sandy open woodland, dry hilly terrain, coastal bushlands, along watercourses, and stony slopes (Albuquerque et al., 2018; Feyssa et al., 2012). Moreover, this tree can be found scattered throughout open terrain, savannas, gallery forests, coastal regions, the understory of dry forests, dry woodlands, and riverbanks. The species can withstand droughts, and its preferred soil types include clays, clay loam, loamy sands, sandy clay loam, and sands. It is capable of growing on altitudes reaching up to 2000 m (Lompo et al., 2021). It is considered one of the essential sources of food among the explored edible fruit plants, for a variety of species such as mammals, with the ability to obtain water and nutrients from other plant species via the root system, however, it not does not rely on this to survive (Albuquerque et al., 2018; Mathalauga, 2020).

Ximenia americana flowering and fruiting seasons are not truly subject or influenced by climate regimes, however flowering usually takes place in the dry season (Albuquerque et al., 2018). It blooms and bears fruit all year round in many locations, and the trees may start to bear fruits after three years of development.

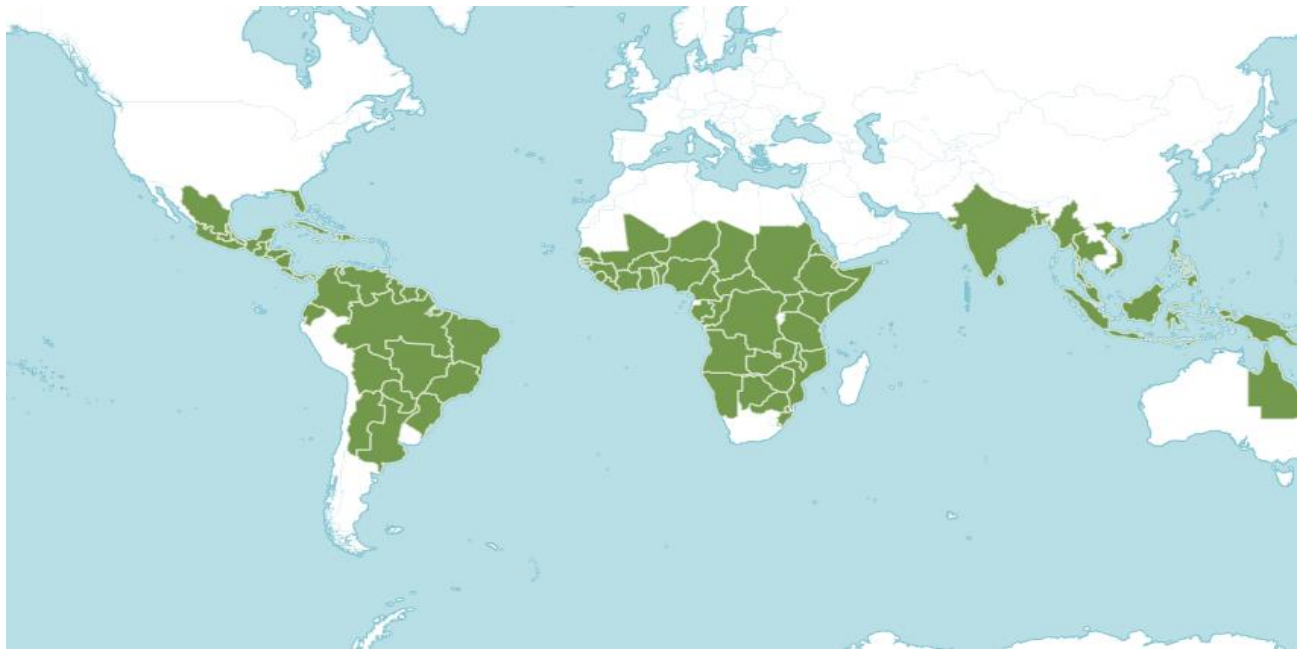


Figure 5: World geographical distribution of *Ximenia americana* (POWO, 2023b)

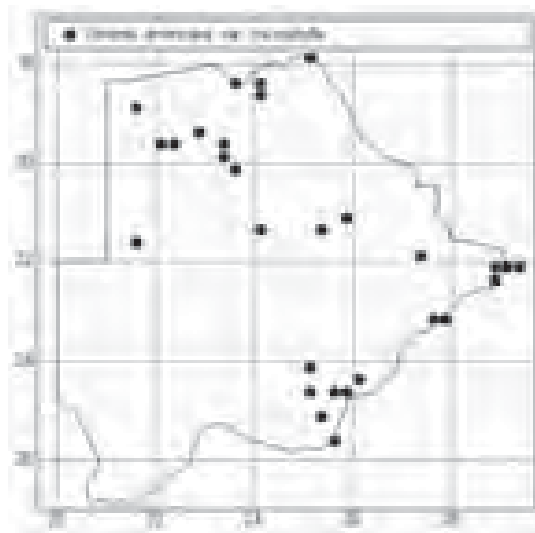


Figure 6: Geographical distribution of *Ximenia americana* in Botswana, Southern Africa (Setshogo & Venter, 2003)

2.4.2. *Ximenia americana* Propagation

Fresh seeds of *Ximenia americana* can be easily grown from utilizing a five is to one combination of sand and compost (Mathalauga, 2020). After sowing, germination will start 14 to 30 days later. The seedling is prepared for transplanting once it has produced its second stage of leaves. Immersing seeds in water for a day and treat with exogenous gibberellic acid, normally enhances germination. Because it may interact with other plants' roots in the ground, this plant, which is semi-parasitic, will thrive there. It can endure mild cold, has a modest growth rate of up to 0.5 m per year, is drought tolerant, and

needs full light (Mathalauga, 2020; Ulian et al., 2019). It can be vegetatively propagated by root suckers (Ulian et al., 2019). Although it can survive temperatures between 10 and 36°C, *Ximenia americana* thrives in environments with yearly daytime temperatures between 14 and 30°C. It can tolerate brief mild frosts on occasion. A pH ranging between five and seven is suitable for its growth, however it can tolerate pH 4.5 to 7.5. Seedlings normally fruit within three and four years (Fern, 2022). Due to the species' dioecious nature, seed germination is extremely low, and identical clones of plants with higher quality cannot be found in nature. *In-vitro* propagation method was developed for *Ximenia americana* using mature plants shoot tip explants to "clonally generate superior genotypes" (Mohamed & Feyissa, 2020). Likewise, micro-propagated *Ximenia americana* from axillary buds obtained from mature plants, shed light on the possibility of rapid clonal propagation "through *in-vitro* culture of nodal explants" (Aloufa et al., 2003).

2.4.3. *Ximenia americana* ethnomedicinal uses

Over the millennia, people have used medicinal plants to maintain and promote health, prevent and treat various ailments (Agyigra et al., 2017). *Ximenia americana* being one of these medicinal plants is commonly utilized in various countries' folk medicine (Urso et al., 2013). According to ethnobotanical studies, this plant has been used for a very long time to make medications to treat conditions like malaria, ulcers, fever, edema, diarrhea, and sexually transmitted infections (Mohamed & Feyissa, 2020). Native healers in Côte-d'Ivoire mostly use the plant for treatment of malarial infections (Aloufa et al., 2003). In Mali, native healers use a decoction of the leaves and stems to treat measles, throat infections and malaria. Root bark powder and macerated solution of roots treat malaria (Le et al., 2012), additionally, roots were also reported to treat dysentery, chest pains, leprosy, schistosomiasis (bilharzia), mental illness, syphilis, hook worm and cough (Sobeh et al., 2017; Ulian et al., 2019). In Tanzania, the root decoction is used to treat anaemia, hernia, and retained placenta (Ruffo et al., 2002). Moreover, leaf decoctions are used as a "vermifuge" (expel parasitic worms), a wash to calm red, inflamed eyes, and "a gargle for tonsillitis" (Sobeh et al., 2017). They are also utilized as headache remedy, particularly in children, angina, and as a poison antidote (Hines & Eckman, 1993). Different tropical nations utilize an infusion of bark or leaves or direct application of chopped leaves as an antidote for snake and scorpion stings (Urso et al., 2013). *Ximenia americana* peelings were also reported to be one of the ingredients in traditional preparations to treat toothaches, mumps and conjunctivitis in some African countries (Sharief et al., 2022). In Brazil, the bark is used to make tea that is popularly used medicinally as an astringent, cicatrizing agent, and as an agent for preventing heavy menstruation (Queiroz Monte et al., 2012). Additionally, it was mentioned that the Dek Island

natives treat rabies illnesses with the plant's bark. Utilization involves soaking the bark in water, which is then consumed orally (Kefelegn & Desta, 2021). In Zimbabwe, a decoction of leafy twigs is used to treat febrile colds, coughs, and as a laxative whereas in Nigeria the same remedy is employed as both a mouthwash and a toothache remedy (Urso et al., 2013). According to (Ruffo et al., 2002), fruits have a vermifuge effect when consumed in large numbers, and they are also helpful in persistent constipation. (Hines & Eckman, 1993). For the treatment of diarrhoea in calves, "a decoction of roots or fruits" is utilized (Hines & Eckman, 1993). The pustules of chickenpox are treated with the juice from decaying fruits. This is based on the fact that fungi that grow on rotting fruit create antibiotics like penicillin (Ulian et al., 2019). Infusions, decoctions, tinctures, syrups, and cataplasms are the methods of preparation that are usually employed (Sharief et al., 2022). However, some native healers claimed that this plant displayed poisonous symptoms including salivation (Albuquerque et al., 2018).

2.4.4. *Ximenia americana* biological activities

A number of *Ximenia americana*'s biological actions have been documented in the literature, supporting some of its ethnomedicinal uses. Other than hepatic and haematological effects, *Ximenia americana* has demonstrated a number of biological activities, including antibacterial, antifungal, anticancer, antineoplastic, antitripanosomal, antirheumatic, antioxidant, analgesic, molluscicidal (Abdalla et al., 2013), and antidiabetic (hypoglycemic) (Sobeh et al., 2017). Fruit and stems isolates have anticancer, antifungal, antiallergic, antiparasitic, antiviral, and antibacterial properties. It is also a very effective antioxidant (Kefelegn & Desta, 2021). The utilization of crude extracts, including whole plants and phytochemicals with recognized antibacterial capabilities, has grown significantly in importance in contemporary medicinal treatments. To scientifically verify their efficacy, numerous research have been carried out in a variety of nations.

2.4.4.1. *Ximenia americana* antibacterial activity

A study was conducted by (Shagal et al., 2013) to elucidate the antibacterial activities of the powdered leaves, stem-bark and roots of *Ximenia americana*. Water and ethanol extracts were tested against three bacterial strains, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella spp.* The ethanol extracts of all the plant parts showed significant inhibitory effects against *Staphylococcus aureus* only whereas water extracts inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* but showed no effect on *Salmonella spp.* The findings of the study suggested that *Ximenia americana* plant has the potential in treatment of microbial ailments. *Staphylococcus aureus* and *Klebsiella pneumoniae* were inhibited by the root, stem, bark, and leaves aqueous and methanolic extracts of *Ximenia americana* while *Shigella flexneri* was exclusively inhibited by the methanolic leaves, aqueous bark, and aqueous leaves

extracts (Queiroz Monte et al., 2012). Another study was conducted (Silva et al., 2012) on *X. americana* bark from the state of Paraíba extracted by ethanol/distilled was active against *Streptococcus oralis*, *Staphylococcus aureus* and *Enterococcus faecalis*. Comparing the zones of inhibition at *Enterococcus faecalis* (14.1 mm) to those at chlorhexidine (positive control, 13.4 mm) revealed statistically different results. Moreover, compared to *Enterococcus faecalis* and *Staphylococcus aureus*, chlorhexidine displayed a better inhibitory for a different bacterium (Silva et al., 2012). The antibacterial activity of *Ximenia americana* bark extracts against *Enterococcus faecalis* was also reported by (Costa et al., 2010) as substitute treatments for endodontic issues. Furthermore, using a 96-well microtiter microplate, the broth microdilution test was used to assess the antibacterial activity of the leaf extract against *Pseudomonas aeruginosa* strain. The study showed a complete inhibition against the growth of *Pseudomonas aeruginosa* at the concentration of 25 mg/mL. The leaf extract showed good dermatocosmeceutical properties that could be linked to its antibacterial and antioxidant properties (Bakrim et al., 2022).

2.4.4.2. *Ximenia americana* antifungal activity

The methanolic leaf extract of *Ximenia americana* demonstrated antifungal activity against *Cryptococcus neoformans* and *Candida albicans* at a concentration of 4000 g/mL (Queiroz Monte et al., 2012). By the method of zone inhibition, antimicrobial activity assessment showed no fungal inhibitory effect against *Candida albicans* strain for ethanolic and aqueous extracts of the powdered leaves, stem-bark and roots (Shagal et al., 2013). In agreement with the aforementioned effect, (Omer & Elnima, 2003) reported *Candida albicans* the least susceptible strain to chloroform, methanolic, and aqueous stem, root, leaves and bark extracts among all the tested fungal strains which included *Aspergillus niger* and *Saccharomyces cerevisiae*. The activity was evaluated by the cup-plate agar diffusion method, and MIC by the agar dilution method. A study on antifungal assessment of *Ximenia americana* bark to develop a tablet from mucoadhesive polymers for treatment of oral candidiasis was evaluated *in-vitro* by the broth microdilution method. The extract was tested against *Candida albicans* fungal strain. The results exhibited "good mucoadhesive forces in mucin disk and pig buccal mucosa" upon mucoadhesive strength evaluation. The study therefore suggested the development of a new alternative for the treatment of buccal candidiasis (Almeida et al., 2019).

2.4.4.3. *Ximenia americana* antiparasitic (Leishmanicidal and Trypanocidal) activity

A study in Brazil (de Menezes et al., 2019) assessed the antiparasitic activity of stem bark hydroethanolic extract against three parasitic protozoal strains namely: *Trypanosoma cruzi*, *Leishmania*

infantum and *Leishmania braziliensis*. The activity was evaluated on a liver infusion tryptose broth in 96-well microplate. Only at a concentration of 1000 µg/mL did the stem bark hydroethanolic extract show efficacy against the *L. infantum* and *L. braziliensis* promastigotes, resulting in mortality rates of 40.66 and 27.62%, respectively. The antiparasitic properties of *Ximenia americana* stem bark hydroethanolic extract against two *Leishmania* species "*infantum* and *braziliensis*" responsible for leishmaniosis, a zoonotic infectious disease transmitted by a female sand fly. This study therefore suggests a fresh viewpoint on an alternate therapeutic possibility for the management of leishmaniosis caused by the protozoan species *L. infantum* in particular (de Menezes et al., 2019).

2.4.5. *Ximenia americana* phytochemistry

Crude alcoholic (ethanolic, methanolic, butanoic), chloroform and aqueous leaves, root, stem and stem bark extracts reportedly contain flavonoids, sterols, terpenoids, tannins, alkaloids, quinones, cyanogenetic glycosides, cardiac glycosides, saponins and carbohydrates in the form of sugars and soluble starch (Queiroz Monte et al., 2012). Steroids (stigmasterol and sitosterol), triterpenoids (betulinic acid, oleanolic acid, 28-O-(-D-glucopyranosyl) oleanolic acid, 3-oxo-oleanolic acid, 3-hydroxycycloart-24(*E*)-ene-26-oic acid) and sesquiterpenoids (furanoic and widdrane type) were recovered in the stem ethanolic extract (Queiroz Monte et al., 2012). The seed oil was composed predominantly of saturated and monounsaturated fatty acids (Kefelegn & Desta, 2021). The ethanolic leaves extract reportedly afforded cyanogenic glycoside, sambunigrin, gallic acid, glucogalline, 1,6-digalloyl glucopyranose, quercetin, quercitrin, avicularin, quercetin-3-O-β-xylopyranoside, quercetin-3-O-(6"-galloyl)-β-glucopyranoside, and kaempferol-3-O-(6"-galloyl)-β-glucopyranoside. Several of them have antioxidant properties, and xanthine oxidase and 15-lipoxygenase inhibitory effects (Bakrim et al., 2022). The methanolic leaves extract HPLC-MS/MS analysis elucidated a total of 23 compounds of classes kaempferol, quercetin, procyanidin B1, epicatechin and catechin (Bakrim et al., 2022).

The bark aqueous ethanolic extract revealed the presence of phenolic derivatives such as gallic acid, chlorogenic acid, caffeic acid, ellagic acid, flavonoids catechin, rutin, quercitrin, quercetin and kaempferol (de Menezes et al., 2019).

2.4.6. *Ximenia americana* economic significance

Ancestral customs have given rise to the utilization of wild food plants and the goods they produce in modern life. This could improve the food security of households in locations where wild plants are consumed, especially in rural areas. It has been determined that *Ximenia americana* L. is among the few plant species that should be included in the domestication process in farming systems in sub-

Saharan Africa to satisfy local populations' needs for revenue, food, and medicine (Kefelegn & Desta, 2021; Maroyi, 2016).

For rural populations in Namibia, *X. americana* together with its closely related specie, *X. caffra*, are commercially significant. In 2008, approximately 5.8 tonnes of *Ximenia* were sold, bringing in US \$117,152, approximately US \$20 per kg (Maroyi, 2016). A survey in four Angolan communities on traditional uses of *Ximenia americana* showed that there was a production of *Ximenia* seed oil in all studied communities offered at local markets for 300 to 1200 kuanza per litre which is approximately 3 to 12 Euros (Urso et al., 2013). In Ethiopia, the key informants' interviews conducted during the course of the study resulted in an overall average output per tree per year of 50.33 kg wet fruits. This was utilized to provide an estimated monetary value equal to US \$186.41 a year, or 2516.50 ETB (Ethiopian Birr), (50.33 x 50 ETB). This money can potentially be utilized to address the needs of many households, including purchasing food, utensils, medicine, and improving livelihoods (Feyssa et al., 2012; Kefelegn & Desta, 2021). According to reports, the Sandawe natives depend on the fruit as a main or staple food (Hines & Eckman, 1993).

CHAPTER 3

3. METHODS

3.1. Reagents

These include; hexane chemically pure Batch No. 030719HE Rochelle Chemicals, methanol 99.0% Batch No. 1054554 Sigma-Aldrich, nitric acid 69-72% Cas No. 7697-37-2 Alpha Chemika, acetone analytical reagent Batch No. 110521AN Rochelle Chemicals, ethanol 99.9% absolute, fully denatured, Cas No. 64-17-5, ethyl acetate HPLC GRADE Cas No. 141-78-6 Alpha Chemika, magnesium metal ribbon Cas No. 7439-95-4 Qualikems, hydrochloric acid 32% AR Batch No. 121002 SKYLABS, cupric sulphate analytical reagent Batch No. 220617CP Rochelle Chemicals, potassium hydroxide pellets analytical reagent Batch No. 201017PT Rochelle Chemicals, ferric chloride hexahydrate lot No. 1039251 MERCK, lead acetate trihydrate 99% Cas No. 6080-56-4 Sigma-Aldrich, sodium nitrite lot No. 1039635, lanthanum nitrate hexahydrate 99.99% Cas No. 10277-43-7 ALDRICH® Chemistry, oxalic acid (99% Alpha Chemika; Cas No. 6153-56-6), potassium permanganate (Alpha Chemika; Cas No. 7722-64-7), sulphuric acid (98% MINEMA; Batch 35858.6044), gallic acid (Alpha Chemika; Cas No. 5995-86-8), folin-ciocalteu reagent (Rochelle Chemicals RC; Batch No. 270917FL), sodium carbonate (MINEMA; Batch No. 41626), polyvinylpyrrolidone K25 (PVP) (ROTH; Cas No. 9003-39-8), arsenic (Supelco Sigma-Aldrich; Lot No. HC16350373).

3.2. Sample Collection

Ripe fruits of wild plum (*X. americana*) were collected fresh from trees in Tshimoyapula in the central district of Botswana, 21°57'56.9"S 27°08'12.7"E, between December 2021 and February 2022 (voucher MZX 001). Leaves and roots of *B. foetida* were collected from Serule, 21°55'49.4"S 27°18'59.7"E, in the central district of Botswana in September 2021 (Voucher MZX 002). The trees of *B. foetida* were high in density in the selected area. The plant species identities were verified by Dr. Andrew Muzila of the University of Botswana Herbarium.

3.3. Study Design and Experimental site

The study samples were collected randomly and subjected to several experiments. Fruits of wild plum (*X. americana*) were obtained from trees in close proximity. Leaves and roots of *B. foetida* were obtained from mature trees at flowering stage. The experiments were conducted in the department of Chemical and Forensic Sciences, Chemistry research laboratory, at the Botswana International University of Science and Technology, Palapye, Botswana.

3.4. Sample Processing

Collected fruits of *X. americana* from trees were pooled to acquire a homogenous sample and separated into three batches. Two batches were frozen at -4°C and analysed at different periods while the other batch was prepared while fresh from harvest for analysis. Prior to analysis, the whole fruit was separated into peels, pulp, coats and kernels by physical means with sterilized glassware and spatulas. The peels and coats were subjected to air drying for three days at room temperature followed by further 30 minutes oven drying at 40°C for homogeneity purposes. The dried samples were pulverized (Model; pulverisette 5 FRITSCH) for 45 minutes. The leaves of *B. foetida* were air dried at room temperature for a week along with the roots which were air dried in a laboratory roof shade. All analyses were done on each batch.

3.5. Gas Chromatography Mass Spectrometry Analysis

3.5.1. Extracts Preparation

The plant materials, *B. foetida* roots and *X. americana* fruit parts, of about 20.0 g were subjected to cold maceration with *n*-hexane, 50.0 mL, at room temperature for two consecutive days to obtain *n*-hexane non-polar extracts. However, the wild plum (*X. americana*) pulp was first extracted with 70% methanol then solvent partitioned with *n*-hexane to obtain a non-polar fraction. All the afforded extracts were kept at 10°C a day prior to GC-MS qualitative analysis.

GC-MS analysis was performed with an Agilent 7890B GC system coupled with an Agilent 5977A mass spectrometer detector. The HP-5MS capillary column (Hewlett-Packard, CA, USA) (30 m × 320 µm × 0.25 µm) coated with 5% phenylmethyl silicone, 95% dimethyl silicone, temperature limit: -60-325°C was used in the stationary phase. Carrier gas used was helium with a constant flow rate of 1 mL/min, pressure of 10.523 psi, and average velocity of 37.293 cm/sec in a ratio 40:0.75 splitless mode. Oven temperature was initially at 100°C for 2 minutes then ramped at 10°C/min to 300°C with a holding time of 30 minutes, amounting to a total run time of 52 minutes. The injection mode was set at 1 µL. The mass spectra were generated by Electron Impact Ionization method at electron potential energy of 70 eV (Phiri et al., 2021).

Identification of compounds was done based on comparison of the acquired sample mass spectra with the known compounds spectra contained in the NIST library and pherobase online library. Mass spectral library scores, match factors and reverse match factors, were also considered in confirmation of compounds identity (Gololo et al., 2016; Phiri et al., 2021). Compounds within the range of 700-800, were considered a fair match, 800-900 was a good match, and 900 upwards was an excellent match, however most compounds were in the range 800-900 and above 900 match factors (Dąbrowski,

2020). Relative percentage compositions of compounds were computed using peak areas, and response factors were not determined (Mothana et al., 2011). All structures were drawn using ChemDraw Ultra 8.0.

Samples were analysed in duplicates and values presented as mean $\pm$ standard deviation.

3.6. Mineral Analysis

3.6.1. Standard Solution Preparation

Stocks of 10 ppm and 100 ppm were prepared using certified reference solutions (Sigma-Aldrich TraceCERT®; MERCK; Supelco® Sigma-Aldrich TraceCERT®) of every element of interest. From the standard stock solutions, the working standards were prepared in the concentration ranges of 0.05-4 ppm for Mn, Co, Pb, Cr, 0.25-4 ppm for Fe, Ni, Cu, Zn, Na, 2-8 and 8-35 ppm for Ca, and 1-80 ppm for K and Mg to establish a linearity curve. Calcium calibration standards were spiked with 1 mL of 0.5 g lanthanum nitrate solution in 50.0 mL volumetric flasks before adjusting to the mark with 1% hydrochloric acid.

3.6.2. Microwave Acid-Assisted Digestion

Digestion was undertaken using CEM microwave digester (CEM One Touch Technology; MARS 6 iLink, serial No. MJ 7260) where about 0.5 g of sample was weighed and transferred into a Teflon vessel followed by the addition of 10.0 mL concentrated nitric acid. Blank solution was prepared in the same manner as the sample, and they were digested concurrently. The digesting conditions were set as follows; power at 290-1800 W, ramped at 20 minutes, holding time at 10 minutes with a maximum temperature of 200°C. The samples were cooled for 20 minutes at room temperature upon removal from the digester. The cooled sample solutions were diluted with 25.0 mL of distilled water in 50.0 mL volumetric flasks then filled up to the 50.0 mL mark with 2% nitric acid (Sibiya et al., 2020).

3.6.3. Sample Preparation for Analysis of Calcium

About 0.5 g of sample was weighed into a digestion vessel followed by the addition of 6 M HCl. The solution was heated at 50°C for 24 hours on a digital hotplate. Upon digestion, sample solutions were transferred into 50.0 mL volumetric flasks (Albakaa et al., 2021). Before adjusting to the mark, the solutions were spiked with 1 mL of lanthanum nitrate solution along with calibration standards.

3.6.4. Flame Atomic Absorption Spectrophotometry Analysis (FAAS)

Mineral contents of 12 elements which include; manganese, cobalt, lead, chromium, iron, nickel, copper, zinc, sodium, potassium, calcium and magnesium were determined using a flame atomic absorption spectrophotometer (contAA 700; analytikjena) with an AS-FD autosampler of 54 positions tray, neon lamp, and ASpect CS 2.2.2.0 Tech: Flame software. air/acetylene was the fuel of choice for

11 elements, and nitrous oxide/acetylene for calcium. Stocks of 10 ppm and 100 ppm were prepared using certified reference solutions (Sigma-Aldrich TraceCERT®; MERCK; Supelco® Sigma-Aldrich TraceCERT®) of every element of interest. Extrapolation from the standards calibration plots was used to determine the concentrations of the samples. The following parameters and conditions were adopted.

Table 1: Instrument Operating Conditions and Parameters

Element	Wavelength (nm)	Fuel Flow Rate (L/h)
Ni	232.003	55
Mg	285.2125	70
Cu	324.754	50
Zn	213.857	50
K	766.4908	55
Na	588.9953	55
Mn	279.4817	80
Co	240.7254	50
Pb	217.0005	65
Cr	357.8687	100
Fe	248.327	60
Ca	422.6728	215

All samples and standards were analysed in triplicates and duplicates, and values were presented as mean $\pm$ standard deviation.

3.6.5. Inductively Coupled Plasma Mass Spectrometry Analysis (ICP-MS)

The ICP-MS (Thermo Scientific™ iCAP Q ICP-MS, Illinois 60060, USA) equipped with CETAC ASX-520 autosampler and argon gas was used to quantify Co and As. The chiller (Thermo Scientific, NESLAB ThermoFlex2500) temperature was set at 18°C, pump speed at 40.0 rpm, spray chamber temperature at 2.70°C, wash and uptake time; 30 minutes, cool, auxiliary and nebulizer gas flow; 14.018, 0.7962 and 1.0147 L/min, forward power; 1548.6 W, interface temperature; 26.86°C, and cooling water flow of 0.55 L/min. A series of calibration solutions ranging within 0.1-5.0 ppm were prepared with 100 ppm (mg/L) mix element standard stock solutions.

3.6.6. Health Risk Assessment of Heavy Metals

Based on an estimated daily intake, hazard quotient, and hazard index, the risk of consuming fruits, nuts, and herbal preparations polluted with heavy metals to human health was assessed.

3.6.6.1 Non-carcinogenic risk assessment

Determining the likelihood that a specific expected amount of negative health effects by single and multiple heavy metals will occur over a specific time period (Mohammadi et al., 2019).

The daily intake dose (EDI) was computed to determine the average daily loading of metals into a consumer's body system at a certain body weight. The following equation was used to determine the estimated daily intake (EDI):

$$EDI = \frac{C_{\text{metal}} \times IR}{BW}$$

Where **C_{metal}** is the concentration of the heavy metal in a sample in (mg/kg); **IR** is the ingestion rate which is the average daily consumption in (g/person/day); **BW** is the average body weight in (Kg). The average daily consumption of fruits, nuts and herbal preparations are 400 g/person/day, 30 g/person/day and 20 g/person/day respectively. To evaluate the non-carcinogenic risk to humans from chronic exposure to heavy metals from vegetables, medicinal plants, and fruits, the hazard quotient is used. The following equation was used to calculate the hazard quotient (HQ):

$$HQ = \frac{EDI}{RfD}$$

Where **EDI** is the daily intake per day (mg/kg/day); **RfD** is the reference dose value of the metal of interest in mg/kg/day. The values for Pb, Cr, Cu, Ni, Co and Zn are 0.004, 0.0015, 0.04, 0.02, 0.02 and 0.3 mg/kg/day respectively. The Hazard Index (HI) tool was further employed to assess the total non-carcinogenic risk to human health posed by multiple heavy metals, and the following equation was used to calculate the hazard index:

$$HI = \sum HQ,$$

if the **$\sum HQ < 1$** , no adverse effects are expected on human health, however, if the **$\sum HQ \geq 1$** , adverse effects on humans are expected (Brown et al., 2021; Gupta et al., 2021; Kandić et al., 2023; Meseret et al., 2020; Pem & Jeewon, 2015).

3.6.6.2 Carcinogenic risk assessment

The carcinogenic risk was estimated by determining the probability of an individual developing any sort of cancer throughout the course of their lifetime as a result of constant daily exposure to heavy metals in the pulp and kernels of the wild plum (*X. americana*) and the roots of *B. foetida*. It was calculated using the Estimated Daily Intake (EDI) and the Cancer Slope Factor (CSF) for a single heavy metal as shown below. The CSF values of Ni and Pb used were 1.7 and 0.38 mg/kg/day respectively (Alsafran et al., 2021; Mohammadi et al., 2019). The Incremental Lifetime Cancer Risk (ILCR) can be understood as the increased likelihood of developing cancer at any point in one's life due to continuous

exposure to a specific carcinogenic substance in a fixed amount every day for the duration of seventy years (Mohammadi et al., 2019).

$$\text{ILCR} = \text{EDI} * \text{CSF}$$

3.6.7. Mineral Bioavailability Estimation

The molar ratios of oxalate to mineral were used to evaluate the relative bioavailability of calcium, iron, magnesium, and potassium. The ratios were calculated using the following equation with 90.03 g/mol as the molar weight of oxalate (Akter et al., 2020):

$$\text{Oxalate to Mineral Molar Ratio} = (\text{Oxalate/Molar weight}) \div (\text{Mineral/Molar weight})$$

3.7. Nutritional Proximate Analyses

3.7.1. Moisture Content

To determine moisture content, a method described by (Sibiya et al., 2021) was adopted. Crucibles were dried in a hot air oven for 35 minutes at 100°C and allowed to cool in a dessicator for 10 minutes and weighed. Approximately 1.5 g of sample was weighed into the dried crucibles and dehydrated in a hot air oven at 100°C for four hours. The samples were allowed to cool in a dessicator for 10 minutes upon drying and weighed. Percent moisture content was calculated as follows:

$$\text{Moisture (\%)} = \frac{M_s - (M_2 - M_1) * 100}{M_s}$$

Where M_s = mass of sample, M_2 = mass of crucible + dried sample, M_1 =mass of crucible

All samples were analysed in triplicates, and values were presented as mean $\pm$ standard deviation.

3.7.2. Ash Content

Empty crucibles were dried in a hot air oven at 100°C for 30 minutes, and allowed to cool in a dessicator followed by weighing. About 1.5 g of sample was weighed directly into the dried crucible and heated in a muffle furnace at 550°C for five hours. The sample was then removed from the furnace and cooled in a dessicator for several minutes to reach room temperature. The sample in the crucible was then weighed. The following equation was used to calculate percent ash.

$$\text{Ash (\%)} = \frac{M_2 - M_1 * 100}{M_s}$$

Where M_2 = mass of crucible + ash, M_1 = mass of crucible, M_s = mass of sample (Sibiya et al., 2021).

All samples were analysed in triplicates, and values presented as mean $\pm$ standard deviation.

3.7.3. Crude Fat Content

A procedure described by (Nyambe et al., 2019) was adopted to determine crude fat content. It was done by extracting the sample, approximately 2 g, with hexane in 200.0 mL sample bottles, with continuous agitation in an orbital shaker for one hour at room temperature. The solution was allowed to settle, and the upper layer was decanted into pre-weighed 50 mL beakers. Hexane was removed in a fume hood at room temperature, and the extract was further dried in a hot air oven at 60°C for 30 minutes and weighed. The following equation was used to calculate percent fat content:

$$\text{Fat (\%)} = \frac{\mathbf{M_2 - M_1 * 100}}{\mathbf{M_s}}$$

Where $\mathbf{M_2}$ = mass of beaker + extract, $\mathbf{M_1}$ = mass of beaker, $\mathbf{M_s}$ = mass of sample.

All samples were analysed in triplicates, and values presented as mean $\pm$ standard deviation.

3.8. Anti-Nutritional Factors

3.8.1. Oxalate Content

A method described by (Mishra et al., 2017) was adopted to determine oxalate content in test samples.

3.8.1.1 Standard Solution Preparations

Oxalic Acid: Approximately 500 mg of oxalic acid was weighed and dissolved in distilled water and diluted to 100.0 mL in a 100.0 mL volumetric flask. The solution was wrapped with an aluminium foil and kept at room temperature. From the standard stock solution, 0.4, 0.5, 0.6, 0.8, 0.9 and 1 mg/mL series of stock calibration standards were prepared in 10.0 mL volumetric flasks to establish a linearity curve.

Potassium Permanganate: 0.047 g of potassium permanganate was weighed and dissolved in 15.0 mL portion of distilled water in a beaker to make 0.003 M concentrated solution. The solution was quantitatively transferred quickly into a 100.0 mL volumetric flask and diluted to the 100.0 mL mark with distilled water. It was then covered with an aluminium foil sheet to establish a dark environment and kept at room temperature.

Sulphuric acid: About 27.2 mL of concentrated sulphuric acid was transferred into a 500.0 mL volumetric flask and diluted to the 500.0 mL mark with distilled water to make 1 M solution of H_2SO_4 .

3.8.1.2 Standard Solution Preparations for Analysis

The prepared series of calibration standards containing 0.4, 0.5, 0.6, 0.8, 0.9 and 1 mg/mL, 1.0 mL volume portion, were mixed with 5.0 mL of 1 M H_2SO_4 and two millilitres of 0.003 M KMnO_4 in

separate test tubes, then incubated at 37°C for 10 minutes on a digital hotplate stirrer (Model; Lasec, Item No. H3760-HSE). The blank solution was however prepared by mixing 5.0 mL of 1 M H₂SO₄ and 2.0 mL of 0.003 M KMnO₄ simultaneously with the calibration standards, and incubated at 37°C for 10 minutes.

3.8.1.3 Sample Preparation for Analysis

Approximately 0.5 g of the fruit material was weighed and mixed with 30.0 mL of 0.25 N HCl in a beaker. The mixture was boiled for 15 minutes, then left to cool for 10 minutes. The extract was filtered into a 50.0 mL volumetric flask using filter disks (BOECO Germany; Diameter: 150 mm), and filled up to the 50.0 mL mark with 0.25 N HCl. From the 50.0 mL extract stock, one millilitre was obtained and mixed with 5.0 mL of 1 M H₂SO₄ and 2.0 mL of 0.003 M KMnO₄ in a test tube, and incubated for 10 minutes at 37°C. The absorbances were measured within 200-525 nm using UV-Vis spectrophotometer (Model; Evolution 201, Serial No. 5A4P051001; ThermoFisher SCIENTIFIC).

All samples were analysed in triplicates, and values presented as mean ± standard deviation.

3.8.2. Alkaloid Content

The alkaloid content was determined by a gravimetric method described by (Adekanmi et al., 2009). About 3 g of the sample was weighed and transferred quantitatively into a 50.0 mL reagent bottle. The sample was immersed in 5% acetic acid solution in ethanol and agitated for three hours on an orbital shaker (Model; Stuart ® SSL1) at 80 rpm. With weighted filter paper (BOECO, Germany; 150 mm), the precipitate was filtered and dried at 60°C for 30 minutes in the hot air oven (SCIENTIFIC OVEN). The residue was weighed, and the following equation was employed to estimate alkaloid content.

$$\text{Percent Weight of alkaloid (\%)} = \frac{W_2 - W_1 * 100}{W_s}$$

Where **W₂** is the weight of filter paper with the residue, **W₁** is the weight of the filter paper, and **W_s** is the weight of the sample.

All samples were analysed in triplicates, and values presented as mean ± standard deviation.

3.8.3. Tannin Content (gallic acid and complex)

3.8.3.1. Standard Solution Preparation

Approximately 0.5 g of gallic acid was dissolved in a portion of distilled water and quantitatively transferred into a 100.0 mL volumetric flask to make a stock of 5 mg/mL. A 5.0 mL portion of the standard was further diluted in another 100.0 mL volumetric flask and then filled up to the mark with

distilled water. From the 5.0 mL stock, 2.0 mL was taken into a 25.0 mL volumetric flask followed by the addition of 1.0 mL of Folin-Ciocalteu reagent and 10.0 mL distilled water. A solution of 25% sodium carbonate (w/v) was used to fill up to the 25.0 mL mark. The mixture was allowed to stand for 30 minutes in a dark place, and the absorbance was measured at 760 nm blanked with distilled water using UV-Vis spectrophotometer (Model; Evolution 201, Serial No. 5A4P051001; ThermoFisher SCIENTIFIC).

3.8.3.2. Sample Preparation

3.8.3.2.1 Total Phenols

The sample, which weighed about 0.7 g, was heated in distilled water on a hot plate (Stuart® SD 500) for 35 minutes then allowed to cool to room temperature. The extract was then filtered into a 50.0 mL volumetric flask and adjusted to the mark with distilled water. From the stock, 5.0 mL was transferred into a 25.0 mL volumetric flask, then adjusted to the mark with distilled water. From the 5.0 mL stock, 2.0 mL of the solution was mixed with 1.0 mL of Folin-Ciocalteu reagent and 10.0 mL of distilled water in a 25.0 mL volumetric flask, and adjusted to the mark with 25% Na₂CO₃ (w/v). The mixture was allowed to stand for 30 minutes in a dark place, and the absorbance was measured at 760 nm blanked with distilled water using UV-Vis spectrophotometer (Model; Evolution 201, Serial No. 5A4P051001; ThermoFisher SCIENTIFIC).

3.8.3.2.2 Non-Complex Residual Phenols

From the original stock, 10.0 mL of the sample solution was mixed with about 3 g of polyvinylpyrrolidone K25 (PVP) in a 250.0 mL conical flask and subjected to agitation for one hour. The solution was then centrifuged (Model; Eppendorf Centrifuge 5804) for 10 minutes at 4200 rpm. The supernatant, 5.0 mL portion, was transferred into a 25.0 mL volumetric flask and adjusted to the mark with distilled water. An amount of 2.0 mL from the 5.0 mL stock was then mixed with 1.0 mL of Folin-Ciocalteu reagent and 10.0 mL of distilled water in a 25.0 mL volumetric flask and adjusted to the mark with 25% Na₂CO₃ (w/v). The mixture was allowed to stand for 30 minutes in a dark place, and the absorbance was measured at 760 nm blanked with distilled water using UV-Vis spectrophotometer (Model; Evolution 201, Serial No. 5A4P051001; ThermoFisher SCIENTIFIC).

The following equation was used to determine the "total tannin content in g% of standard";

$$\text{Total Tannin (g\%)} = \frac{62.5 \times (A_1 - A_2) \times m_2}{A_3 \times m_1}$$

Where A_1 is the absorbance of the sample solution of total phenols; A_2 , the absorbance of the sample solution of non-complex residual phenols; m_1 , mass of the sample used in grams; m_2 , mass of the standard in grams; A_3 , absorbance of the standard solution (Amorim et al., 2008; Galvão et al., 2018).

All samples were analysed in triplicates, and values presented as mean $\pm$ standard deviation.

3.9. Statistical Analysis

All data were expressed as mean $\pm$ standard deviation ($n=3$, $n=2$) and subjected to One-Way Analysis of Variance using Microsoft Excel 365 followed by Fisher's Least Significant Difference (LSD) post-hoc test (Meier, 2006) using R 4.2.1 software to determine statistical significant differences between mean values of samples, however, comparison was not required for *B.foetida* samples. Pearson's Product Moment Correlation was also performed to determine correlation between metals and antinutrients of the wild plum (*X. americana*) fruit parts. P-Values ≤ 0.05 were considered significant. The significance level was kept at $\alpha=0.05$.

3.10. Qualitative Preliminary Phytochemical Screening of *Boscia foetida* Schinz. Leaves and Roots

The phytochemical screening was done following the standard methods summarised by (Mazimba et al., 2015; Mukherjee, 2019; Shaikh & Patil, 2020; Sheel et al., 2014; Ugochukwu et al., 2013; Vimalkumar et al., 2014).

3.10.1. Alkaloids

3.10.1.1. Dragendorff's Reagent Test:

N-hexane and ethyl acetate extracts, 0.4 mL, dissolved in acetone, were acidified with 1 M hydrochloric acid solution followed by 5 drops of the reagent. An orange and brownish colouration appeared in the extracts, respectively, indicating the presence of alkaloids. About 0.01 g of alcoholic extracts, pure and 70% methanol, were dissolved directly in 3.0 mL 1 M hydrochloric acid solution, filtered and treated with 3 drops of the reagent. A colour change or precipitate formation in extracts indicates the presence of alkaloids.

3.10.1.2. Wagner's Test:

N-hexane and ethyl acetate extracts, 0.4 mL, dissolved in acetone were acidified with 1 M hydrochloric acid solution followed by 5 drops of the reagent. An orange inclining towards golden brown and reddish-brown colouration appeared in the extracts, respectively, indicating the presence of alkaloids. About 0.01 g of alcoholic extracts were dissolved directly in 1 M hydrochloric acid solution, filtered, and treated with 3 drops of the reagent. A turbid solution and a reddish-brown precipitate appeared in the extracts, respectively, indicates the presence of alkaloids.

3.10.2. Phenols

3.10.2.1. Ferric Chloride Test:

N-hexane and ethyl acetate extracts, 1.0 mL, (dissolved in acetone) were treated with 3 drops of aqueous 5% ferric chloride, respectively. Positive indicative: deep blue or black colour, none of the colours formed indicating the absence of phenolics. Alcoholic extracts, 1.0 mL, all dissolved in their mother solvents were treated with alcoholic 5% ferric chloride (90% ethanol). A bluish black colour indicates the presence of phenols.

3.10.2.2. Lead Acetate Test:

All the four extracts, 1.0 mL portions into the test tubes, followed by 3.0 mL of distilled water were treated with 1.0 mL 10% (w/v) lead acetate solution. Positive indicator: white, yellowish, orange precipitate. White and yellowish precipitates formed in the extract confirms the presence of phenolic compounds.

3.10.2.3. Ellagic Acid Test:

All the four extracts, 1.0 mL portions into the test tubes, were treated with 3 drops of 5% (v/v) glacial acetic acid solution followed by 5% (w/v) sodium nitrite solution. Muddy or niger brown precipitate indicates a positive result for phenols.

3.10.3. Saponins

Foam Test: The 1.0 mL portion of each extract was vigorously agitated with 5.0 mL of distilled water for 2 minutes and left to rest for 20 minutes long. The formation of a froth that stabilizes for atleast 20 minutes indicates the presence of saponins.

3.10.4. Terpenoids

All the four extracts, 2.0 mL, were treated each with 2.0 mL acetic anhydride and 3 drops of concentrated sulphuric acid. A greenish blue colouration indicates the presence of terpenoids.

3.10.5. Coumarins

Sodium Hydroxide Test: All the extracts, 1.0 mL, were treated with 1.0 mL of 10% (w/v) sodium hydroxide solution. Yellow colouration indicates the presence of coumarins.

3.10.6. Proteins and Ammino Acids

3.10.6.1. Ninhydrin Test:

All the extracts, 1.0 mL each, were treated with 4 drops of 1% (w/v) ninhydrin solution, dissolved in acetone, and placed in a boiling water bath for 1 minute. The formation of a purple colour indicates the presence of proteins.

3.10.6.2. Biuret Test:

Extracts, 2.0 mL each, were treated with 2 drops of 2% (w/v) copper sulphate solution, 1.0 mL of 95% (v/v) ethanol solution and 2.0 mL of 5% (w/v) potassium hydroxide solution. A pink colour that disappeared towards orange upon quick agitation shows the presence of proteins.

3.10.7. Quinones

3.10.7.1. Alcoholic Potassium Hydroxide:

All the extracts, 1.0 mL, were treated with 0.5 mL of 1% (w/v) alcoholic potassium hydroxide (90% ethanol solution). Expectation: A colouration ranging from red to blue indicates the presence of quinones. Observation: An orange colour appeared.

3.10.7.2. Concentrated Hydrochloric Acid:

The extract solutions, 1.0 mL each, were treated with 3 drops of concentrated hydrochloric acid. The formation of yellow colouration or precipitate or green colouration indicates the presence of quinones.

3.10.8. Tannins

Braymer's Test: All the extracts, about 0.1 g solvent free, were subjected to boiling in 7.0 mL of 45% (v/v) ethanol for 5 minutes and left for cooling at room temperature and pressure then filtered. About 1.0 mL of each extract/ filtrate was diluted with 0.5 mL of distilled water followed by 2 drops of alcoholic 5% (w/v) ferric chloride solution (90% ethanol solution). A blue-black to black colour with a thick emulsion upon shaking indicates the presence of tannins (hydrolysable tannins).

3.10.9. Flavonoids

3.10.9.1. Shinoda's Test:

Every extract, about 1.0 mL, was treated with 0.2 mL of concentrated sulphuric acid followed by the addition of about 0.03 g of magnesium ribbon to the solutions. An intense red and orange colouration indicates the presence of flavonoids.

3.10.9.2. Lead Acetate Test:

All the extracts, 1.0 mL, were treated with 0.2 mL of 10% (w/v) lead acetate solution. A yellow precipitate formed indicated the presence of flavonoids. An "orange to crimson colour" is an indication of the presence of a class of flavonoids called flavonones.

3.10.10. Cardiac Glycosides

Keller-Kiliani's Test: All the extracts, 1.0 mL, were treated with 1.5 mL of glacial acetic acid, a drop of 5% (w/v) ferric chloride solution followed by 2 drops of concentrated sulphuric acid along the sides of the test tube. A blue colouration indicates the presence of cardiac glycosides.

3.10.11. Sterols

3.10.11.1. Libermann-Burchard's Test:

All the extracts, 1.0 mL, were treated with 1.0 ml chloroform, 2.0 mL acetic anhydride followed by 2 drops of concentrated sulphuric acid along the sides of the test tube. A first blue-green to brick red colouration indicates the presence of sterols.

3.10.11.2. Salkowski's Test:

All the extracts, 3.0 mL, were treated with 2.0 mL of chloroform followed by 1.0 mL of concentrated sulphuric acid along the sides of the test tube. A reddish colouration shows the presence of sterols.

3.10.12. Triterpenoids

Salkowski's Test: All the extracts, 3.0 mL, were treated with 2.0 mL of chloroform followed by 1.0 mL of concentrated sulphuric acid along the sides of the test tube. A golden yellow bottom layer indicates the presence of triterpenoids.

3.10.13. Reducing Sugars

Benedict's Test: All the extracts, 0.5 mL, were treated with 0.5 mL of Benedict's reagent then subjected to boiling in a water bath for 2 minutes. A yellowish-green colouration with a precipitate, and a green colour formed exhibits the presence of reducing sugars.

CHAPTER 4

4. RESULTS AND DISCUSSION

4.1. *Boscia foetida* preliminary qualitative phytochemical screening of leaves and roots, and mineral element analysis

4.1.1. Preliminary qualitative phytochemical screening

Table 2: Preliminary phytochemical screening of *Boscia foetida* leaf and root extracts.

PHYTOCHEMICAL CLASS	TEST	LEAVES			ROOTS
		<i>Hexane</i>	<i>Ethyl Acetate</i>	<i>Methanol</i>	<i>70% Methanol</i>
Alkaloids	<i>Dragendorff's Reagent</i>	+	+	-	-
	<i>Wagner's Reagent</i>	+	+	+	+
Phenols	<i>Ferric Chloride</i>	-	-	++	-
	<i>Lead Acetate</i>	+	+	+	+
	<i>Ellagic Acid</i>	-	-	-	-
Saponins	<i>Foam</i>	-	-	+	++
Terpenoids		+	+	+	-
Coumarins	<i>Sodium Hydroxide</i>	+	+	++	-
Proteins and Amino Acids	<i>Ninhydrin</i>	-	-	++	++
	<i>Biuret</i>	+	+	-	-
Quinones	<i>Concentrated Hydrochloric Acid</i>	+	++	+	-
	<i>Alcoholic Potassium Hydroxide</i>	+	++	+	-

Tannins	<i>Braymer</i>	-	-	++	-
Flavonoids	<i>Shinoda</i>	++	++	+	-
	<i>Lead Acetate</i>	+	+	+	+
Cardiac Glycosides	<i>Keller-Kiliani</i>	+	+	+	-
Sterols	<i>Libermann-Burchard</i>	+	+	+	-
	<i>Salkowski</i>	++	-	+	-
Triterpenoids	<i>Salkowski</i>	++	+	+	-
Oxalate	<i>Glacial Acetic Acid</i>	-	-	-	-
Reducing Sugars	<i>Benedict's</i>	-	-	++	+

*(++) **Strongly Present**; (+) **Present**; (-) **Absent**

The preliminary qualitative phytochemical screening of *Boscia foetida* leaves and roots revealed a broad spectrum of phytochemicals. These include phenols, alkaloids, terpenoids, quinones, triterpenoids, coumarins, proteins, reducing sugars, sterols, flavonoids, tannins, glycosides and saponins across all four extracts, *n*-hexane, ethyl acetate, methanol, and 70% methanol. Leaves: alkaloids, phenols, terpenoids, coumarins, quinones, flavonoids, cardiac glycosides, sterols and triterpenoids were present in both the *n*-hexane and ethyl acetate extracts. Roots: reducing sugars, alkaloids, saponins and flavonoids were present in both methanol and 70% methanol extracts, while triterpenoids, steroids, phenols, terpenoids, coumarins, quinones, tannins and cardiac glycosides were only present in methanol extracts (**Table 2**).

Essential phytochemicals responsible for "anti-hyperglycemic, anti-inflammatory, anti-diabetic, and anti-microbial" actions, as well as other recognized bioactivities, are reportedly abundant in medicinal plants and herbs (Singh et al., 2022). These phytoconstituents may explain why different plant preparations exhibit medicinal properties (Konappa et al., 2020). Phytochemical screening is effective in identifying bioactive compounds that can be used in the production of therapeutic pharmaceuticals (Pant et al., 2017).

One of the key constituents revealed in our study upon screening *B. foetida* leaves and roots was alkaloids. Studies have shown that these compounds possess a vast array of pharmacological activities, such as antiasthma, anticancer, analgesic, antimalarial, antibacterial, antihyperglycemic, and antiarrhythmic activities, thus playing a significant role in the defence system of the body (Awuchi,

2019; Dey et al., 2020). Furthermore, they possess stimulating properties (Kurek, 2019). A recent study reported 11 alkaloids with antiviral activity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Ferreira, 2022).

Tannins have also been revealed, only in methanol root extract, and they play a crucial role through their diverse antimicrobial activities (Chung et al., 1998), such as inhibition of food-borne bacteria, and prevention of human immunodeficiency virus (HIV) infections (Chung et al., 1998; Tong et al., 2022). Moreover, (Aganga & Adogla-Bessa, 1999) quantitatively screened *B. foetida* leaves for tannins to evaluate their effect on crude protein digestibility in domesticated browsers in Botswana. The findings showed fairly low manageable quantities. Screening revealed the presence of flavonoids in both leaves and roots which have proven to be good health promoters with strong antioxidant effects against adverse environmental conditions, such as sunlight (Awa et al., 2020; Chaves et al., 2020). They also exhibit anticarcinogenic, anti-inflammatory, and antimutagenic activities (Panche et al., 2016). Triterpenoids, except for the methanol root extract, were present in both the leaves and roots. These compounds have been reported to possess antimicrobial, anticancer, antioxidant, and anti-inflammatory activities (Bishayee et al., 2011). Terpenoids were also present in both leaves and roots, except for the methanol root extract. This group of phytochemicals plays an important role in human health by preventing chronic conditions, such as cancer (Masyita et al., 2022; Thoppil & Bishayee, 2011). Coumarins were also detected in both the plant parts. These secondary metabolites have been reported to play a notable role in plant nutrition, particularly in the uptake of iron (Fe) from the soil into plant tissues (Robe et al., 2021); therefore, the strong presence of coumarins in the methanol root extract of *B. foetida* (**Table 2**) could explain the observed amount of iron in the roots of *B. foetida* (**Table 4**). They also possess antileishmanial, antioxidant, antimicrobial and antidepressant properties (Gonçalves et al., 2020; Heghes et al., 2022).

Cardiac glycosides were detected in both leaves and roots but were absent in the methanol root extract. Studies have noted their capabilities, such as preventing "cardiac insufficiency and congestive heart failure" (Fu et al., 2019). They also inhibit HIV infections, curb stress, and possess antibacterial properties (Samatha et al., 2012). Saponins were only present in root extracts. Research on this phytochemical group is increasing owing to its various biological and pharmacological applications. The anti-inflammatory, antiviral, antifungal, and antibacterial activities of saponins have been previously reported (Ashour et al., 2019). Sterols were present in both the leaves and roots. These compounds have demonstrated good inhibitory activity against rapid cholesterol absorption into the

human body, which is a prevalent dietary health concern (Berger et al., 2004). A study by (Osuala et al., 2022) also showed different degrees of phytochemical constituents in the roots of *Boscia senegalensis*, which is comparable to our findings. Constituents such as saponins were strongly present in the roots of both species. Furthermore, quinones were present in both roots and leaves, except for the 70% methanol root extract. These compounds have gained much attention in the medical field because of their reported anticancer properties, antioxidant properties, and ability to treat mitochondrial infections; however, some quinones have deleterious effects (Madeo et al., 2013; Q. Zhang et al., 2009). The presence of these phytochemical constituents preliminarily validates the ethnomedicinal uses of *B. foetida* plant.

4.1.2. GC-MS analysis of *B. foetida*

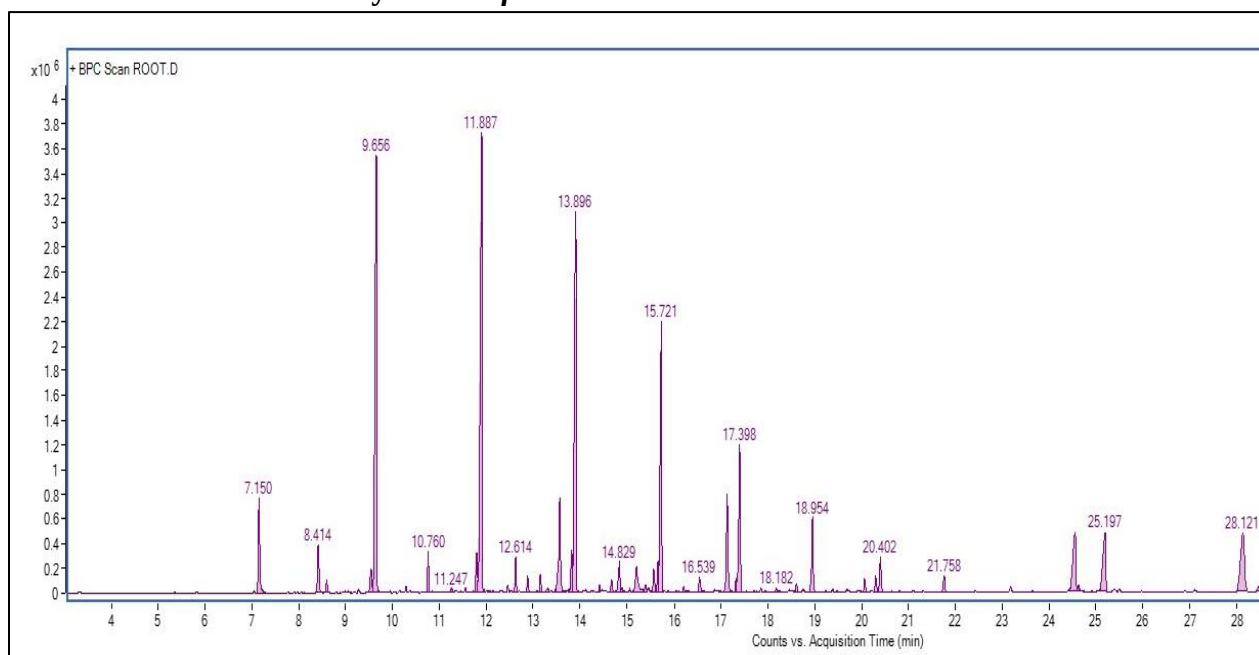


Figure 7: GC-MS profile of *n*-hexane extract of *Boscia foetida* roots.

Phytochemical analysis of *B. foetida* roots using GC-MS led to the identification of 11 compounds as shown in **Table 3**, all of which belong to the secondary metabolite groups of triterpenoids, phytosterols, alkane hydrocarbons, nitrogen-containing compounds, and esters. Octadecane (14.15%) was the major component followed by eicosane (11.90%), hexadecane (10.50%), tetracosane (4.80%), γ -sitosterol (2.21%), lup-20(29)-en-3-one (lupenone) (2.13%), 9-octadecenamide, (Z) (oleic acid amide) (2.00%), tetradecane (1.73%), 9-octadecenoic acid (Z)-, methyl ester (methyl oleate) (1.09%), heptadecane (0.98%), and phthalic acid, butyl tetradecyl ester (0.89%). These compounds were also found in varying compositions in other plant species, and their biological properties have been reported.

4.1.2.1. Lup-20(29)-en-3-one (Lupenone)

Lupenone (2.13%) is a secondary metabolite belonging to lupane-type triterpenoids found in many plant families (Xu et al., 2018) and possesses good anti-diabetic activity and tumor suppression activity, particularly against "breast and prostate cancer" (Rodrigues Da Silva et al., 2022). A study by (Villareal et al., 2013) demonstrated that lupenone from *Erica multiflora* ethyl acetate leaf extract stimulates melanogenesis by increasing the expression of the tyrosinase enzyme through the inhibition of mitogen-activated protein kinase phosphorylated extracellular signal-regulated kinases 1 and 2, which suggests that it may be used to treat hypopigmentation-inducing diseases. Furthermore, this compound has been isolated from the roots of *Adenophora triphylla* var. *japonica* which are used in Chinese medicine to formulate an anti-inflammatory and cough relieving agent (Ahn & Oh, 2013).

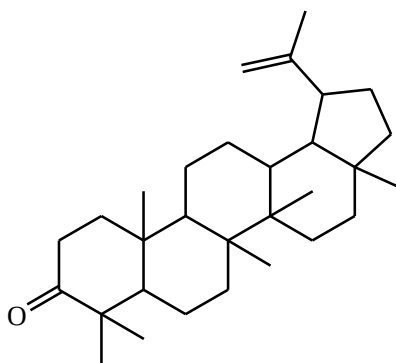


Figure 8: Lupenone

4.1.2.2. γ -Sitosterol

It is an epimer of β -Sitosterol with antihyperglycemic and anticancer activities (Sirikhansaeng et al., 2017). This compound was previously isolated from the leaves of *Acacia nilotica* and exhibited notable anticancer activity against human cancer cells (MCF-7 and A549 cell lines) (Sundarraaj et al., 2012). The same compound has been isolated from *Girardinia heterophylla* (Decne) leaves and *Lippia nodiflora* L. whole plant, and its antidiabetic activity has been demonstrated (Balamurugan et al., 2011; Tripathi et al., 2013). The roots and herb of *Girardinia heterophylla* (Decne) and *Lippia nodiflora* L. are used as soap for washing and for the treatment of ulcers, respectively (Balamurugan et al., 2011; Tripathi et al., 2013).

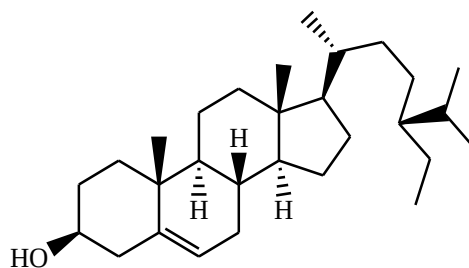


Figure 9: γ -Sitosterol

4.1.2.3. Tetracosane

Tetracosane (4.80%) is found in many volatile plant oils. The compound was reportedly isolated from *Acrostichum aureum* plant aerial parts and exhibited cytotoxic activity against cancer cell lines upon subjection to cytotoxicity evaluation (Uddin et al., 2012). Additionally, tetracosane isolated from *Acacia pinnata* twigs demonstrated strong inhibition against β -amyloid aggregation which plays a significant role in the development of Alzheimer's disease (Lomarat et al., 2015).

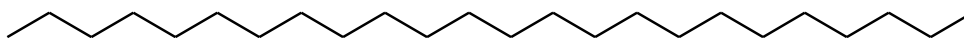


Figure 10: Tetracosane

4.1.2.4. 9-Octadecenamide, (Z) (Oleic acid amide)

A long chain fatty acid amide (2.00%) recognized for its potential role in mediating the drive to sleep, particularly in mammalian species, and various neuropharmacological activities (Cheng et al., 2010). It also exhibits antibacterial, anti-inflammatory, and analgesic activities (Olaoluwa et al., 2018; Salah et al., 2015). The compound was isolated from the hexane crude extract of the fermentation broth of the fungus *Diaporthe schini*. The resultant fraction exhibited antifungal activity against the multi-drug resistant fungus *Candida krusei* (Dos Reis et al., 2019).

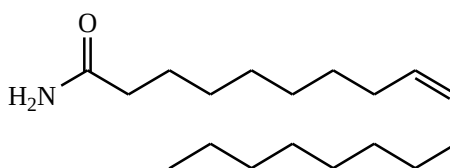


Figure 11: 9-Octadecenamide, (Z) (Oleic acid amide)

4.1.2.5. 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)

Methyl oleate (1.09%) is a fatty acid methyl ester of oleic acid reported to possess antioxidant and antihypertensive activities. It also exhibits antitumor effect (Hagr et al., 2021; Momodu et al., 2022; Yusuf et al., 2023).

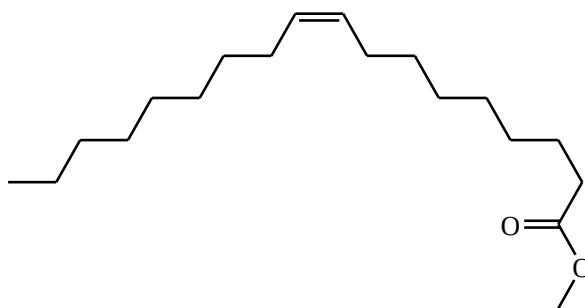


Figure 12: 9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)

4.1.2.6. Eicosane

An aliphatic hydrocarbon (11.90%) resourceful in cosmetics, as a food additive and plasticizer. It exhibited an inhibition rate of 60% against *Candida albicans* biofilm in an in-vitro antibiofilm evaluation (Beema Shafreen et al., 2022). Furthermore, it has shown cytotoxic activity against HeLa and MCF-7 cells and exhibits appreciable wound healing activity "in a diabetic rat wound model" (Balachandran et al., 2023; Saleh-E-In et al., 2021). This compound exhibited antifungal activity against *Rhizoctonia solani* AG-3, which is responsible for the target spot disease in tobacco leaves (Ahsan et al., 2017).

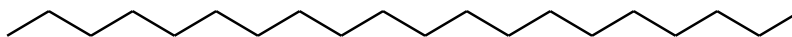


Figure 13: Eicosane

4.1.2.7. Phthalic acid, butyl tetradecyl ester

This is a lipophilic compound (0.89%) belonging to phthalates commonly used as plasticizers during plastic making. Low molecular weight phthalates are normally used in cosmetics and inks for printers (Huang et al., 2021). This compound was detected in leaf essential oil of *Euphorbia hirta* Linn. and reported to possess anti-insecticidal activity (Huang et al., 2021; Ogunlesi et al., 2009).

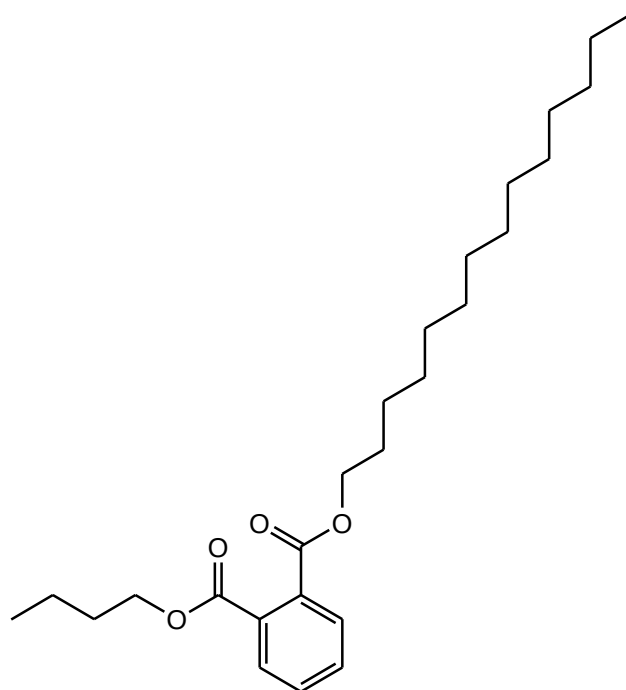


Figure 14: Phthalic acid, butyl tetradecyl ester

4.1.2.8. Octadecane

Octadecane (14.15%), an aliphatic hydrocarbon, was identified as a major component in the current study and was also reported in a large proportion in the Tunisian *Allium nigrum* L. bulb (30.5%) essential oil (Rouis-Soussi et al., 2014). This compound was previously reported to possess moderate antibacterial activity (Kant et al., 2019).

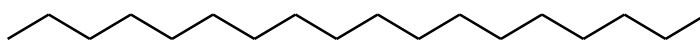


Figure 15: Octadecane

4.1.2.9. Heptadecane

Heptadecane (0.98%), a volatile substance, reportedly prevents the synthesis of fatty acids and treats several conditions linked to oxidative stress (Kim et al., 2013). Besides, (Kim et al., 2013) demonstrated the anti-inflammatory activity mechanism of heptadecane in "aged kidney tissues." This hydrocarbon has previously shown inhibitory effects on bacterial strains (Kumari et al., 2019). Furthermore, (Naeim et al., 2020) reported heptadecane as one of the major alkane hydrocarbons in the chloroform extract of *Centaurea pumilio* L. aerial part (16.05%), and its antimicrobial properties have been described in literature.

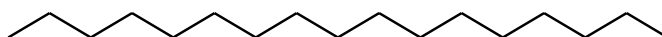


Figure 16: Heptadecane

4.1.2.10. Hexadecane

A six-carbon-atom long chain hydrocarbon (10.50%) reported to possess antibacterial and antioxidant properties (Ferdosi et al., 2023; Valarmathi et al., 2023; Yogeswari et al., 2012). Its analogs, trifluoro-acetoxy hexadecane and 2,6,11,15-tetramethyl hexadecane, reportedly exhibit antifungal and flavouring properties (François et al., 2020; Ibnouf et al., 2022).

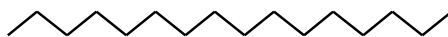


Figure 17: Hexadecane

4.1.2.11. Tetradecane

Tetradecane (1.73%) is an aliphatic hydrocarbon reported as a component in the cockroach *Blattella craniifer*'s aggregation pheromone (Francke & Schulz, 1999). On the other hand, it is found in plant parts of many plant species. It was extracted in its pure form from *Pediococcus acidilactici* DSM: 20284-CM endophytic bacterial isolate extract. The tetradecane extract exhibited strong antibacterial activity against gram-positive *Staphylococcus aureus* (Nasr et al., 2021). It has also showed fungal inhibition against *Candida albicans*, *Aspergillus flavus* and *Rhizoctonia solani* (Girija et al., 2014; Khan & Javaid, 2019). Furthermore, tetradecane has been reported to possess antioxidant properties (Khan & Javaid, 2019).



Figure 18: Tetradecane

Based on prior studies on the biological activities of compounds detected in *B. foetida* *n*-hexane root extract in this study using GC-MS, it is therefore presumably a potential antimicrobial agent against various bacterial and fungal strains.

Table 3: Phytochemical compounds identified in *n*-hexane root extract of *Boscia foetida*

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
11	Lup-20(29)-en-3-one (Lupenone)	25.197	2.13 ± 0.75	424.7	C ₃₀ H ₄₈ O
10	γ-sitosterol	24.550	2.21 ± 0.74	414.7	C ₂₉ H ₅₀ O
9	Tetracosane	17.398	4.80 ± 1.82	338.7	C ₂₄ H ₅₀
8	9-Octadecenamide, (Z) (Oleic acid amide)	17.134	2.00 ± 0.63	281.5	C ₁₈ H ₃₅ NO
7	9-Octadecenoic acid (Z)-, methyl ester (Methyl oleate)	14.829	1.09 ± 0.41	296.27	C ₁₉ H ₃₆ O ₂
6	Eicosane	13.896	11.90 ± 3.03	282.3 (282.328651340)	C ₂₀ H ₄₂
5	Phthalic acid, butyl tetradecyl ester	12.614	0.89 ± 0.28	418.3	C ₂₆ H ₄₂ O ₄
4	Octadecane	11.887	14.15 ± 1.63	254.5	C ₁₈ H ₃₈
3	Heptadecane	10.760	0.98 ± 0.21	240.2	C ₁₇ H ₃₆
2	Hexadecane	9.656	10.50 ± 1.56	226.44	C ₁₆ H ₃₄
1	Tetradecane	7.150	1.73 ± 0.81	198.39	C ₁₄ H ₃₀

4.1.3. *B. foetida* mineral element analysis

Mineral elements are often present in fruits, plants, fish, and other meat types, and several of them play an essential part in "nutrient metabolism," including other important cellular processes (Shah et al., 2023). Quantification of mineral elements in this study revealed the following in different concentrations; sodium, potassium, manganese, zinc, iron, nickel, copper, chromium, magnesium, calcium, cobalt, and lead (**Table 4**).

Table 4: *Boscia foetida* roots mineral element profile showing both trace and major elements

Elements	Concentration (mg/kg)/(ppm)	Permissible Concentration levels (mg/kg)/(ppm)*
Sodium (Na)	358.90 ± 19.68	-
Manganese (Mn)	61.34 ± 3.03	200 (plants)
Cobalt (Co)	38.30 ± 2.38	0.05-0.1
Arsenic (As)	ND	1
Lead (Pb)	3.76 ± 0.03	0.3
Chromium (Cr)	ND	2.30
Iron (Fe)	104.30 ± 3.40	450
Nickel (Ni)	4.67 ± 0.78	2.70
Copper (Cu)	1.09 ± 0.04	10
Zinc (Zn)	69.04 ± 3.83	50 60 (fruits)
Magnesium (Mg)	2348.00 ± 173.21	2000
Potassium (K)	7239.00 ± 386.37	-
Calcium (Ca)	2871.33 ± 46.49	-

*Permissible limits by FAO/WHO and ATSDR for plants, fruits and vegetables (AlKhatib et al., 2022; Amer et al., 2019; Bawwab et al., 2022; “Compendium of Chemical Hazards: Arsenic,” 2016; Das et al., 2004; Eissa & Negim, 2018; Lere et al., 2021; Mekassa & Chandravanshi, 2015; Nisa et al., 2020; A. Sharma et al., 2016; Sultana et al., 2022; Varol et al., 2022; World Health Organization, 2011; Yami et al., 2016; Yaqub et al., 2021)

**ND, Not Detected

The data is expressed as mean ± standard deviation (n=3, except for nickel and lead n=2)

The roots contained significant high amounts of potassium, calcium, magnesium, sodium, iron, copper and zinc, comparatively higher than *B. variabilis* and closely related species, *B. albitrunca* (Maroyi, 2019; Mon, 2018; Wehmeyer, 1986). Calcium plays a pivotal role in numerous aspects such as, muscle contractions, cellular differentiation, neuronal activities, strong bones and teeth, and apoptosis (Pu et al., 2016; Radha et al., 2021). Moreover, probable mechanisms of calcium cholesterol-lowering activity has been demonstrated (Mulet-Cabero & Wilde, 2021). Prior studies have also revealed the potential effect of potassium in regulating intracellular pH, and synthesizing DNA and proteins. It has also been reported to possess antihypertensive effect engendered by its synergistic relationship with sodium (Kettritz & Loffing, 2023; Kwak & Kim, 2023).

The World Health Organization recommended potassium intake of more than 3.5 g/day and less than 2 g/day of sodium to facilitate the blood pressure-lowering effect (Reddin et al., 2023). Magnesium is one essential element with the ability to stabilize lipids and protein membranes. Higher dietary magnesium intake is linked to a decreased risk of Alzheimer’s disease, suggesting that Mg intake or supplementation may have a neuroprotective impact (Shah et al., 2023). On the other hand, sodium is

required for body fluid balance and cellular homeostasis (Farquhar et al., 2015). Iron is an essential element for humans and plants alike with several functions such as, oxygen transport, immunity, and cell differentiation (Piskin et al., 2022). Copper plays a crucial role as a cofactor for various enzymes that regulate blood coagulation and control blood pressure (Burkhead & Collins, 2022). Furthermore, zinc is also a crucial element in human health required in oxidative stress, and immune functions (Chasapis et al., 2012). These elements are considered essential, therefore, these elevated concentrations makes *B. foetida* roots a good source of these nutrients (**Table 4**). In comparison to the mineral content of *Paeonia lactiflora* and *Boscia senegalensis* roots, *Boscia foetida* roots has been found to contain lower concentration of manganese with respect to *Boscia senegalensis*, and higher concentration with respect to *Paeonia lactiflora* (Li et al., 2020; Maroyi, 2019d). Comparatively, *B. foetida* roots has shown increased level of lead (Pb), than the *B. senegalensis* roots (Maroyi, 2019d), indicating potential toxic heavy metal contamination. Lead is a toxic heavy metal even at very low levels, with neurotoxic effects such as, mental and intelligence breaking down and "behavioural abnormalities" (Hamurcu et al., 2010; Parveen et al., 2020). Cobalt was also found at higher concentration than the permissible value. Although cobalt is regarded an essential element, its excess may disrupts the plant nutrition system by interfering in the uptake of other essential elements, and cause adverse effects to humans which are linked to its interaction with sulfhydryl group and antagonistic effects in the calcium ion channel (Sharma et al., 2016).

This contamination could be attributed to environmental pollution therefore, this study suggests that regular monitoring of toxic heavy metals be implemented in this region due to the fact that heavy metal accumulation can be dangerous to users of *B. foetida* plant parts when present in excess. All this highlights the fact that the mineral content of plants may vary due to various factors such as the type of soil, plant species, and environmental conditions (Radha et al., 2021).

4.2. *X. Americana* (wild plum) mineral content and the effect of long-term frozen storage on mineral content

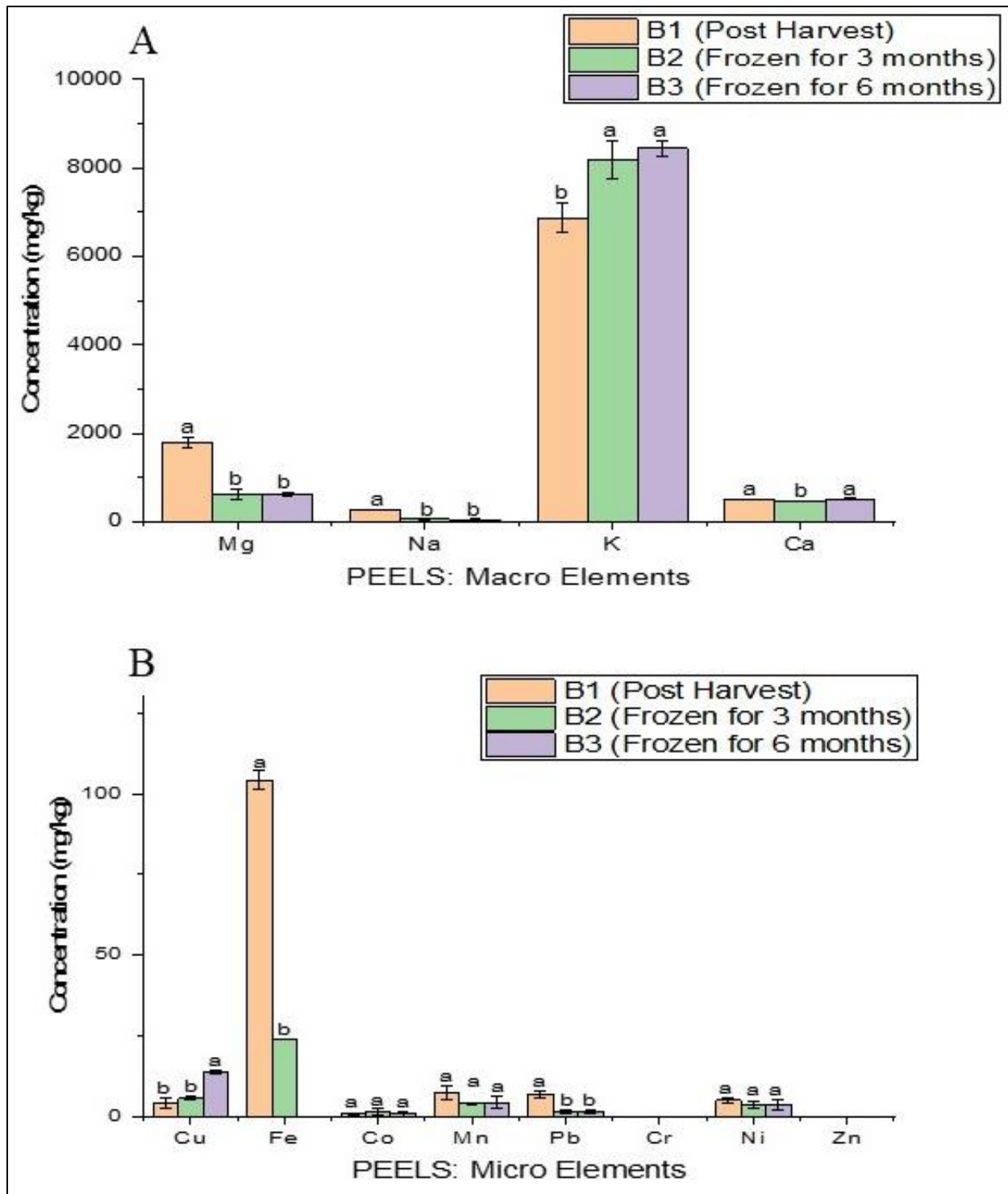


Figure 19: Changes in macro and micro mineral elements content of the wild plum fruit peels after harvest and during storage at -4°C . Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

Table 5: Wild plum (*X. americana*) fruit peels mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.

PEELS				Permissible Concentration Levels (mg/kg/ ppm)
	B1 (Post Harvest)	B2 (After Three Months)	B3 (After Six Month)	
Sodium (Na)	276.90 ± 2.55 ^a	54.32 ± 6.51 ^b	43.70 ± 9.31 ^b	-
Manganese (Mn)	7.46 ± 2.17 ^a	3.95 ± 0.07 ^a	4.46 ± 1.82 ^a	200 (plants)
Cobalt (Co)	0.94 ± 0.28 ^a	1.50 ± 0.84 ^a	0.80 ± 0.66 ^a	0.05-0.1
Lead (Pb)	6.96 ± 1.13 ^a	1.76 ± 0.52 ^b	1.70 ± 0.61 ^b	0.10
Chromium (Cr)	ND	ND	ND	0.10
Iron (Fe)	104.10 ± 2.97 ^a	23.98 ± 0.21 ^b	ND	425
Nickel (Ni)	4.99 ± 0.95 ^a	3.70 ± 0.86 ^a	3.74 ± 1.63 ^a	0.20
Copper (Cu)	4.24 ± 1.41 ^b	5.61 ± 0.45 ^b	13.61 ± 0.54 ^a	10
Zinc (Zn)	ND	ND	ND	60
Magnesium (Mg)	1794 ± 129 ^a	619.50 ± 110.80 ^b	617.30 ± 24.44 ^b	2000
Potassium (K)	6877 ± 334 ^b	8186 ± 428 ^a	8438 ± 167 ^a	-
Calcium (Ca)	517.07 ± 3.84 ^a	456.43 ± 4.40 ^b	519.37 ± 7.99 ^a	-

*ND, Not detected

**All values within a row with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

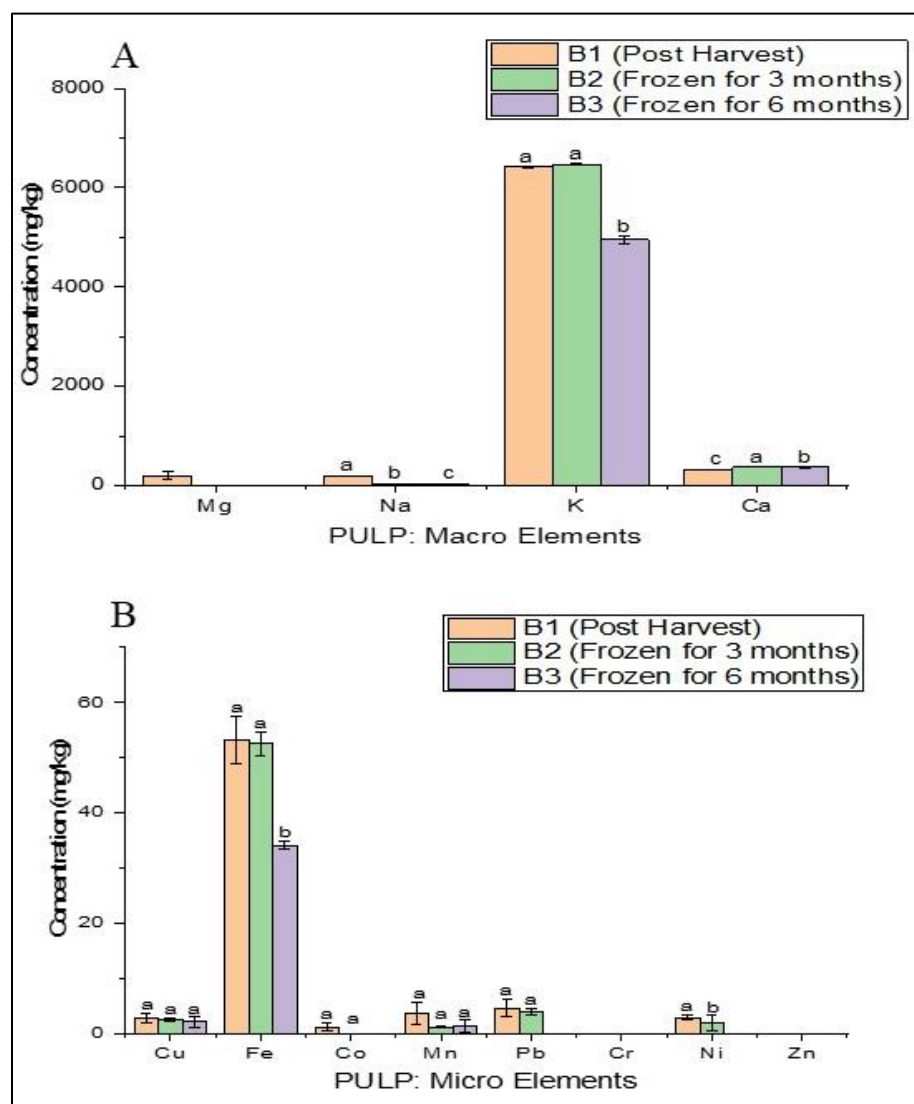


Figure 20: Changes in macro and micro mineral elements content of wild plum fruit pulp after harvest and during storage at -4°C . Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

Table 6: Wild plum (*X. americana*) pulp mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.

PULP				Permissible Concentration Levels (mg/kg/ ppm)
	B1 (Post Harvest)	B2 (After Three Months)	B3 (After Six Months)	
Sodium (Na)	195.30 ± 4.31 ^a	38.33 ± 6.42 ^b	18.00 ± 7.72 ^c	-
Manganese (Mn)	3.69 ± 2.05 ^a	1.16 ± 0.11 ^a	1.30 ± 1.08 ^a	200 (plants)
Cobalt (Co)	1.13 ± 0.77 ^a	0.22 ± 0.08 ^a	ND	0.05-0.1
Lead (Pb)	4.56 ± 1.51 ^a	3.89 ± 0.64 ^a	ND	0.10
Chromium (Cr)	ND	ND	ND	0.10
Iron (Fe)	53.29 ± 4.27 ^a	52.60 ± 2.17 ^a	34.13 ± 0.76 ^b	425
Nickel (Ni)	2.85 ± 0.46 ^a	1.96 ± 1.37 ^b	ND	0.20
Copper (Cu)	2.82 ± 0.82 ^a	2.54 ± 0.33 ^a	2.07 ± 0.89 ^a	10
Zinc (Zn)	ND	ND	ND	60 (fruits)
Magnesium (Mg)	196.9 ± 74.39	ND	ND	2000
Potassium (K)	6413.00 ± 16.97 ^a	6468.50 ± 21.92 ^a	4955.50 ± 77.07 ^b	-
Calcium (Ca)	328.90 ± 3.27 ^c	381.97 ± 4.61 ^a	368.87 ± 6.77 ^b	-

*ND, Not Detected

**All values within a row with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

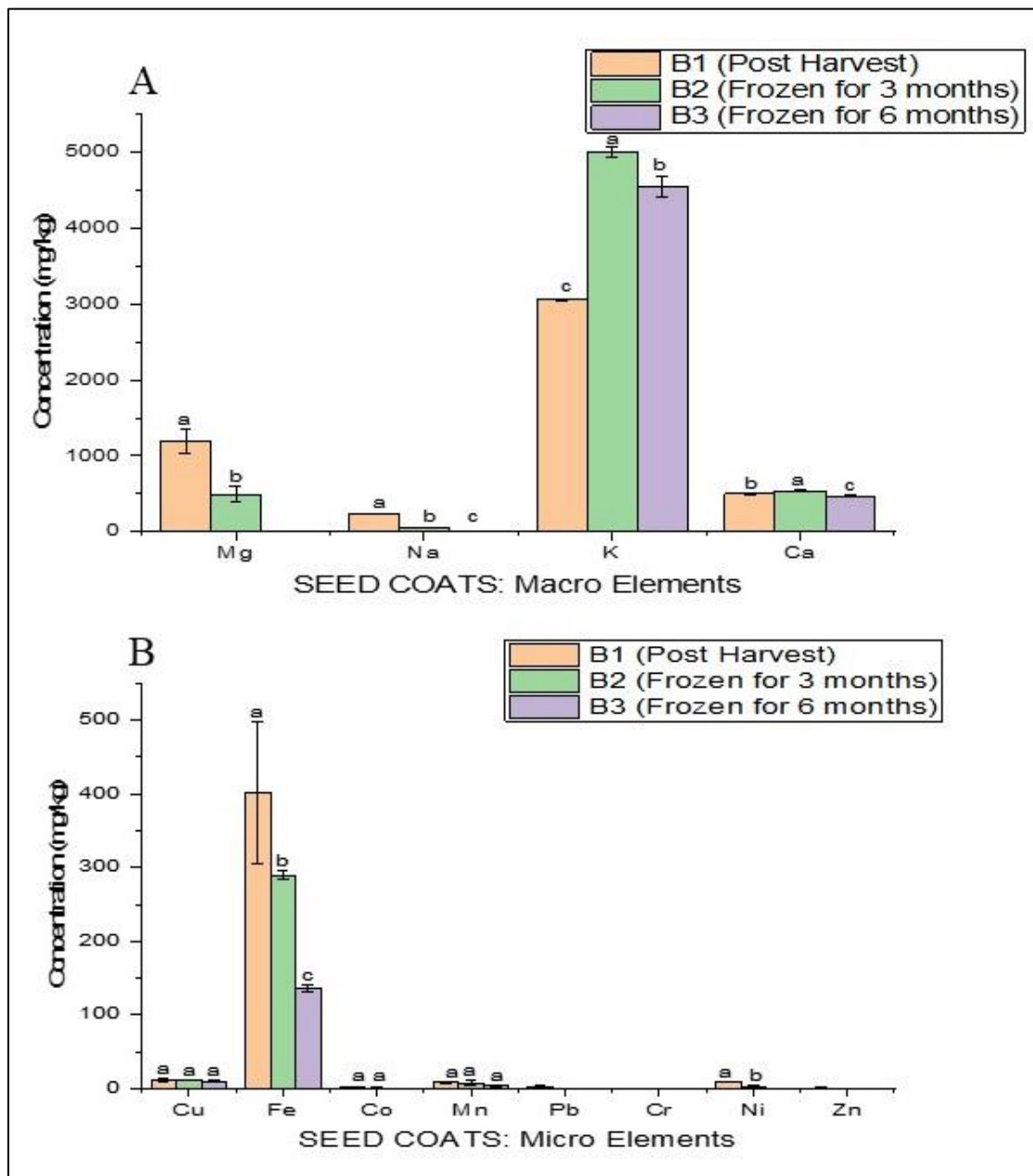


Figure 21: Changes in macro and micro mineral elements content of wild plum fruit seed coats after harvest and during storage at -4°C . Different lower-case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

Table 7: Wild plum (*X. americana*) seed coats mineral element profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.

SEED COAT				Permissible Concentration Levels (mg/kg/ ppm)
	B1 (Post Harvest)	B2 (After Three Month)	B3 (After Three Month)	
Sodium (Na)	229.2 ± 3.54 ^a	57.42 ± 2.69 ^b	5.23 ± 0.36 ^c	-
Manganese (Mn)	9.15 ± 1.10 ^a	7.75 ± 3.26 ^a	3.51 ± 0.96 ^a	200 (plants)
Cobalt (Co)	1.63 ± 0.18 ^a	1.05 ± 0.29 ^a	ND	0.05-0.1
Lead (Pb)	2.59 ± 0.97	ND	ND	0.10
Chromium (Cr)	ND	ND	ND	0.10
Iron (Fe)	402.25 ± 96.38 ^a	289.80 ± 6.65 ^b	137.00 ± 4.81 ^c	425
Nickel (Ni)	9.91 ± 0.45 ^a	3.27 ± 0.61 ^b	ND	0.20
Copper (Cu)	11.70 ± 2.36 ^a	11.51 ± 0.53 ^a	10.41 ± 0.09 ^a	10
Zinc (Zn)	1.16 ± 1.10	ND	ND	60 (fruits)
Magnesium (Mg)	1192 ± 157 ^a	495.40 ± 106.39 ^b	ND	2000
Potassium (K)	3053.33±18.61 ^c	5000.00±63.15 ^a	4550.67±138.66 ^b	-
Calcium (Ca)	505.93 ± 9.47 ^b	536.57 ± 10.97 ^a	473.37 ± 7.05 ^c	-

*ND, Not Detected

**All values within a row with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

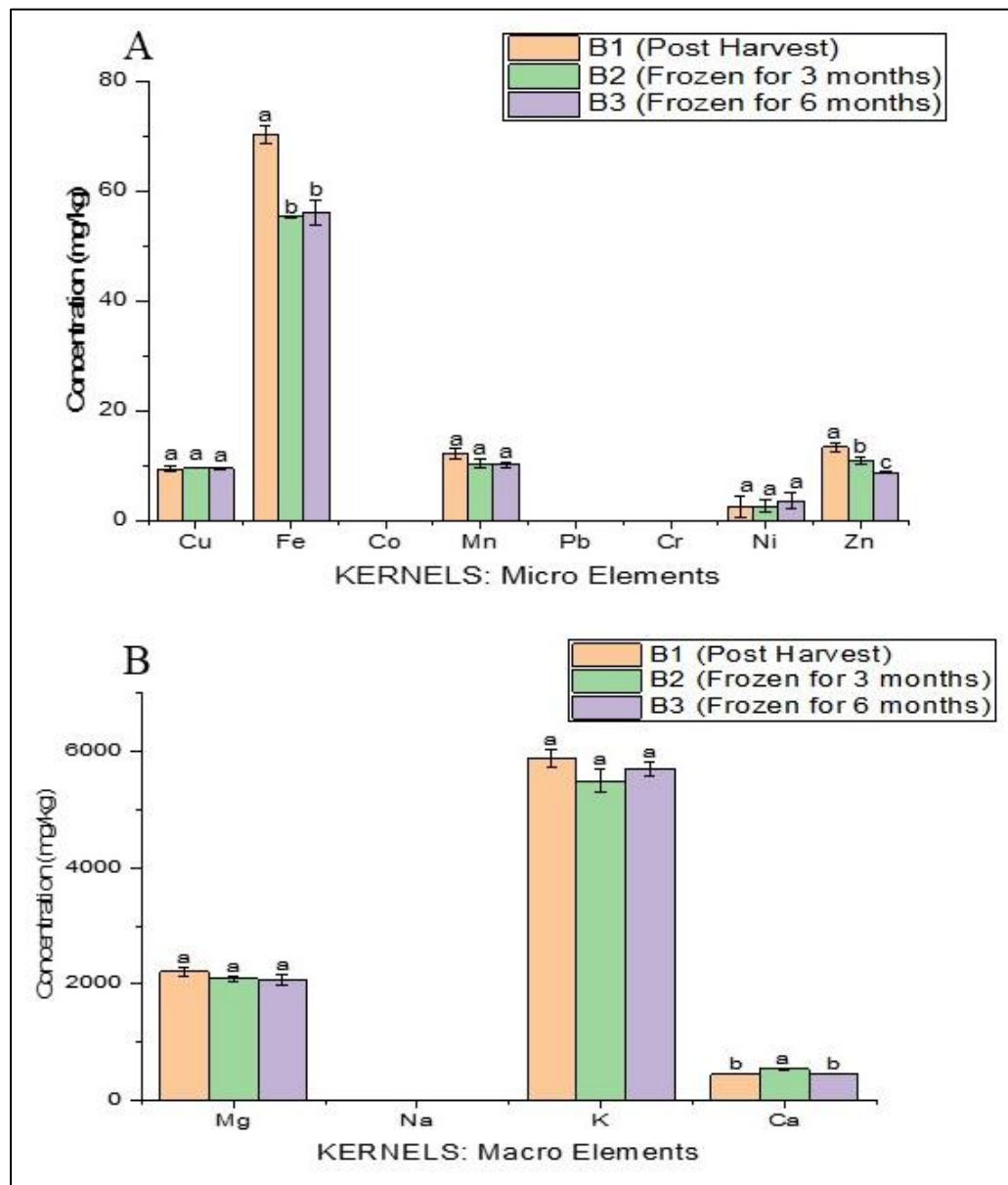


Figure 22: Changes in macro and micro mineral elements content of the wild plum fruit kernels after harvest and during storage at -4°C . Different lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

Table 8: Wild plum (*X. americana*) kernels mineral elements profile showing both trace and major elements after harvest and after 3 and 6 months of frozen storage.

KERNELS (NUTS)				Permissible Concentration Levels (mg/kg/ppm)
	B1 (Post Harvest)	B2 (After Three Month)	B3 (After Six Month)	
Sodium (Na)	8.70 ± 0.25	ND	ND	-
Manganese (Mn)	12.21 ± 0.84 ^a	10.44 ± 0.95 ^a	10.23 ± 0.52 ^a	200 (plants)
Cobalt (Co)	ND	ND	ND	0.05-0.1
Lead (Pb)	ND	ND	ND	0.10 (Fruits)
Chromium (Cr)	ND	ND	ND	0.10
Iron (Fe)	70.41 ± 1.53 ^a	55.35 ± 0.06 ^b	56.20 ± 2.27 ^b	425
Nickel (Ni)	2.51 ± 2.04 ^a	2.58 ± 1.14 ^a	3.59 ± 1.49 ^a	0.20
Copper (Cu)	9.46 ± 0.59 ^a	9.52 ± 0.01 ^a	9.49 ± 0.16 ^a	10
Zinc (Zn)	13.37 ± 0.70 ^a	10.97 ± 0.54 ^b	8.76 ± 0.21 ^c	60 (fruits)
Magnesium (Mg)	2210.00 ± 74.48 ^a	2097.67 ± 46.80 ^a	2069.67 ± 91.49 ^a	2000
Potassium (K)	5881.33 ± 141.99 ^a	5493.00 ± 201.41 ^a	5704.67 ± 120.09 ^a	-
Calcium (Ca)	451.43 ± 3.79 ^b	546.83 ± 13.45 ^a	450.43 ± 4.31 ^b	-

*ND, Not Detected

**All values within a row with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

The results of this study were first treated with analysis of variance to determine a group test for the null hypothesis. Where differences are statistically significant, Fischer's least significant differences test was employed.

Mineral elements play a crucial role in the human body and are essential components of food we consume on daily basis (Weyh et al., 2022). However, content alterations may occur during storage and processing.

Minerals are generally able to withstand storage conditions and other processes, and for this reason, changes in their content are typically less noticeable (Bouzari et al., 2015; Rickman et al., 2007). According to (Rickman et al., 2007), some of the major processes capable of mineral loss in vegetables and fruits include, leaching, and blanching, a pre-treatment procedure usually employed before freezing where a hot water, steam or microwave is used to remove undesirable materials on the surface of the product, things like oxidising enzymes e.g ascorbic acid oxidase and peroxidase with the aim of

maintaining the product quality (Celli et al., 2016). In this study, normal washing with water was applied.

Despite the less susceptibility of minerals to various treatments, our results showed a significant decrease in iron content in seed coats, pulp and peels after 3 and 6 months of freezing. However, kernels showed insignificant change in iron content after 3 and 6 months of frozen storage (**Figure 19, 20, 21, 22**). The data also demonstrated a significant decrease in magnesium (Mg), zinc (Zn), sodium (Na), and potassium (K) contents of the seed coats, kernels and pulp over 6 months of frozen storage (**Figure 20, 21, 22**). Similar findings were reported by (Oulai et al., 2015) who observed a decrease in calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), iron (Fe), sodium (Na), and zinc (Zn) contents of five vegetable species stored at -18°C over a period of 3 months. Likewise, a study by (Mudau et al., 2015) demonstrated a similar trend in magnesium (Mg), iron (Fe), and zinc (Zn) contents of spinach leaves stored at 4°C over a period of 12 days, and the effect was observed after 8 days. The decrease might be due to the physical injuries of the fruit cells during processing and after freezing, which may result in the release of cell content comprising of mineral elements (Fellows, 2000; Oulai et al., 2015). However, there were no significant differences in magnesium (Mg), potassium (K) and sodium (Na) contents of the peels and kernels (**Figure 19, 22**). Calcium was relatively well retained across all fruit parts despite significant differences noted after harvest and at frozen storage (**Figure 19, 20, 21, 22**). Furthermore, a significant decrease in nickel (Ni) content of the pulp and coats was observed. These results were in agreement with the findings of (López et al., 2004) who found a decrease in nickel content of green asparagus frozen at -18°C over a period of 90 days.

Manganese (Mn) remained almost stable across all fruit parts, no significant differences in content. Chromium (Cr) was not detected in all fruit parts. Moreover, copper (Cu) showed a significant increase in the peels while observed no significant changes in pulp, coats and kernels (**Figure 19, 20, 21, 22**). The increase in copper (Cu) content of the peels could be due to dehydration associated with storage at freezing temperatures (Malik et al., 2021) which leads to concentrated analyte, and surface contaminants such as soil left on the peels after washing. Lead showed no significant change in peels over a period of 6 months, however, we observed a significant decrease in the concentration of pulp. Coats and kernels recorded no presence of lead. Differences in the concentration levels of mineral elements in fresh and frozen fruits and vegetables are reportedly a result of procedures such as washing and blanching before exposure to freezing temperatures and storage. Another possible cause of these changes could be the freezing process rather than storage alone, as far as this study is concerned.

Damage to cells and tissues during frozen storage could be attributed to observable decreases in mineral content since abundant exuded liquid during thawing is highly possible and may result in significant mineral losses (López et al., 2004). Instead of the "biochemical or physical loss of the analyte," it is perceived that declines in mineral levels after storage are most likely caused by variations in how easily particular minerals are removed from the sample matrix during analysis (Bouzari et al., 2015). Crude sample variabilities may also be responsible for observable increases and declines in mineral content (Bouzari et al., 2015). Additionally, the process of turning water into pure ice by removing it from plasma or colloidal solution during freezing may account for mineral losses. Osmotic imbalance is caused by this event because the residual solution becomes extremely concentrated with dissolved salts (Malik et al., 2021). The effect of long-term frozen storage on mineral elements content differed depending on studied mineral element, and storage treatment.

The nutritional information of the wild plum (*X. americana*) pulp and kernels was determined in terms of five most essential dietary elements. The following tables shows the amounts of Fe, Mg, Ca, Na and K per 100 g serving of the wild plum pulp and kernels (nuts) as compared to common commercial watermelon, red apple, almonds and peanuts.

Table 9: Nutritional information of the wild plum pulp (essential elements) (mg/100 g per serving)

	Wild plum pulp	Watermelon (USDA, 2019)	Red apple (USDA, 2019)
K	641.3	112.0	107.0
Ca	32.9	7.0	6.0
Fe	5.3	0.24	0.11
Na	19.5	1.0	1.0
Mg	19.7	10.0	5.0

Table 10: Nutritional information of the wild plum kernels (essential elements) (mg/100 g per serving)

	Wild plum kernels	Almonds (USDA, 2019)	Peanuts (USDA, 2019)
K	588.1	728.0	705.0
Ca	45.1	248.0	92.0
Fe	7.0	3.71	4.58
Na	0.87	1.0	18.0
Mg	22.1	270.0	168.0

The findings presented above (**Table 9, 10**) show, K, Ca, and Mg were the most abundant elements, followed by Na and Fe. It was observed that Ca was higher in the wild plum pulp (329 mg/kg) compared to commercial popular cultivars, watermelon (70 mg/kg) and red apple (60 mg/kg) (USDA, 2019). Calcium is one essential mineral to bone development, strength and function (Nguyen & Murimi, 2021). Children within the ages of 4-8 years require 1000 mg/day of calcium (IOM (Institute of Medicine), 2011), as they are in their early stages of growth and development, and this mineral is critical at that stage of life. The wild plum (*X. americana*) pulp in the current study was reported to contain 329 mg/kg of calcium suggesting that 100 g of the pulp will have extra 32.9 mg of calcium. This proves the assertion that edible native fruits from the wild could close the nutrition gap in rural communities. Potassium and sodium are also essential for their important roles in muscle contractions and regulating and maintaining plasma volume (Nascimento et al., 2021). The two minerals were also observed to be higher in the wild plum pulp compared to red apple and watermelon. Likewise, magnesium was higher in the wild plum pulp in comparison to the selected common fruits. Adults, both male and female, require daily intake ranging from 300-400 mg/day of magnesium (Vormann & Anke, 2002). In that light, the experimental 197 mg/kg magnesium content of the wild plum pulp will provide 19.7 mg of magnesium per 100 g serving. Iron has shown to be higher in both the pulp and kernels (nut) than the selected commercial fruits and nuts suggesting the potential of the wild plum (*X. americana*) as a dietary supplement.

4.3. Health risk assessment of heavy metals in edible parts of *B. foetida* and *X. americana*

Table 11: Estimated daily intake (EDI) of heavy metals in average adults through the consumption of root preparations of *B. foetida*, pulp and kernels of wild plum (*X. americana*)

Sample	Pb	Cr	Cu	Zn	Ni	Co
Roots (<i>B.foetida</i>)	0.00116	-	0.000335	0.0212	0.00144	0.01178
Pulp	0.0281	-	0.0174	-	0.0175	0.00695
Kernels	-	-	0.00436	0.00617	0.00116	-

Table 12: Hazard quotient values (HQ) through the consumption of root preparations of *B. foetida*, pulp and kernels of wild plum (*X. americana*) from the study areas

Sample	Pb	Cr	Cu	Zn	Ni	Co
Roots (<i>B.foetida</i>)	0.289	-	0.00838	0.0708	0.072	0.5892
Pulp	7.02	-	0.434	-	0.875	0.348
Kernels	-	-	0.109	0.0206	0.058	-

*HQ<1, pose no risk **HQ>1, pose a risk

Table 13: Hazard indices (HI) through the consumption of root preparations of *B. foetida*, pulp and kernels of wild plum (*X. americana*) from the study areas

Sample	Values
Roots (<i>B.foetida</i>)	1.03
Pulp	8.68
Kernels	0.188

*HI<1, pose no risk **HI>1, pose a risk

Table 14: Carcinogenic risk assessment values for lead and nickel in the wild plum (*X. americana*) pulp and kernels and *B. foetida* roots

Sample	Pb	Ni
Roots (<i>B.foetida</i>)	4.41×10^{-4}	2.45×10^{-3}
Pulp	1.07×10^{-2}	2.98×10^{-2}
Kernels	-	1.97×10^{-3}

The estimated daily intake (EDI), hazard quotient (HQ), and hazard index (HI) values of Pb, Cr, Cu, Zn, and Ni contents in *B. foetida* roots and *X. americana* pulp and kernels are presented in **Table 11, 12, 13**. Estimated daily intake is an essential health risk assessment tool. It accounts for the exposure frequency, time, and exposed person's body weight. The daily intake computation was followed by the estimation of the hazard quotient (HQ) which is the measurement of the non-carcinogenic risk from long-term exposure to heavy metals by humans. If HQ is less than 1, the product does not pose health risk to consumers, it is assumed to be safe. On the other hand, if the HQ is greater than 1, the product poses a potential risk to consumers. The results have shown the HQ values of Pb, Cu, Zn, Co and Ni of the roots to be less than 1, suggesting that the consumption of these roots may pose no health risk. The Cu, Zn, and Ni HQ values were also below 1 in kernels, indicating no potential health risk to the consumers. Furthermore, Cu, Co and Ni HQ values in the pulp were less than 1, however, the HQ of Pb in the same sample was found to be greater than 1, suggesting a potential health risk because of the long-term exposure to Pb (**Table 12**).

The hazard indices of the studied heavy metals (HI) were also assessed. The HI value of the kernels of the wild plum was less than 1, indicating that the overall effect of the studied heavy metals in the samples may pose no health risk to the consumers in the long term. However, the HI values of *B. foetida* roots and the wild plum pulp were greater than 1, suggesting that consumers are susceptible to non-carcinogenic health risks of these metals upon consumption in the long-term (Brown et al., 2021; Kandić et al., 2023; Meseret et al., 2020; Pem & Jeewon, 2015). This indicates that in their collection and use the heavy metals analysis should be a quality control parameter. The carcinogenic risk assessment has shown that the values for lead (Pb) and nickel (Ni) ranged from 4.41×10^{-4} - 2.98×10^{-2} across all the studied samples, were above the specified permissible limits by the United States Environmental Protection Agency (USEPA) of 1×10^{-6} and 1×10^{-4} (**Table 14**) (Bello et al., 2019; M. Zhang et al., 2020) demonstrating the possibility of cancer development when the wild plum fruit pulp and kernels and *B. foetida* roots from the selected sampling sites are continuously consumed.

4.4. X. Americana (wild plum) fruit extracts GC-MS analysis and the effect of storage on the extracts composition

4.4.1. GC-MS analysis of wild plum fruit pulp non-polar extracts

The GC-MS *n*-hexane pulp extract analysis results are shown in **Figure 23**.

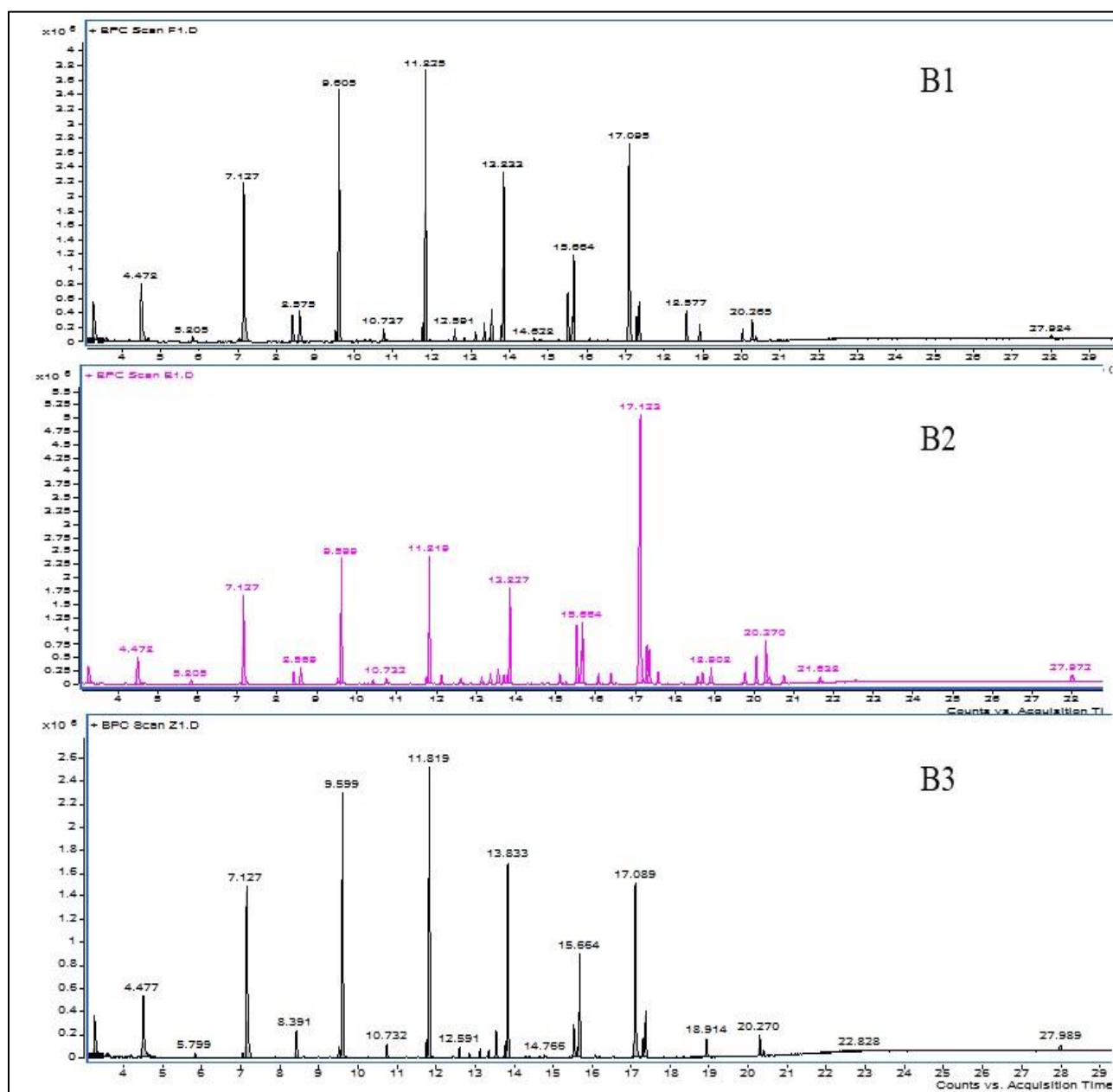


Figure 23: Influence of long-term frozen storage on *n*-hexane pulp extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles after 3 months and 6 months of storage respectively.

Table 15: Compounds identified in *n*-hexane pulp extract after harvest (**B1**)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
21	13-Docosenamide, (Z), (Erucamide)	20.265	0.98 ± 0.42	337.6	C ₂₂ H ₄₃ NO
20	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, (Bis(2-ethylhexyl) isophthalate)	20.030	0.52 ± 0.11	390.6	C ₂₄ H ₃₈ O ₄
19	Heptacosane	18.914	0.93 ± 0.16	380.7	C ₂₇ H ₅₆
18	Diisooctyl phthalate	18.577	1.45 ± 0.21	390.6	C ₂₄ H ₃₈ O ₄
17	Tetracosane	17.352	2.27 ± 0.10	338.7	C ₂₄ H ₅₀
16	9-Octadecenamide, (Z) (Oleic Acid Amide)	17.095	12.20 ± 0.57	281.5	C ₁₈ H ₃₅ NO
15	Hexadecanamide (Palmitic Amide)	15.510	2.98 ± 0.01	255.44	C ₁₆ H ₃₃ NO
14	Eicosane	13.833	8.84 ± 0.59	282.5	C ₂₀ H ₄₂
13	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester (Butyl isodecyl phthalate)	13.530	1.77 ± 0.09	362.5	C ₂₂ H ₃₄ O ₄
12	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	13.341	0.98 ± 0.03	292.4	C ₁₈ H ₂₈ O ₃
11	Hexadecanoic acid, methyl ester (Methyl Palmitate)	13.118	0.45 ± 0.04	270.5	C ₁₇ H ₃₄ O ₂
10	Phthalic acid, isobutyl octadecyl ester (Isobutyl octadecyl phthalate)	12.591	0.67 ± 0.02	474.3	C ₃₀ H ₅₀ O ₄
9	Octadecane	11.825	13.44 ± 0.08	254.5	C ₁₈ H ₃₈
8	Heptadecane	10.737	0.73 ± 0.03	240.2	C ₁₇ H ₃₆
7	Hexadecane	9.605	14.34 ± 0.20	226.44	C ₁₆ H ₃₄
6	Phenol, 2,4-bis(1,1-dimethylethyl)	8.575	2.03 ± 0.02	206.167	C ₁₄ H ₁₂ O
5	Pentadecane	8.391	1.70 ± 0.04	212.25	C ₁₅ H ₃₂
4	Tetradecane	7.127	10.95 ± 0.07	198.39	C ₁₄ H ₃₀
3	Tridecane	5.805	0.37 ± 0.03	184.2	C ₁₃ H ₂₈
2	Dodecane	4.478	5.02 ± 0.00	170.3	C ₁₂ H ₂₆
1	Undecane	3.236	3.63 ± 0.03	156.3	C ₁₁ H ₂₄

Table 16: Compounds identified in *n*-hexane pulp extract frozen for 3 months (B2)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
20	13-Docosenamide, (Z)- (Erucamide)	20.270	3.95 ± 0.52	337.6	C ₂₂ H ₄₃ NO
19	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, (Bis(2-ethylhexyl) isophthalate)	20.030	2.08 ± 0.16	390.6	C ₂₄ H ₃₈ O ₄
18	Heptacosane	18.908	1.34 ± 0.07	380.7	C ₂₇ H ₅₆
17	Diisooctyl phthalate	18.571	0.44 ± 0.03	390.6	C ₂₄ H ₃₈ O ₄
16	Octadecanamide	17.283	3.49 ± 0.45	283.5	C ₁₈ H ₃₇ NO
15	9-Octadecenamide,(Z)- (Oleic acid amide)	17.123	28.35 ± 3.46	281.5	C ₁₈ H ₃₅ NO
14	Hexadecanamide (Palmitic amide)	15.515	5.05 ± 0.37	255.44	C ₁₆ H ₃₃ NO
13	Eicosane	13.827	6.40 ± 0.25	282.5	C ₂₀ H ₄₂
12	1,2-Benzenedicarboxylic acid, butyl octyl ester (Butyl octyl phthalate)	13.530	1.15 ± 0.03	334.4	C ₂₀ H ₃₀ O ₄
11	Benzenepropanoic acid, 3,5-bis (1,1-dimethyl)-4-hydroxy-, methyl ester	13.341	0.70 ± 0.01	292.4	C ₁₈ H ₂₈ O ₃
10	Hexadecanoic acid, methyl ester (Methyl palmitate)	13.118	0.49 ± 0.004	270.5	C ₁₇ H ₃₄ O ₂
9	Phthalic acid, butyl tetradecyl ester	12.591	0.50 ± 0.004	418.6	C ₂₆ H ₄₂ O ₄
8	Octadecane	11.819	8.26 ± 0.30	254.5	C ₁₈ H ₃₈
7	Heptadecane	10.732	0.46 ± 0.01	240.2	C ₁₇ H ₃₆
6	Hexadecane	9.599	8.70 ± 0.36	226.4	C ₁₆ H ₃₄
5	Phenol, 2,4-bis (1,1-dimethylethyl)-	8.569	1.35 ± 0.06	206.167	C ₁₄ H ₁₂ O
4	Tetradecane	7.127	6.59 ± 0.08	198.39	C ₁₄ H ₃₀
3	Tridecane	5.805	0.39 ± 0.01	184.2	C ₁₃ H ₂₈
2	Dodecane	4.478	2.99 ± 0.09	170.3	C ₁₂ H ₂₆
1	Undecane	3.236	2.00 ± 0.10	156.3	C ₁₁ H ₂₄

Table 17: Compounds identified in *n*-hexane pulp extract frozen for 6 months (**B3**)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
17	13-Docosenamide, (Z)- (Erucamide)	20.270	1.07 ± 0.08	337.6	C ₂₂ H ₄₃ NO
16	Heptacosane	18.914	0.94 ± 0.08	380.7	C ₂₇ H ₅₆
15	9-Octadecenamide, (Z)- (Oleic acid amide)	17.089	10.75 ± 0.49	281.5	C ₁₈ H ₃₅ NO
14	Hexadecanamide (Palmitic amide)	15.515	2.10 ± 0.04	255.44	C ₁₆ H ₃₃ NO
13	Eicosane	13.833	9.83 ± 0.39	282.5	C ₂₀ H ₄₂
12	1,2-Benzenedicarboxylic acid, dibutyl ester (Dibutyl phthalate)	13.530	1.46 ± 0.01	278.34	C ₁₆ H ₂₂ O ₄
11	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	13.341	0.33 ± 0.01	292.4	C ₁₈ H ₂₈ O ₃
10	Hexadecanoic acid, methyl ester (Methyl palmitate)	13.118	0.43 ± 0.02	270.5	C ₁₇ H ₃₄ O ₂
9	Tetradecane, 2,6,10-trimethyl-	12.843	0.24 ± 0.02	240.5	C ₁₇ H ₃₆
8	Octadecane	11.819	15.00 ± 0.14	254.5	C ₁₈ H ₃₈
7	Heptadecane	10.732	0.75 ± 0.02	240.2	C ₁₇ H ₃₆
6	Hexadecane	9.599	15.60 ± 0.85	226.4	C ₁₆ H ₃₄
5	Pentadecane	8.391	1.79 ± 0.11	212.4	C ₁₅ H ₃₂
4	Tetradecane	7.127	12.40 ± 1.48	198.39	C ₁₄ H ₃₀
3	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	5.799	0.34 ± 0.03	366.7	C ₂₆ H ₅₄
2	Dodecane	4.477	5.46 ± 0.35	170.3	C ₁₂ H ₂₆
1	Undecane	3.230	4.05 ± 0.53	156.3	C ₁₁ H ₂₄

A total of 21, 20 and 17 components were identified at post-harvest, after 3 and 6 months of storage respectively (**Table 15, 16, 17**). At harvest, a total of 6 components were considered major based on their estimated relative compositions of at least 5%. On the other hand, a total of 6 major components were identified both in samples frozen for 3 and 6 months. Eicosane, 9-Octadecenamide, (Z)- (oleic acid amide), octadecane, hexadecane, and tetradecane appeared to be present across, after harvest, and after 3 and 6 months of frozen storage. Sample B1 and B3 had the same major compounds which only differed in relative abundances. Oleic acid amide is the only component that showed a decline in composition after 6 months when compared to B1 while others showed an increment. From samples

B1 to B2, 9-Octadecenamide, (Z)- (oleic acid amide) showed an increase in both composition and retention time, however, from B2 to B3, a decrease was observed. Eicosane decreased in composition and retention time from B1 to B2 and increased from B2 to B3. There was an observable fluctuation (decrease, increase) in octadecane composition from B1 to B3 (after harvest, and after 3 and 6 months of frozen storage). In terms of retention times, the component appeared after a longer time (11.825 min) after harvest (B1) than after 3 and 6 months of frozen storage (B2 and B3), where the retention time remained stable (11.819 min). The relative composition of hexadecane declined from B1 to B2, that is, after 3 months of frozen storage, and showed an increase from B2 to B3, that is, after 6 months of frozen storage. There was a change in retention time after 3 months of frozen storage (from 9.605 to 9.599 min) of which remained stable onwards. Tetradecane composition fluctuated from B1 to B3 (decreased and increased), however, the retention time remained unchanged. Dodecane composition decreased after 3 months of frozen storage from 5.02 to 2.99% then subsequently increased from 2.99 to 5.46%. There was a slight change in retention time after 6 months of frozen storage, otherwise the retention time remained stable.

4.4.2. GC-MS analysis of wild plum fruit peels non-polar extracts

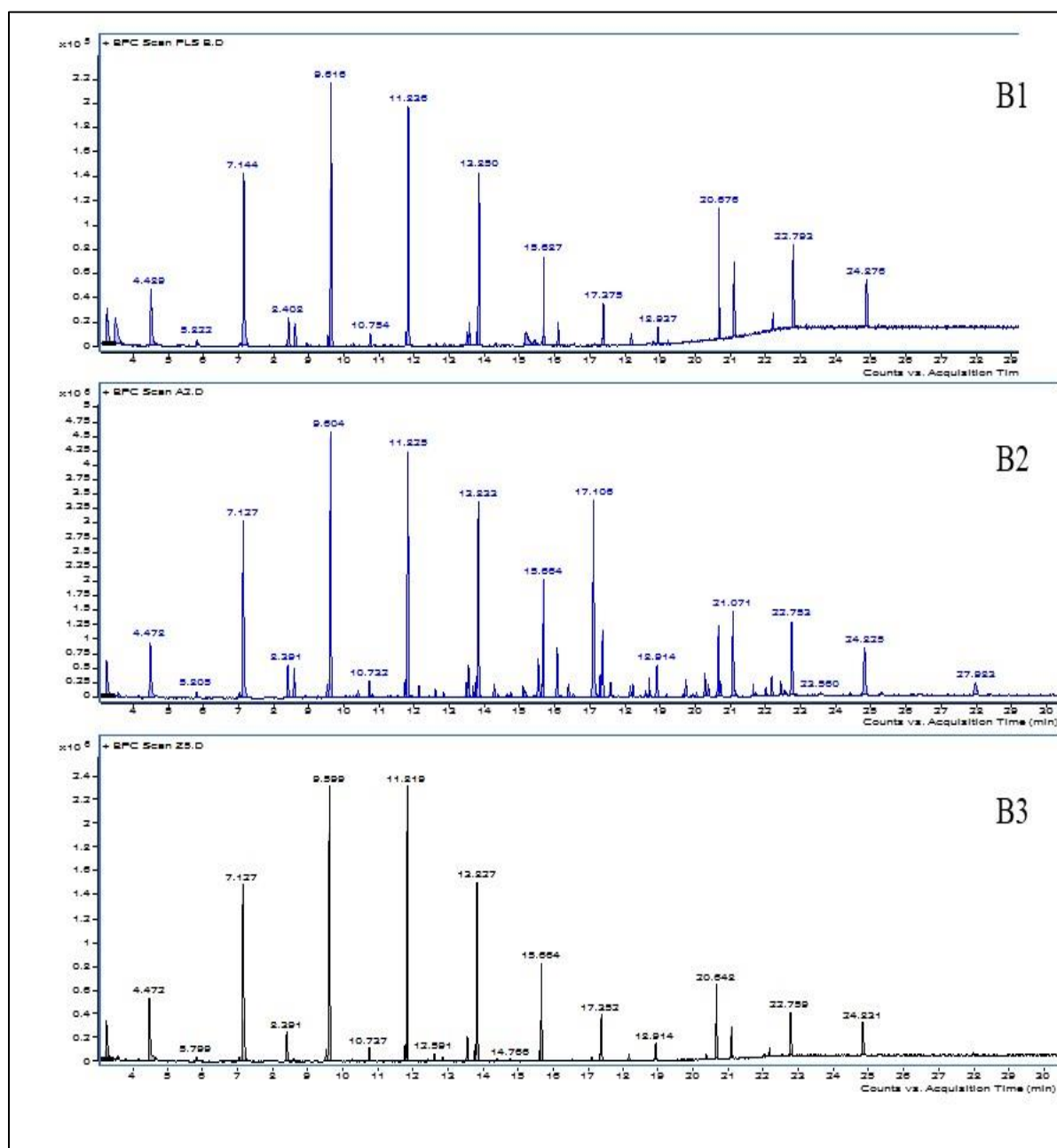


Figure 24: Influence of long-term frozen storage on *n*-hexane peel extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles for extracts stored for 3 and 6 months respectively.

Table 18: Compounds identified in *n*-hexane peels extract after harvest (B1)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
11	β -Amyrin	24.876	3.40 ± 0.30	426.7	C ₃₀ H ₅₀ O
10	Vitamin E (α -Tocopherol)	22.793	4.08 ± 0.73	430.7	C ₂₉ H ₅₀ O ₂
9	γ -Tocopherol	22.210	0.82 ± 0.38	416.7	C ₂₈ H ₄₈ O ₂
8	Docosane	15.687	3.91 ± 1.03	310.6	C ₂₂ H ₄₆
7	<i>Cis</i> -Vaccenic acid	15.183	3.01 ± 0.18	282.5	C ₁₈ H ₃₄ O ₂
6	Eicosane	13.850	6.78 ± 2.18	282.5	C ₂₀ H ₄₂
5	Octadecane	11.836	9.94 ± 2.77	254.5	C ₁₈ H ₃₈
4	Hexadecane	9.616	11.80 ± 2.97	226.4	C ₁₆ H ₃₄
3	Phenol, 2,4-bis(1,1-dimethylethyl)-	8.597	1.36 ± 0.10	206.32	C ₁₄ H ₂₂ O
2	Tetradecane	7.144	8.99 ± 1.99	198.39	C ₁₄ H ₃₀
1	Undecane	3.247	2.91 ± 0.52	156.3	C ₁₁ H ₂₄

A total of 11, 16, and 10 components were identified at post-harvest, after 3 and 6 months of frozen storage respectively, as shown in **Table 18, 19, 20**. Four, five, and five major components were identified at post-harvest, after 3 and 6 months respectively. Eicosane, octadecane, hexadecane, and tetradecane were common in all 3 samples. An increase in eicosane composition (B1-6.78%, B2-8.79%, B3-10.30%) was observed at post-harvest and during storage. In terms of the retention time, we observed a decrease between samples B1 and B2, and B2 and B3. The composition of octadecane increased after 3 months of frozen storage until the end of 6 months whereas the retention time showed a decline after 3 and 6 months of storage. Furthermore, a similar trend was observed with hexadecane, with an increase in composition and a decrease in retention time from harvest until the end of the storage period. The tetradecane composition increased after 3 and 6 months of storage. The retention time decreased from B1 to B2 (7.144-7.127 min) and remained stable to the last sample.

Table 19: Compounds identified in *n*-hexane peels extract frozen for 3 months (**B2**)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
16	β -Amyrin	24.825	2.09 ± 0.16	426.7	C ₃₀ H ₅₀ O
15	Vitamin E (α -Tocopherol)	22.753	2.65 ± 1.36	430.7	C ₂₉ H ₅₀ O ₂
14	γ -Tocopherol	22.170	0.56 ± 0.26	416.7	C ₂₈ H ₄₈ O ₂
13	13-Docosenamide, (Z)-	20.270	1.08 ± 0.10	337.6	C ₂₂ H ₄₃ NO
12	Tetracosane	17.352	2.68 ± 0.20	338.7	C ₂₄ H ₅₀
11	9-Octadecenamide, (Z)- (Oleic acid amide)	17.106	9.78 ± 0.59	281.5	C ₁₈ H ₃₅ NO
10	Hexadecanamide	15.515	1.97 ± 0.35	255.44	C ₁₆ H ₃₃ NO
9	Eicosane	13.833	8.79 ± 1.99	282.5	C ₂₀ H ₄₂
8	Phthalic acid, butyl tetradecyl ester	12.591	0.31 ± 0.05	418.6	C ₂₆ H ₄₂ O ₄
7	Octadecane	11.825	11.73 ± 3.63	254.5	C ₁₈ H ₃₈
6	Hexadecane	9.604	13.00 ± 4.24	226.4	C ₁₆ H ₃₄
5	Phenol, 2,4-bis (1,1-dimethylethyl)-	8.569	1.26 ± 0.01	206.32	C ₁₄ H ₂₂ O
4	Tetradecane	7.127	9.37 ± 2.59	198.39	C ₁₄ H ₃₀
3	Tridecane	5.805	0.25 ± 0.09	184.36	C ₁₃ H ₂₈
2	Dodecane	4.478	2.83 ± 0.33	170.33	C ₁₂ H ₂₆
1	Undecane	3.230	1.71 ± 0.75	156.3	C ₁₁ H ₂₄

Table 20: Compounds identified in *n*-hexane peels extract frozen for 6 months (**B3**)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
10	β -Amyrin	24.831	3.66 ± 0.26	426.7	C ₃₀ H ₅₀ O
9	Vitamin E (α -Tocopherol)	22.759	3.11 ± 0.16	430.7	C ₂₉ H ₅₀ O ₂
8	γ -Tocopherol	22.176	0.44 ± 0.07	416.7	C ₂₈ H ₄₈ O ₂
7	Eicosane	13.827	10.30 ± 0.14	282.5	C ₂₀ H ₄₂
6	Phthalic acid, butyl tetradecyl ester	12.591	0.49 ± 0.003	418.6	C ₂₆ H ₄₂ O ₄
5	Octadecane	11.819	15.40 ± 0.14	254.5	C ₁₈ H ₃₈
4	Hexadecane	9.599	17.20 ± 0.00	226.4	C ₁₆ H ₃₄
3	Tetradecane	7.127	13.30 ± 0.57	198.39	C ₁₄ H ₃₀
2	Dodecane	4.472	5.75 ± 0.27	170.33	C ₁₂ H ₂₆
1	Undecane	3.230	4.00 ± 0.18	156.3	C ₁₁ H ₂₄

4.4.3. GC-MS analysis of wild plum fruit coat non-polar extracts

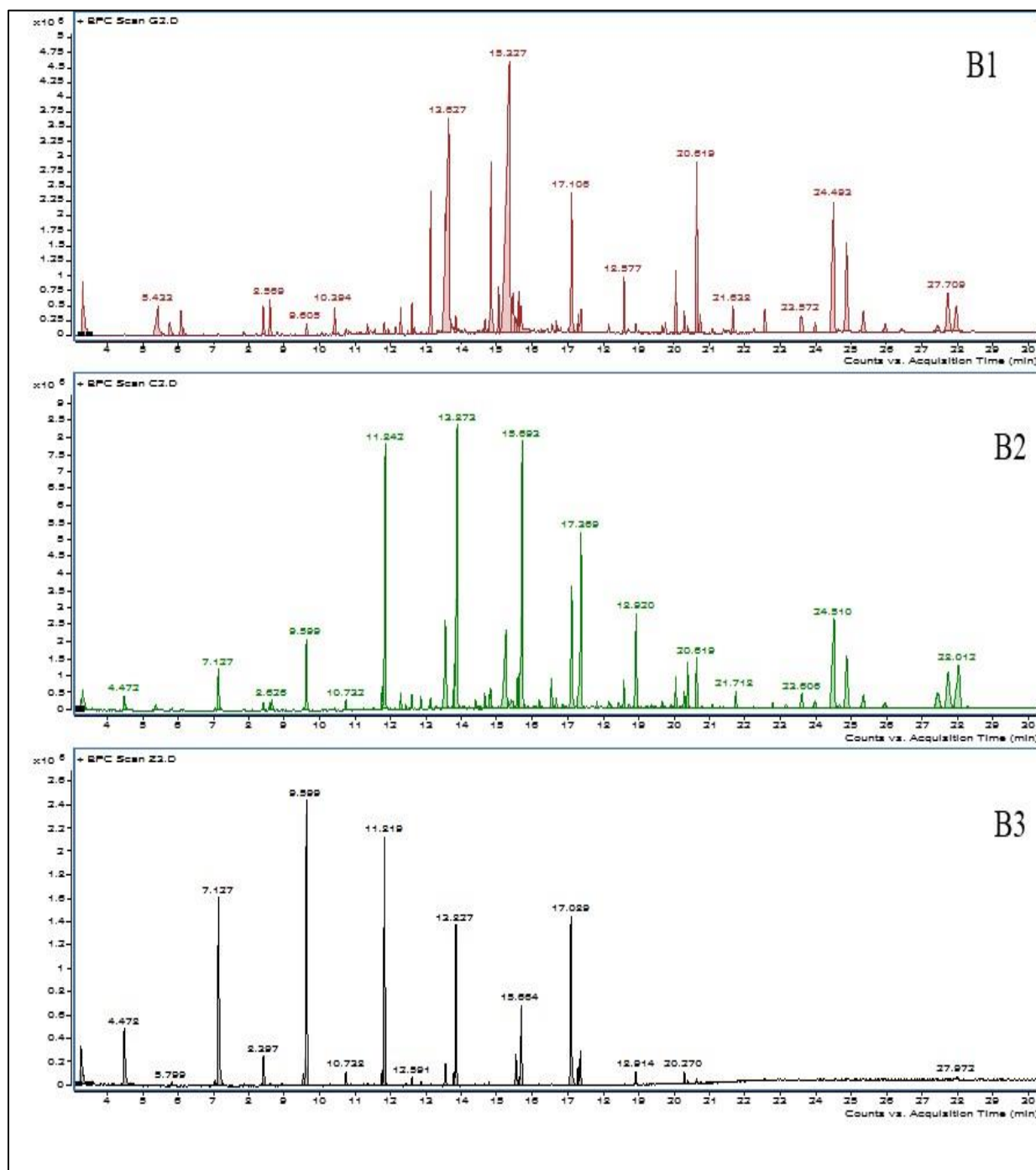


Figure 25: Influence of long-term frozen storage on *n*-hexane coats extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles of extracts stored for 3 and 6 months respectively.

Table 21: Compounds identified in *n*-hexane coats extract after harvest (B1)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
21	Lupeol	25.403	1.45 ± 0.63	426.7	C ₃₀ H ₅₀ O
20	β-Amyrin	24.922	5.92 ± 2.18	426.7	C ₃₀ H ₅₀ O
19	γ-sitosterol	24.562	9.33 ± 3.21	414.7	C ₂₉ H ₅₀ O
18	Lanosterol	24.035	0.41 ± 0.10	426.7	C ₃₀ H ₅₀ O
17	Campesterol	23.658	1.02 ± 0.08	400.7	C ₂₈ H ₄₈ O
16	Squalene	20.659	5.34 ± 1.25	410.7	C ₃₀ H ₅₀
15	13-Docosenamide, (Z)	20.310	0.79 ± 0.33	337.6	C ₂₂ H ₄₃ NO
14	Bis(2-ethylhexyl) isophthalate	20.070	1.65 ± 0.13	390.6	C ₂₄ H ₃₈ O ₄
13	Bis(6-methylheptyl) phthalate	18.611	1.25 ± 0.09	390.6	C ₂₄ H ₃₈ O ₄
12	Octadecanamide	17.318	0.60 ± 0.27	283.5	C ₁₈ H ₃₇ NO
11	9-Octadecenamide, (Z)- (Oleic acid amide)	17.129	5.80 ± 2.41	281.5	C ₁₈ H ₃₅ NO
10	Hexadecanamide	15.572	1.36 ± 0.60	255.44	C ₁₆ H ₃₃ NO
9	9-Octadecenoic acid (E)	15.246	18.20 ± 6.36	282.5	C ₁₈ H ₃₄ O ₂
8	9-Octadecenoic acid, methyl ester (E)	14.834	5.04 ± 0.04	296.5	C ₁₉ H ₃₆ O ₂
7	n-Hexadecanoic acid (Palmitic acid)	13.553	12.54 ± 4.90	256.42	C ₁₆ H ₃₂ O ₂
6	Hexadecanoic acid, methyl ester (methyl palmitate)	13.146	3.36 ± 0.02	270.5	C ₁₇ H ₃₄ O ₂
5	Butyl octyl phthalate	12.614	0.59 ± 0.14	334.4	C ₂₀ H ₃₀ O ₄
4	4,8,12-tetradecatrienal, 5,9,13-trimethyl	12.299	0.64 ± 0.04	248.4	C ₁₇ H ₂₈ O
3	Phenol, 2,4-bis(1,1-dimethylethyl)	8.597	0.94 ± 0.26	206.32	C ₁₄ H ₂₂ O
2	2,4-Decadienal	6.091	0.70 ± 0.32	152.23	C ₁₀ H ₁₆ O
1	Nonanal	3.305	2.01 ± 0.62	142.24	C ₉ H ₁₈ O

Table 22: Compounds identified in *n*-hexane coats extract frozen for 3 months (B2)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
23	Lupeol	25.329	0.61 ± 0.23	426.7	C ₃₀ H ₅₀ O
22	β-Amyrin	24.865	2.78 ± 0.86	426.7	C ₃₀ H ₅₀ O
21	γ-sitosterol	24.510	5.16 ± 1.51	414.7	C ₂₉ H ₅₀ O
20	Lanosterol	23.972	0.26 ± 0.15	426.7	C ₃₀ H ₅₀ O
19	Campesterol	23.606	0.79 ± 0.01	400.7	C ₂₈ H ₄₈ O
18	Squalene	20.619	1.34 ± 0.36	410.7	C ₃₀ H ₅₀
17	Bis(2-ethylhexyl) isophthalate	20.030	0.79 ± 0.17	390.6	C ₂₄ H ₃₈ O ₄
16	Bis(6-methylheptyl) phthalate	18.577	0.76 ± 0.14	390.6	C ₂₄ H ₃₈ O ₄
15	Tetracosane	17.369	5.75 ± 1.02	338.7	C ₂₄ H ₅₀
14	9-Octadecenamide, (Z)- (Oleic acid amide)	17.112	5.04 ± 0.83	281.5	C ₁₈ H ₃₅ NO
13	9-Octadecenoic acid (E)	15.235	4.64 ± 0.33	282.5	C ₁₈ H ₃₄ O ₂
12	11-Octadecenoic acid, methyl ester	14.800	0.55 ± 0.03	296.5	C ₁₉ H ₃₆ O ₂
11	Eicosane	13.873	12.25 ± 1.06	282.5	C ₂₀ H ₄₂
10	n-Hexadecanoic acid (Palmitic acid)	13.530	4.24 ± 0.12	256.42	C ₁₆ H ₃₂ O ₂
9	Hexadecanoic acid, methyl ester (methyl palmitate)	13.112	0.31 ± 0.07	270.5	C ₁₇ H ₃₄ O ₂
8	Phthalic acid, butyl tetradecyl ester	12.586	0.47 ± 0.12	418.6	C ₂₆ H ₄₂ O ₄
7	4,8,12-tetradecatrienal, 5,9,13-trimethyl	12.277	0.63 ± 0.10	248.4	C ₁₇ H ₂₈ O
6	Octadecane	11.842	10.30 ± 1.99	254.5	C ₁₈ H ₃₈
5	Hexadecane	9.599	2.59 ± 0.81	226.44	C ₁₆ H ₃₄
4	Butylated hydroxytoluene	8.626	0.38 ± 0.09	220.35	C ₁₅ H ₂₄ O
3	Phenol, 2,4-bis(1,1-dimethylethyl)	8.569	0.31 ± 0.09	206.32	C ₁₄ H ₂₂ O
2	Tetradecane	7.127	1.68 ± 0.49	198.39	C ₁₄ H ₃₀
1	Nonanal	3.288	1.15 ± 0.18	142.24	C ₉ H ₁₈ O

Table 23: Compounds identified in *n*-hexane coats extract frozen for 6 months (B3)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
11	13-Docosenamide, (Z)	20.270	0.77 ± 0.01	337.6	C ₂₂ H ₄₃ NO
10	9-Octadecenamide, (Z)- (Oleic acid amide)	17.089	12.25 ± 0.21	281.5	C ₁₈ H ₃₅ NO
9	Docosane	15.664	4.86 ± 0.16	310.6	C ₂₂ H ₄₆
8	Hexadecanamide	15.515	2.28 ± 0.00	255.44	C ₁₆ H ₃₃ NO
7	Eicosane	13.827	9.99 ± 1.01	282.5	C ₂₀ H ₄₂
6	Butyl isodecyl phthalate	13.530	1.55 ± 0.13	362.5	C ₂₂ H ₃₄ O ₄
5	Butyl octyl phthalate	12.591	0.46 ± 0.01	334.4	C ₂₀ H ₃₀ O ₄
4	Octadecane	11.819	14.25 ± 0.49	254.5	C ₁₈ H ₃₈
3	Hexadecane	9.599	17.05 ± 0.78	226.44	C ₁₆ H ₃₄
2	Pentadecane	8.397	2.08 ± 0.12	212.41	C ₁₅ H ₃₂
1	Tetradecane	7.127	13.35 ± 0.49	198.39	C ₁₄ H ₃₀

In fruit coats, a total of 21, 23 and 11 compounds were identified after harvest, after 3 and 6 months of frozen storage respectively. Sample B1 contained a total of 7 components considered major followed by sample B2 and B3 with a total of 5 components. Among all components that were considered major, only 9-Octadecenamide, (Z)- (oleic acid amide) appeared across all the samples. The composition of 9-octadecenamide, (Z)- (oleic acid amide) after harvest, and after 3 and 6 months of storage was fluctuating (decreasing and increasing). On the other hand, the retention time varied among the samples, it showed a decline after 3 and 6 months of storage. The retention time variations are usually caused by column temperature fluctuations, leaks and flow rate instabilities, and column contaminations (Letter, 2015). The coexistence of monomers with their trimers and dimers caused several compounds, including 1-hexanol, 1-pentanol, 3-octanone, heptanal, and hexanal, described by (Dai et al., 2022), to exhibit some variations in retention times.

4.4.4. GC-MS analysis of wild plum fruit kernels non-polar extracts

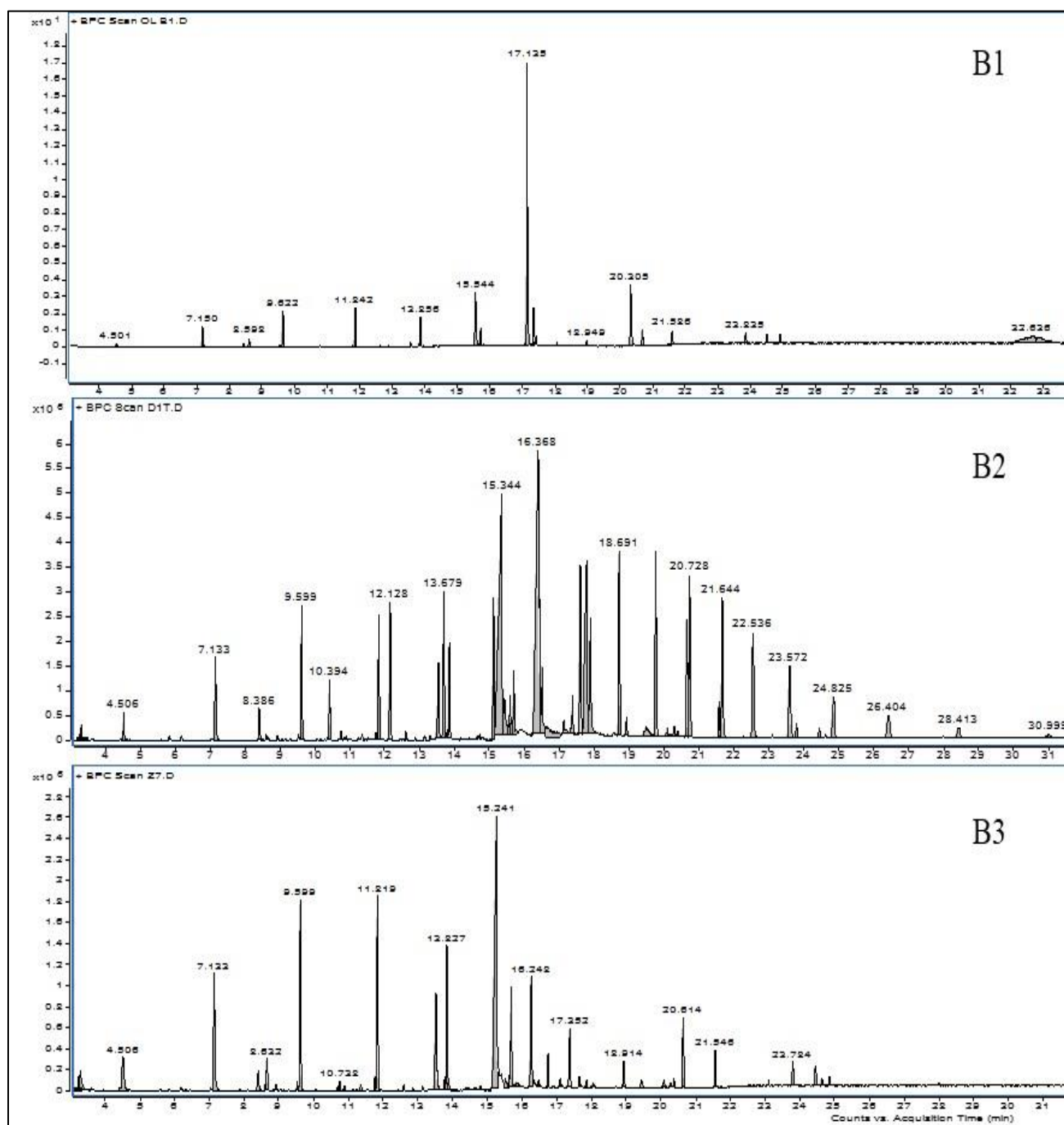


Figure 26: Influence of long-term frozen storage on *n*-hexane kernels extract, both immediately following harvest and at 3- and 6-months post-harvest. B1 shows a profile after harvest, whereas B2 and B3 shows profiles frozen stored for 3 months and 6 months respectively.

Table 24: Compounds identified in *n*-hexane kernels extract after harvest (B1)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
12	β -Amyrin	24.899	0.95 ± 0.37	426.7	C ₃₀ H ₅₀ O
11	γ -sitosterol	24.487	0.81 ± 0.35	414.7	C ₂₉ H ₅₀ O
10	Squalene	20.654	1.92 ± 0.37	410.7	C ₃₀ H ₅₀
9	13-Docosenamide, (Z)	20.305	6.55 ± 1.74	337.6	C ₂₂ H ₄₃ NO
8	Octadecanamide	17.318	3.73 ± 1.22	283.5	C ₁₈ H ₃₇ NO
7	9-Octadecenamide, (Z) (Oleic acid amide)	17.135	35.15 ± 3.04	281.5	C ₁₈ H ₃₅ NO
6	Hexadecanamide	15.544	5.51 ± 1.59	255.44	C ₁₆ H ₃₃ NO
5	Eicosane	13.856	3.55 ± 0.92	282.3	C ₂₀ H ₄₂
4	Octadecane	11.842	4.92 ± 1.27	254.5	C ₁₈ H ₃₈
3	Hexadecane	9.622	5.11 ± 1.32	226.44	C ₁₆ H ₃₄
2	Phenol, 2,4-bis(1,1-dimethylethyl)	8.598	1.38 ± 0.29	206.32	C ₁₄ H ₂₂ O
1	Tetradecane	7.150	3.32 ± 0.66	198.39	C ₁₄ H ₃₀

Table 25: Compounds identified in *n*-hexane kernels extract frozen for 3 months (B2)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
12	γ -sitosterol	24.436	0.64 ± 0.15	414.7	C ₂₉ H ₅₀ O
11	Squalene	20.625	2.21 ± 0.21	410.7	C ₃₀ H ₅₀
10	Methyl Octadeca-9-yn-11-trans-enoate (methyl Santalbate/ Ximenynic acid methyl ester)	16.482	2.00 ± 0.37	292.5	C ₁₉ H ₃₂ O ₂
9	9-Octadecynoic acid (stearolic acid)	15.573	0.84 ± 0.03	280.4	C ₁₈ H ₃₂ O ₂
8	9-Octadecenoic acid (E)	15.344	14.90 ± 0.71	282.5	C ₁₈ H ₃₄ O ₂
7	Eicosane	13.833	1.51 ± 0.01	282.3	C ₂₀ H ₄₂
6	n-Hexadecanoic acid (Palmitic acid)	13.530	2.08 ± 0.05	256.42	C ₁₆ H ₃₂ O ₂
5	Octadecane	11.819	2.07 ± 0.01	254.5	C ₁₈ H ₃₈
4	Hexadecane	9.599	2.35 ± 0.06	226.44	C ₁₆ H ₃₄
3	Tetradecane	7.133	1.65 ± 0.04	198.39	C ₁₄ H ₃₀
2	Dodecane	4.506	0.80 ± 0.03	170.33	C ₁₂ H ₂₆
1	Undecane	3.287	0.41 ± 0.06	156.31	C ₁₁ H ₂₄

Table 26: Compounds identified in *n*-hexane kernels extract frozen for 6 months (B3)

Peak No.	Name of Compound	Retention Time (min)	Relative Percent Composition (%)	Molecular Weight (g/mol)	Molecular Formula
14	β -Amyrin	24.836	0.38 ± 0.12	426.7	C ₃₀ H ₅₀ O
13	γ -sitosterol	24.425	0.94 ± 0.18	414.7	C ₂₉ H ₅₀ O
12	Squalene	20.614	2.38 ± 0.24	410.7	C ₃₀ H ₅₀
11	9-Octadecenamide, (Z) (Oleic acid amide)	17.089	1.13 ± 0.20	281.5	C ₁₈ H ₃₅ NO
10	Methyl Octadeca-9-yn-11-trans-enoate (Methyl Santalbate/ Ximenynic acid methyl ester)	16.248	5.65 ± 0.97	292.5	C ₁₉ H ₃₂ O ₂
9	9-Octadecenoic acid (E)	15.241	23.70 ± 2.83	282.5	C ₁₈ H ₃₄ O ₂
8	Eicosane	13.827	5.07 ± 0.21	282.3	C ₂₀ H ₄₂
7	n-Hexadecanoic acid (Palmitic acid)	13.501	5.25 ± 0.45	256.42	C ₁₆ H ₃₂ O ₂
6	Octadecane	11.819	6.55 ± 0.06	254.5	C ₁₈ H ₃₈
5	Hexadecane	9.599	6.82 ± 0.08	226.44	C ₁₆ H ₃₄
4	Butylated hydroxytoluene	8.632	1.26 ± 0.01	220.35	C ₁₅ H ₂₄ O
3	Tetradecane	7.133	5.34 ± 0.24	198.39	C ₁₄ H ₃₀
2	Dodecane	4.506	2.66 ± 0.25	170.33	C ₁₂ H ₂₆
1	Undecane	3.282	1.48 ± 0.18	156.31	C ₁₁ H ₂₄

A total of 12, 12 and 14 components were identified at post-harvest, 3 and 6 months of frozen storage respectively, as shown in **Table 24, 25, 26**. Sample B1 had 4 major components followed by sample B2 with 1 and B3 with 7 major components. Apart from major components, components such as γ -sitosterol, squalene, eicosane, octadecane, hexadecane and tetradecane were present after harvest, and after 3 and 6 months of frozen storage with varying compositions, and retention times. The composition of γ -sitosterol decreased from B1 and B2 followed by an increase in B3. Sample B1 recorded more time (RT) followed by B2 and B3. An increase in the squalene composition was observed throughout the samples. However, with retention time, a decline was observed. A similar trend was observed for eicosane with respect to the retention time. The relative composition decreased from B1 to B2, and then increased from B2 to B3. Octadecane, hexadecane and tetradecane all revealed a similar trend in both retention time and composition from sample B1 to B2 and B3.

Besides, the wild plum fruit parts have generally been shown to contain various phytochemical classes such as triterpenoids, esters, aldehydes, phenolics, amides, phytosterols, tocopherols and alkane

hydrocarbons which in essence would respond differently to long-term frozen storage, as previously observed and described, or any storage treatment employed. For instance, campesterol, lanosterol and γ -sitosterol which are all phytosterols were all present in coats after harvest and after 3 months of frozen storage and then disappeared after 6 months of freezing storage in terms of percent composition. Previously, (Puupponen-Pimiä et al., 2003) observed a similar trend in potatoes and peas stored at -20°C for 6 and 12 months. The findings revealed a slight decrease and about 40% decline of sterol content in peas and potatoes respectively. These changes may be attributed to the oxidation of a steroid ring of sterols either by enzymatic reactions or reactive oxygen species producing new phytosterol derivatives (Gachumi et al., 2021; Menéndez-Carreño et al., 2008; Puupponen-Pimiä et al., 2003).

The γ -sitosterol composition in kernels have shown a decrease, followed by an increase to a lesser extent after 3 and 6 months of frozen storage respectively. However, unlike in the coats, the compound remained appreciably stable over time. The slight increase after 6 months could be due to the reduced catalysis of enzymes by ice over time. Sample B2 of the kernels showed a different profile with numerous newly emerged peaks (**Figure 26**). Some of the peaks were unidentified and some were found to be siloxanes which typically results from the depolymerization of the linear polysiloxane chain of the GC column at high temperatures (Kumar & Sharma, 2022). Aldehydes (nonanal, 2,4-decadienal and 4,8,12-tetradecatrienal, 5,9,13-trimethyl) detected in coats generally shows a decline over 6 months of frozen storage as seen from **Table 21, 22, 23**. However, 2,4-decadienal vanished during the entire storage whereas nonanal and 4,8,12-tetradecatrienal, 5,9,13-trimethyl disappeared after 6 months of storage. Comparatively, (Yan et al., 2020) demonstrated the effect of cold storage (0°C) on the content of hexanal and E-2-hexenal in blueberries, which showed a gradual decrease in E-2-hexenal, while hexanal fluctuated after 15 and 60 days of cold storage. In addition, (Muto et al., 2020) pointed out the fact that aldehydes could reduce in content whenever exposed to low temperatures.

It is noticeable from **Table 21, 22** that aldehyde composition was higher after harvest as compared to after 3 and 6 months freezing storage, and this could be linked to chilling injuries engendered by freezing storage (Yan et al., 2020; Zhang et al., 2011). Besides, the observed decline in aldehyde composition may be linked to their oxidation by aldehyde dehydrogenase, an enzyme responsible for the oxidation of aldehydes to the corresponding carboxylic acids and esters, during aerobic respiration taking place in the cells (Messner et al., 2012; S. Singh et al., 2013). Moreover, esters were among the identified components across the fruit parts. Fruits generally undergo an esterification reaction catalysed by LOX (lipoxygenase) enzymes as they ripen, consuming a lot of acid in the process upon

oxidation or degradation of fatty acids (Muto et al., 2020; Yang et al., 2019) as shown in **Tables 15, 16, 19, 20, 23, 25, 26**, where methyl octadeca-9-yn-11-trans-enoate (ximenynic acid methyl ester) in kernels, 1,2-benzenedicarboxylic acid, dibutyl ester (dibutyl phthalate) in pulp, phthalic acid, butyl tetradecyl ester in peels, and butyl isodecyl phthalate in coats showed an increase composition after 3 and 6 months of frozen storage but were not detected at post-harvest. Similarly, (Yang et al., 2019) observed that esters were scarce in fresh samples (after harvest) of jujube fruits but were found in large quantities towards the end of cold storage. It is imperative to note that during freezing ripening proceeds but at a reduced rate. These findings imply that low temperatures could favour preservation and synthesis of essential esters and prevent their drastic degradation during storage.

A total of four fatty acids of the categories monounsaturated and saturated were also detected. These comprised three monounsaturated fatty acids (9-octadecanoic acid, 9-octadecynoic acid and cis-vaccenic acid), and one saturated fatty acid (*n*-hexadecanoic acid). The *n*-hexadecanoic acid (palmitic acid), 9-octadecanoic acid (E) (*trans*-oleic acid) and 9-octadecynoic acid (stearolic acid) in kernels appeared after 3 months of freezing and then showed an increase in compositions after 6 months of frozen storage except 9-octadecynoic acid (stearolic acid) which vanished after 6 months. In contrast, the same components except 9-octadecynoic acid (stearolic acid) in coats showed a decrease in terms of composition (**Table 18, 21, 22, 25, 26**).

In agreement with our findings, (Choi et al., 2022) observed a similar trend with oleic acid found in green ‘Hayward’ kiwifruit cultivar after 90 days over a period of 4 months at 0°C. Previous studies have reported that these variations may be linked to high ratio of unsaturated to saturated fatty acids which is indicative of chilling injury resistance by the lipid membrane. The fatty acid unsaturation have been reported to directly influence membrane permeability at low temperatures, as the fatty acid unsaturation increases membrane permeability also increases in order to prevent chilling injury (physiological damage of cellular tissues) (Antunes & Sfakiotakis, 2008).

Plant species cultivated at low temperatures have indicated that there is an increase in lipid unsaturation as an adaptation measure to low temperatures (Abdala et al., 1996) as observed in the current study as 9-octadecynoic acid and 9-octadecanoic acid (E) (*trans*-oleic acid) increased after 3 and 6 months of frozen storage in kernels of the wild plum. This fact implies that the more unsaturated fatty acids present the less susceptible the fruit may be to essential fatty acid losses during freezing storage. A decline in compositions of 9-octadecanoic acid (E) (*trans*-oleic acid) and *n*-hexadecanoic acid (palmitic acid) was observed after 3 months of frozen storage, however they both vanished after 6 months. A plausible

reason for this observation could be the fact that saturated fatty acids do not undergo peroxidation but they play a role in the induction of oxidative stress in cells resulting in the peroxidation of unsaturated fatty acids. The damage is usually imposed by free radicals or ROS. A typical example of such saturated fatty acids is palmitic acid (Ayala et al., 2014; Onyango, 2021).

Vitamin E (α -tocopherol) which is a biologically active fat-soluble antioxidant was detected in peels of the wild plum. A decrease in its composition was observed after 3 months of frozen storage followed by a increase after 6 months. This fluctuation may be due to tocopherol oxidase aided oxidation of α -tocopherol to a tocopheryl radical which could also be reversed by being re-reduced back to tocopherol by ascorbic acid (Krieger-Liszkay, 2006; Power & Koutsos, 2019; Samsel & Meghani, 2021).

4.5. Influence of long-term frozen storage on proximate composition of *X. americana* (wild plum) fruit parts

In the food sector, proximate analysis is frequently used, especially when describing nutritional information on food labels. It entails the determination of the main food components such as, protein, ash, fat, moisture, fibre and carbohydrates (Abdul-Hamid et al., 2020; Aja et al., 2015). The proximate analysis of wild plum fruit parts after harvest and after 3 and 6 months of frozen storage is summarized in **Table 27, 28, 29, 30**.

Ash is the term used to describe the inorganic residue left behind after the complete oxidation (acid-facilitated) of organic materials in food or after combustion. It is representative of the total mineral content in a food sample. Ashing is usually the first step in proximate analysis (Harris & Marshall, 2017). In the present study, the wild plum (*X. americana*) edible parts (Pulp and kernels) were found to contain ash ranging from 2.62 ± 0.31 to $4.76 \pm 0.42\%$ (**Table 27**) and 1.65 ± 0.47 to $2.14 \pm 0.43\%$ (**Table 30**) respectively, which is somewhat higher than the anticipated ranges of ash content in fruits and vegetables which ranges from 0.2 to 3.5%. On the other hand, peels and coats were found to contain 5.33 ± 0.14 to $8.21 \pm 1.16\%$ (**Table 28**) and 0.86 ± 0.42 to $1.39 \pm 1.01\%$ (**Table 29**) of ash content respectively. Ash content is inversely linked to moisture content (Harris & Marshall, 2017; Pomeranz & Meloan, 1994). A previous study reported lower ash content in *X. americana* seed (kernel) and higher content in pulp as compared to the current study (Bazezew et al., 2021). Moreover, (Sarmiento et al., 2015) reported 3.65% and 1.31% of ash in pulp and seed (kernel) of wild plum from the semiarid region of Brazil respectively which is comparable to the values obtained in this study. In another report, the ash content was 2.3% for *Syzygium cordatum* (water berry) and 1.4% for *Ximenia caffra* Sond. (the other variety of *Ximenia*) comparatively lower than the *X. americana* pulp in the current study (Sibiya et al., 2021). The pulp, peels and kernels may be rich suppliers of minerals given their high ash content.

There were no significant changes in ash content across all fruit parts over a period of 6 months of frozen storage indicating that ash was not susceptible to long term frozen storage as noted by (Zhang et al., 2017) who observed a similar outcome. The degree of moisture in food products is also a crucial factor in preventing food from spoiling and consequently influences consumers' taste (Abdul-Hamid et al., 2020). Nonetheless, moisture content also determines how fresh the fruit is (Oladele et al., 2021). In the present study we reported the highest moisture content in the pulp with no significant changes observed after 3 and 6 months of frozen storage according to Fischer's Least Square difference test. This implies that the wild plum pulp is prone to microbial spoilage, therefore may have short shelf-life if not exposed to treatments such as freezing. Peels, coats and kernels showed significant differences in moisture content after 3 and 6 months of frozen storage, however, the moisture content was relatively lower than that of the pulp indicating that microbial spoilage susceptibility is low and may have potentially long shelf life (Osei et al., 2023). According to (Zhang et al., 2017) water loss of more than 5% in many horticultural items would result in a loss of freshness, a wilting appearance, and even a loss of market value.

Fat in proximate analysis, which is the total fat comprising of fatty acids, fat-soluble vitamins, steroids, and oil-soluble dyes, is also a crucial food component in food analysis (Ganogpichayagrai & Suksaard, 2020; Liu et al., 2017). The World Health Organization (WHO) recommends a total fat intake ranging from 20 to 35% of total calories (Liu et al., 2017). In this study, the fat content across all fruit parts in all three samples (B1, B2, B3) were found in compliance with the recommended intakes, to even lower. The wild plum kernels showed the highest amount of fat as compared to other studied parts in the current study as expected. Similarly, (Lesten et al., 2018) reported high amount of fat (46.05%) in *Parinari curatellifolia* (Maula) fruit kernels, significantly higher than values obtained for the wild plum. In another previous study by (Magaia et al., 2013), higher fat content values in *Adansonia digitata* (35-39.9%) and *Sclerocarya birrea* (58.3-63.1%) kernels were reported. This is true because kernels are generally good sources of fat (Magaia et al., 2013). During frozen storage, there were no statistically significant changes in crude fat content across all fruit parts of the wild plum after 3 and 6 months of frozen storage indicating that the influence of long-term frozen storage of crude fat content was minimal. However, there was a significant decrease in crude fat content observable between B1 (after harvest) and B2 (3 months frozen storage) samples of kernels (**Table 30**). This could be due to the fact that fats are high in unsaturated fatty acids, which are vulnerable to oxidative degradation and may be the cause of the observed loss (Zhang et al., 2017).

Table 27: Fruit pulp proximate analysis

PULP			
(%)	B1 (Post harvest)	B2 (After Three Months)	B3 (After Six Months)
Moisture Content	77.73 ± 0.55 ^a	78.40 ± 1.44 ^a	77.60 ± 0.46 ^a
Ash Content	4.76 ± 0.42 ^a	2.91 ± 0.25 ^b	2.62 ± 0.31 ^b
Crude Fat Content	0.59 ± 0.06 ^a	0.65 ± 0.22 ^a	0.74 ± 0.06 ^a

Values are mean ± standard deviation of triplicate determinations

Table 28: Fruit peels proximate analysis

PEELS			
(%)	B1 (Post harvest)	B2 (After Three Months)	B3 (After Six Months)
Moisture Content	11.20 ± 0.17 ^a	9.02 ± 0.03 ^b	7.79 ± 0.17 ^c
Ash Content	8.21 ± 1.16 ^a	6.37 ± 0.47 ^b	5.33 ± 0.14 ^b
Crude Fat Content	2.87 ± 0.15 ^a	3.05 ± 0.24 ^a	2.54 ± 0.84 ^a

Values are mean ± standard deviation of triplicate determinations

Table 29: Fruit seed coats proximate analysis

COATS			
(%)	B1 (Post harvest)	B2 (After Three Months)	B3 (After Six Months)
Moisture Content	8.71 ± 0.17 ^a	7.45 ± 0.06 ^c	8.11 ± 0.08 ^b
Ash Content	1.39 ± 1.01 ^a	0.86 ± 0.42 ^a	0.90 ± 0.04 ^a
Crude Fat Content	0.39 ± 0.08 ^a	0.35 ± 0.05 ^a	0.30 ± 0.11 ^a

Values are mean ± standard deviation of triplicate determinations

Table 30: Fruit kernels proximate analysis

KERNELS			
(%)	B1 (Post harvest)	B2 (After Three Months)	B3 (After Six Months)
Moisture Content	2.90 ± 0.09 ^b	5.09 ± 0.09 ^a	2.71 ± 0.04 ^c
Ash Content	1.83 ± 0.02 ^a	1.65 ± 0.47 ^a	2.14 ± 0.43 ^a
Crude Fat Content	33.90 ± 1.97 ^a	18.70 ± 0.21 ^b	21.10 ± 3.86 ^b

Values are mean ± standard deviation of triplicate determinations

*All values within a row with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

4.6. Antinutritional factors in *Ximenia americana* (wild plum) fruit parts

Antinutrients are compounds that exist naturally or synthesized. They tend to limit nutritional intake by interfering with nutrient absorption, and may additionally have negative effects (Popova &

Mihaylova, 2019). Oxalate, tannin, and alkaloid levels in wild plum (*X. americana*) fruit parts after harvest, and after 3 and 6 months of frozen storage are summarized in **Table 32**.

Oxalic acid is one of the organic acids with antinutritional effects. It is capable of forming both soluble; potassium and sodium, and insoluble; calcium, magnesium and iron salts or esters known as oxalates. Insoluble calcium oxalate is the common salt of concern, which has been reported to inhibit the uptake and utilization of calcium by the body resulting in certain conditions such as kidney stones and rickets (Popova & Mihaylova, 2019; Umaru et al., 2007). In the present study, the bioavailability of calcium, iron, magnesium and potassium to oxalate were estimated and presented in **Table 31**. It was observed that, the values obtained across all the fruit parts were below the reported critical values of 1.0, 2.0, and 2.5 (Akter et al., 2020; Barde et al., 2012; Gemedede et al., 2016) indicating low levels of oxalates, therefore no expected negative effects on the bioavailability of the studied mineral elements (Gemedede et al., 2016). In comparison to the studied fruit pulp and seed coats, the oxalate levels determined by (Akter et al., 2020) were very high in the pulp and seed coats of *Terminalia ferdinandiana* fruit ranging from 1120 to 1420 mg/100g and 313 to 327 mg/100g respectively. Levels greater than 50 mg/100 g DW of oxalate in various foods are regarded as high (Massey, 2007).

Table 31: Oxalate: Mineral molar ratios of *Ximenia americana* (wild plum) fruit parts

Sample	(Oxalate/Magnesium)	(Oxalate/Potassium)	(Oxalate/Iron)	(Oxalate/Calcium)	Critical Value *
PULP					
B1	-	-	-	-	1.0, 2.0, 2.5
B2	-	-	-	-	
B3	-	-	-	-	
PEELS					
B1	0.000254	0.000107	0.0101	0.00145	1.0, 2.0, 2.5
B2	0.000349	0.0000425	0.0207	0.000782	
B3	0.000459	0.0000540	-	0.000899	
COATS					
B1	0.000139	0.0000875	0.00115	0.000541	1.0, 2.0, 2.5
B2	0.000201	0.0000320	0.000749	0.000306	
B3	-	-	-	-	
KERNELS					
B1	0.0000567	0.0000343	0.00409	0.000458	1.0, 2.0, 2.5
B2	0.0000435	0.0000267	0.00379	0.000275	
B3	0.0000503	0.0000294	0.00426	0.000381	

*Source: (Akter et al., 2020; Barde et al., 2012; Gemedet et al., 2016)

A significant decrease in oxalate content between sample B1 (after harvest) and B2 (3 months frozen storage) in peels, coats and kernels have been noted (**Figure 27**). The observed effect could be due to the physical damage of tissue cells by ice crystals, resulting in the release of cell content to the surrounding materials. This is in agreement with a study by (Musa & Ogbadoyi, 2014) who observed a similar trend. We observed a significant increase in oxalate content in the peels and kernels after 6 months of frozen storage, which could be attributed to gradual frozen storage dehydration as described by (Malik et al., 2021). We however observed no oxalates in the fruit pulp.

Tannins contained in fruits are responsible for astringent flavour that inhibits appetite. Their nutritional effects are mostly influenced by their interactions with proteins and are known to hinder the activities of digestive enzymes. Because of the insoluble nature of tannin-protein complexes, the protein digestion is reduced (Muhammad et al., 2019; Popova & Mihaylova, 2019; Samtiya et al., 2020). The Food and Agriculture Organization of the United Nations previously proposed a temporary acceptable daily intake of 0-0.6 mg/kg (*FAO Nutrition Meetings Report Series 48a*, 1970) although the current studies reported there is no permissible limit for tannins because of insufficient data available "on biochemical aspects, reproductive and developmental toxicity" for tannins (FAO/WHO, 2017; Ojo, 2022). Tannin content decreased significantly from sample B1 (after harvest) to B2 (3 months frozen) across all fruit parts (**Figure 28**). The decrease progressed to sample B3 (6 months frozen) except in the kernels where a slight increase was observed. Like other phenolic compounds, tannins are susceptible to oxidative degradation, therefore, the apparent decrease in tannin content could be attributed to tannin hydrolysis and polymerization into complex tannins (Silva et al., 2013). Furthermore, enzymatic reactions during freezing do not completely cease, but progress gradually (Poiana et al., 2010). This could have also contributed to the decrease in tannin content after 3 and 6 months of frozen storage (-4°C). A slight increase in tannin content after 6 months of frozen storage is attributable to loss of water (Mulot et al., 2019; Silva et al., 2013).

Alkaloids act as antinutritional factors and pharmacological components. They are characterized by the bitter taste of some vegetables (Essack et al., 2017). They are deemed antinutrients because of their interference with the normal functioning of the nervous system by the disruption of cell signaling (Pathaw et al., 2022; Sinha & Khare, 2017). Steroidal glycoalkaloids are typical examples of toxic alkaloids (Itkin et al., 2013). . As frozen storage duration increased from 3 months to 6 months alkaloid

content significantly decreased in pulp and peels with significant increase in coats. There was no statistical difference in kernels in terms of alkaloidal content through time (**Figure 29**), which implies that long-term frozen storage effect was minimal. Accelerated respiration and the use of stored carbohydrates may be the cause of the ensuing rise in alkaloid content after 6 months of freezing storage in seed coats (Rosa et al., 1984). In contrast, (Odufuwa, 2013) reported an increase in alkaloid content in vegetables upon blanching. The apparent decreases may be due to alkaloid decomposition upon exposure to moisture, air and light (Nantongo et al., 2018). Sample processing procedures such as grinding and milling could also impact alkaloid content as described by (Hossain et al., 2016).

Table 32: *X. americana* antinutritional factors

Sample	Oxalates (mg/ml)	Tannins (g%)	Alkaloids (%)
PULP			
B1	ND	3.59 ± 0.04 ^a	9.78 ± 0.64 ^a
B2	ND	1.76 ± 0.02 ^c	8.75 ± 1.86 ^a
B3	ND	2.97 ± 0.09 ^b	3.79 ± 2.37 ^b
PEELS			
B1	1.69 ± 0.01 ^a	8.18 ± 0.07 ^c	13.80 ± 0.89 ^b
B2	0.80 ± 0.003 ^c	9.05 ± 0.08 ^b	17.90 ± 1.99 ^a
B3	1.05 ± 0.01 ^b	9.83 ± 0.13 ^a	16.10 ± 1.61 ^{ab}
COATS			
B1	0.62 ± 0.01 ^a	3.24 ± 0.05 ^c	4.35 ± 0.22 ^b
B2	0.37 ± 0.03 ^b	3.68 ± 0.07 ^b	2.69 ± 0.35 ^c
B3	ND	6.89 ± 0.08 ^a	5.09 ± 0.36 ^a
KERNELS			
B1	0.46 ± 0.01 ^a	0.92 ± 0.01 ^b	9.89 ± 2.26
B2	0.34 ± 0.01 ^c	0.68 ± 0.01 ^a	10.80 ± 1.46
B3	0.39 ± 0.01 ^b	1.32 ± 0.03 ^c	9.76 ± 0.84

*All values within a column with different lower case letter superscripts indicate significant differences between the means ($P \leq 0.05$)

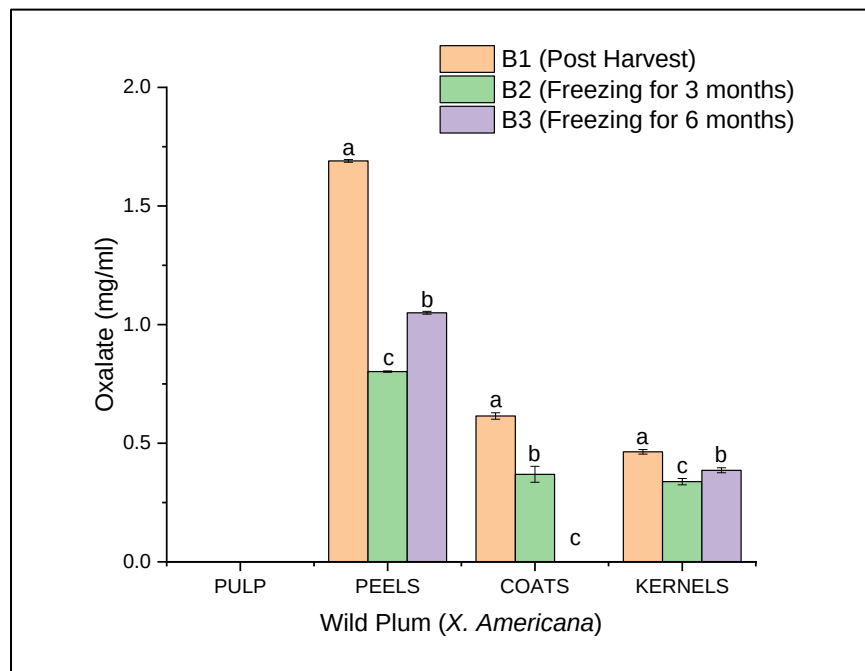


Figure 27: Changes in oxalate content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C . Lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

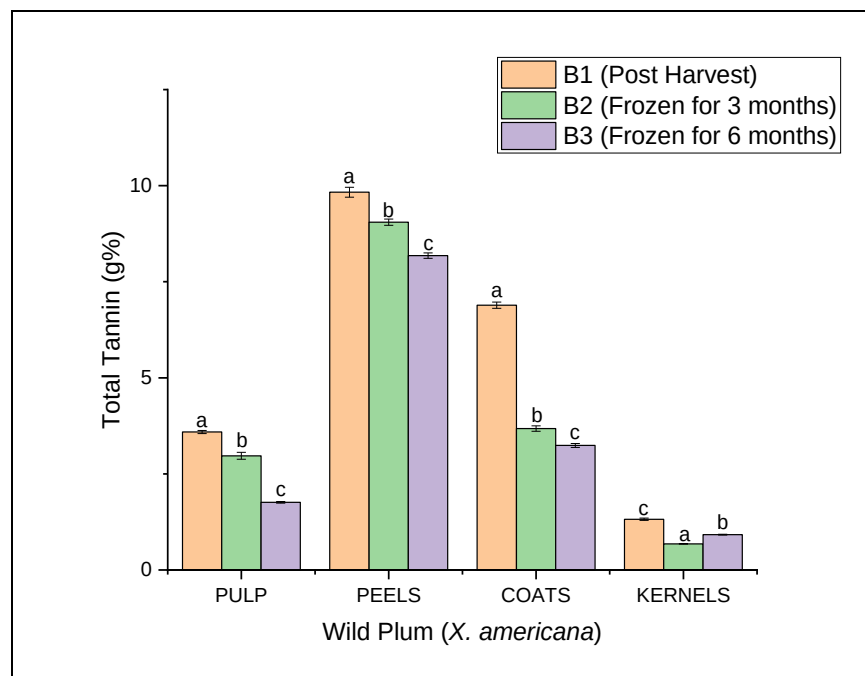


Figure 28: Changes in tannin content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C . Lower case letters indicate significant differences between mean values of elements at ($p \leq 0.05$).

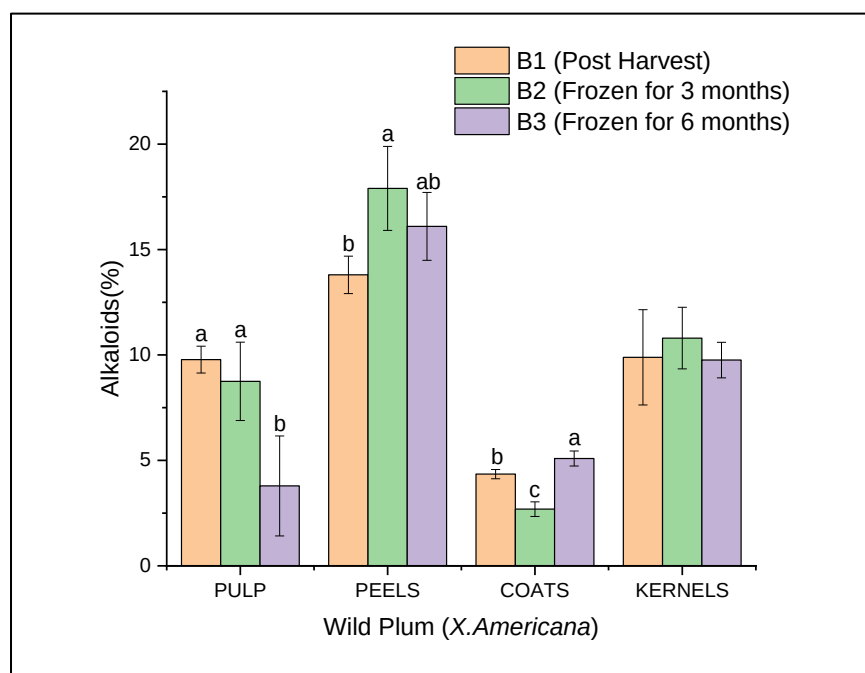


Figure 29: Changes in alkaloid content of the wild plum fruit across all fruit parts after harvest and during storage at -4°C . Lower case letters indicate significant differences between mean concentrations of elements at ($p \leq 0.05$).

4.7. Pearson correlation between mineral elements and antinutrients

The Pearson correlation matrices using correlation coefficients were used to establish an interrelation between the concentration of each mineral element and antinutrients in wild plum (*X. americana*) parts. The correlation coefficients are presented in **Tables 33, 34, 35, 36**.

For the fruit pulp, Na, Mg, Cu, Mn, Ni, and Co showed a negative correlation with tannins, showing a strong correlation with Mn and Mg, moderate correlation with Na and Co and weak correlation with Ni and Cu while K, Fe, Pb, and Ca showed a positive correlation, with Ca being strongly correlated, K and Fe weakly correlated and Pb which showed a very weak correlation. Furthermore, all elements correlated positively with alkaloids except Ca which showed a moderate negative correlation. Elements such as K, Fe, Cu, Ni and Pb showed a very strong positive correlation with alkaloids while correlated strongly with Na and Co followed by Mg and Mn were moderately correlated. No correlation was found with Cr and Zn (**Table 33**).

For the fruit peels, oxalates correlated positively with Na, Mg, Fe, Mn, Ni, Pb and Ca, and showed a negative correlation with K, Cu and Co. Mineral elements such as Na, Mg, Mn, Ni and Pb showed a very strong positive correlation with oxalates whereas with Fe showed a strong correlation and with Ca ;and moderate negative correlation respectively. Tannins showed a positive interaction with Na, Mg, Fe, Mn, Ni, Co and Pb. Na, Mg, Fe, Ni and Pb showed a strong correlation while Mn and Co showed

moderate and weak correlations respectively. The Cu, Ca and K were negatively correlated with tannins, showing a very strong, weak, and strong negative correlations respectively. A positive correlation was observed in K, Co, and Cu with respect to alkaloids, showing a strong positive correlation in K and Co, and weak positive correlation in Cu. In the case of Na, Mg, Fe, Pb, Ca, Ni and Mn with respect to alkaloids, a negative correlation was observed with a strong negative correlation with Na, Mg, Fe, Pb and Ca, and a very strong negative correlation with Ni and Mn (**Table 34**).

For the fruit coats, oxalates showed a positive correlation with Na, Mg, Fe, Cu, Mn, Ni, Co, Zn, Pb, and Ca. From the before mentioned elements, Na, Mg, Fe, Cu, Mn, Ni, and Co showed a very strong positive correlation with oxalates whereas Zn and Pb showed a strong correlation. Moderate positive correlation was observed in Ca. Lastly, K showed a moderate negative correlation. Tannins also showed some correlations with the studied mineral elements. A very strong positive correlation was observed with Fe, Cu, Mn and Co, strong positive correlation with Na, Mg, Ni and Ca, and moderate positive correlation with Zn and Pb. Weak negative correlation with K was observed. Alkaloids showed a negative correlation with Ca, Co, Mn, Cu, Fe, K, Na, Mg and Ni with a very strong correlation with Ca, moderate correlation with Co, Mn, Cu, Fe and K, a very weak correlation with Na, and a weak negative correlation with Mg and Ni. They showed a weak positive correlation with Pb and Zn and no correlation with Cr (**Table 35**).

Finally in kernels, oxalates positively correlated with Na, K, Fe, Mg, Zn and Mn, showing a very strong correlation with Na, K and Fe, and strong correlation with Mg and Mn. With Zn a moderate positive correlation was observed. Oxalates further showed a very strong negative correlation with Cu, strong negative correlation with Ca, and a weak negative correlation with Ni. Tannins correlated positively with Mg, Zn, Cu, Mn, Ca, Mg, Fe and Na. The correlations showed a strong positive correlation with Ca, moderate positive correlation with Zn, weak positive correlations with Na, Mg, Cu and Mn, and very weak correlation with Fe. Strong and moderate negative correlation with Ni and K was observed respectively. Co, Cr and Pb showed no correlation. Alkaloids correlated positively with Ca, Cu and Zn. The correlation appeared very strong, strong, and very weak with Ca, Cu and Zn respectively. They showed a weak negative correlation with Na, Mg and Mn, moderate negative correlation with Ni and Fe, and strong negative correlation with K. No correlation with Cr, Co and Pb (**Table 36**).

Nearly all correlations were insignificant ($P>0.05$) except alkaloids with Pb in pulp, oxalates with Co in coats, tannins with Cu in coats, and oxalates with Cu in kernels which showed significant correlations ($P<0.05$). Both strong and moderate positive correlations might be linked to similarity in chemical

properties of the studied variables as well as natural sources such as biochemical pathways. These correlations might indicate proportional absorption and distribution of the studied antinutrients and mineral elements across the fruit parts of the wild plum (*X. americana*). Strong and moderate negative correlations could indicate that one variable may affect the other in terms of absorption rates. Weak negative and positive correlations indicate that the effect of one variable's presence or absence on the other is less significant (Dar et al., 2017; Mekassa & Chandravanshi, 2015; Papageorgiou, 2022; Schober et al., 2018; Yami et al., 2016)

Table 33: Pearson correlation coefficients for antinutrients to mineral elements in pulp samples

	<i>Tannins</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Tannins</i>	1												
<i>Na</i>	-0.69	1											
<i>Mg</i>	-0.76	0.99	1										
<i>K</i>	0.20	0.56	0.47	1									
<i>Fe</i>	0.14	0.61	0.52	0.99	1								
<i>Zn</i>	-	-	-	-	-	1							
<i>Cu</i>	-0.20	0.84	0.78	0.91	0.94	-	1						
<i>Mn</i>	-0.79	0.98	0.99	0.42	0.48	-	0.75	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.13	0.80	0.73	0.94	0.96	-	0.99	0.70	-	1			
<i>Co</i>	-0.63	0.99	0.98	0.62	0.67	-	0.88	0.97	-	0.84	1		
<i>Pb</i>	0.04	0.69	0.61	0.98	0.99	-	0.97	0.57	-	0.98	0.74	1	
<i>Ca</i>	0.89	-0.94	-0.97	-0.24	-0.31	-	-0.61	-0.98	-	-0.55	-0.91	-0.40	1
	<i>Alkaloids</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Alkaloids</i>	1												
<i>Na</i>	0.71	1											
<i>Mg</i>	0.63	0.99	1										
<i>K</i>	0.98	0.56	0.47	1									
<i>Fe</i>	0.99	0.61	0.52	0.99	1								
<i>Zn</i>	-	-	-	-	-	1							
<i>Cu</i>	0.97	0.84	0.78	0.91	0.94	-	1						
<i>Mn</i>	0.59	0.98	0.99	0.42	0.48	-	0.75	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	0.98	0.80	0.73	0.94	0.96	-	0.99	0.70	-	1			
<i>Co</i>	0.76	0.99	0.98	0.62	0.67	-	0.88	0.97	-	0.84	1		
<i>Pb</i>	0.99 ^a	0.69	0.61	0.98	0.99	-	0.97	0.57	-	0.98	0.74	1	
<i>Ca</i>	-0.43	-0.94	-0.97	-0.24	-0.31	-	-0.61	-0.98	-	-0.55	-0.91	-0.40	1

where ^a indicates a significant correlation at 0.05 confidence level.

Table 34: Pearson correlation coefficients for antinutrients to mineral elements in peel samples.

	<i>Oxalate</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Oxalate</i>	1												
<i>Na</i>	0.95	1											
<i>Mg</i>	0.96	0.99	1										
<i>K</i>	-0.91	-0.99	-0.98	1									
<i>Fe</i>	0.87	0.98	0.97	-0.99	1								
<i>Zn</i>	-	-	-	-	-	1							
<i>Cu</i>	-0.11	-0.41	-0.37	0.51	-0.57	-	1						
<i>Mn</i>	0.99	0.98	0.99	-0.95	0.93	-	-0.24	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	0.96	0.99	0.99	-0.98	0.96	-	-0.35	0.99	-	1			
<i>Co</i>	-0.57	-0.29	-0.33	0.18	-0.11	-	-0.74	-0.45	-	-0.35	1		
<i>Pb</i>	0.95	0.99	0.99	-0.99	0.98	-	-0.40	0.98	-	0.99	-0.30	1	
<i>Ca</i>	0.69	0.43	0.47	-0.33	0.26	-	0.63	0.58	-	0.49	-0.98	0.44	1
	<i>Tannins</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Tannins</i>	1												
<i>Na</i>	0.80	1											
<i>Mg</i>	0.77	0.99	1										
<i>K</i>	-0.86	-0.99	-0.98	1									
<i>Fe</i>	0.89	0.98	0.97	-0.99	1								
<i>Zn</i>	-	-	-	-	-	1							
<i>Cu</i>	-0.97	-0.64	-0.61	0.72	-0.77	-	1						
<i>Mn</i>	0.68	0.98	0.99	-0.95	0.93	-	-0.50	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	0.75	0.99	0.99	-0.98	0.96	-	-0.59	0.99	-	1			
<i>Co</i>	0.33	-0.29	-0.33	0.18	-0.11	-	-0.53	-0.45	-	-0.35	1		
<i>Pb</i>	0.79	0.99	0.99	-0.99	0.98	-	-0.63	0.98	-	0.99	-0.30	1	
<i>Ca</i>	-0.18	0.43	0.47	-0.33	0.26	-	0.40	0.58	-	0.49	-0.98	0.44	1
	<i>Alkaloids</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Alkaloids</i>	1												
<i>Na</i>	-0.88	1											
<i>Mg</i>	-0.89	0.99	1										
<i>K</i>	0.82	-0.99	-0.98	1									
<i>Fe</i>	-0.78	0.98	0.97	-0.99	1								
<i>Zn</i>	-	-	-	-	-	1							
<i>Cu</i>	0.20	-0.64	-0.61	0.72	-0.77	-	1						
<i>Mn</i>	-0.94	0.98	0.99	-0.95	0.93	-	-0.50	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.91	0.99	0.99	-0.98	0.96	-	-0.59	0.99	-	1			
<i>Co</i>	0.71	-0.29	-0.33	0.18	-0.11	-	-0.53	-0.45	-	-0.35	1		
<i>Pb</i>	-0.88	0.99	0.99	-0.99	0.98	-	-0.63	0.98	-	0.99	-0.30	1	
<i>Ca</i>	-0.81	0.43	0.47	-0.33	0.26	-	0.40	0.58	-	0.49	-0.98	0.44	1

where ^a indicates a significant correlation at 0.05 confidence level.

Table 35: Pearson correlation coefficients for antinutrients to mineral elements in coats samples.

	<i>Oxalate</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Oxalate</i>	1												
<i>Na</i>	0.91	1											
<i>Mg</i>	0.97	0.97	1										
<i>K</i>	-0.65	-0.90	-0.79	1									
<i>Fe</i>	0.98	0.82	0.91	-0.49	1								
<i>Zn</i>	0.80	0.97	0.91	-0.97	0.67	1							
<i>Cu</i>	0.96	0.77	0.88	-0.42	0.99	0.61	1						
<i>Mn</i>	0.98	0.83	0.92	-0.51	0.99	0.69	0.99	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	0.95	0.99	0.99	-0.85	0.87	0.94	0.83	0.88	-	1			
<i>Co</i>	0.99 ^a	0.89	0.96	-0.61	0.98	0.77	0.97	0.99	-	0.93	1		
<i>Pb</i>	0.80	0.97	0.91	-0.97	0.67	1	0.61	0.69	-	0.94	0.77	1	
<i>Ca</i>	0.61	0.23	0.42	0.20	0.75	0.01	0.80	0.73	-	0.34	0.64	0.01	1
	<i>Tannins</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Tannins</i>	1												
<i>Na</i>	0.75	1											
<i>Mg</i>	0.87	0.97	1										
<i>K</i>	-0.39	-0.90	-0.79	1									
<i>Fe</i>	0.99	0.82	0.91	-0.49	1								
<i>Zn</i>	0.59	0.97	0.91	-0.97	0.67	1							
<i>Cu</i>	0.99 ^a	0.77	0.88	-0.42	0.99	0.61	1						
<i>Mn</i>	0.99	0.83	0.92	-0.51	0.99	0.69	0.99	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	0.82	0.99	0.99	-0.85	0.87	0.94	0.83	0.88	-	1			
<i>Co</i>	0.96	0.89	0.96	-0.61	0.98	0.77	0.97	0.99	-	0.93	1		
<i>Pb</i>	0.59	0.97	0.91	-0.97	0.67	1	0.61	0.69	-	0.94	0.77	1	
<i>Ca</i>	0.81	0.23	0.42	0.20	0.75	0.01	0.80	0.73	-	0.34	0.64	0.01	1
	<i>Alkaloids</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Alkaloids</i>	1												
<i>Na</i>	-0.0067	1											
<i>Mg</i>	-0.20	0.97	1										
<i>K</i>	-0.42	-0.90	-0.79	1									
<i>Fe</i>	-0.57	0.82	0.91	-0.49	1								
<i>Zn</i>	0.21	0.97	0.91	-0.97	0.67	1							
<i>Cu</i>	-0.63	0.77	0.88	-0.42	0.99	0.61	1						
<i>Mn</i>	-0.55	0.83	0.92	-0.51	0.99	0.69	0.99	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.11	0.99	0.99	-0.85	0.87	0.94	0.83	0.88	-	1			
<i>Co</i>	-0.45	0.89	0.96	-0.61	0.98	0.77	0.97	0.99	-	0.93	1		
<i>Pb</i>	0.21	0.97	0.91	-0.97	0.67	1	0.61	0.69	-	0.94	0.77	1	
<i>Ca</i>	-0.97	0.23	0.42	0.20	0.75	0.01	0.80	0.73	-	0.34	0.64	0.01	1

where ^a indicates a significant correlation at 0.05 confidence level

Table 36: Pearson correlation coefficients for antinutrients to mineral elements in kernel samples.

	<i>Oxalate</i>	<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Oxalate</i>	1												
<i>Na</i>	0.92	1											
<i>Mg</i>	0.83	0.98	1										
<i>K</i>	0.98	0.83	0.72	1									
<i>Fe</i>	0.94	0.99	0.97	0.86	1								
<i>Zn</i>	0.63	0.87	0.95	0.47	0.85	1							
<i>Cu</i>	-0.99 ^a	-0.92	-0.83	-0.98	-0.94	-0.62	1						
<i>Mn</i>	0.88	0.99	0.99	0.78	0.98	0.91	-0.88	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.19	-0.54	-0.69	-0.005	-0.50	-0.88	0.18	-0.62	-	1			
<i>Co</i>	-	-	-	-	-	-	-	-	-	-	1		
<i>Pb</i>	-	-	-	-	-	-	-	-	-	-	-	1	
<i>Ca</i>	-0.78	-0.49	-0.31	-0.88	-0.53	-0.01	0.78	-0.40	-	-0.45	-	-	1
<i>Tannins</i>		<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Tannins</i>	1												
<i>Na</i>	0.11	1											
<i>Mg</i>	0.29	0.98	1										
<i>K</i>	-0.44	0.83	0.72	1									
<i>Fe</i>	0.06	0.99	0.97	0.86	1								
<i>Zn</i>	0.57	0.87	0.95	0.47	0.85	1							
<i>Cu</i>	0.27	-0.92	-0.83	-0.98	-0.94	-0.62	1						
<i>Mn</i>	0.21	0.99	0.99	0.78	0.98	0.91	-0.88	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.89	-0.54	-0.69	-0.005	-0.50	-0.88	0.18	-0.62	-	1			
<i>Co</i>	-	-	-	-	-	-	-	-	-	-	1		
<i>Pb</i>	-	-	-	-	-	-	-	-	-	-	-	1	
<i>Ca</i>	0.80	-0.49	-0.31	-0.88	-0.53	-0.01	0.78	-0.40	-	-0.45	-	-	1
<i>Alkaloids</i>		<i>Na</i>	<i>Mg</i>	<i>K</i>	<i>Fe</i>	<i>Zn</i>	<i>Cu</i>	<i>Mn</i>	<i>Cr</i>	<i>Ni</i>	<i>Co</i>	<i>Pb</i>	<i>Ca</i>
<i>Alkaloids</i>	1												
<i>Na</i>	-0.39	1											
<i>Mg</i>	-0.21	0.98	1										
<i>K</i>	-0.83	0.83	0.72	1									
<i>Fe</i>	-0.44	0.99	0.97	0.86	1								
<i>Zn</i>	0.09	0.87	0.95	0.47	0.85	1							
<i>Cu</i>	0.71	-0.92	-0.83	-0.98	-0.94	-0.62	1						
<i>Mn</i>	-0.30	0.99	0.99	0.78	0.98	0.91	-0.88	1					
<i>Cr</i>	-	-	-	-	-	-	-	-	1				
<i>Ni</i>	-0.54	-0.55	-0.69	-0.005	-0.50	-0.88	0.18	-0.62	-	1			
<i>Co</i>	-	-	-	-	-	-	-	-	-	-	1		
<i>Pb</i>	-	-	-	-	-	-	-	-	-	-	-	1	
<i>Ca</i>	0.99	-0.49	-0.31	-0.88	-0.53	-0.01	0.78	-0.40	-	-0.45	-	-	1

where ^a indicates a significant correlation at 0.05 confidence level.

CHAPTER 5

5. CONCLUSIONS AND RECOMMENDATIONS

Ximenia americana (wild plum) fruits contain a great deal of dietary nutrients essential for human health, therefore postharvest storage duration is an important factor in ensuring their quality and market potential. Freezing is one fruits treatment that generally allows the retention of nutritive qualities for longer periods, thus prolonging their availability. This study demonstrated that when the wild plum fruits are frozen at -4°C for 3 and 6 months, their mineral elements, phytochemicals, antinutrients and proximate contents varied according to the fruit part. Edible parts such as pulp and kernel showed a significant decrease in essential macro-mineral elements content such as sodium. However, the pulp showed a decline in magnesium content after 3 months and potassium content after 6 months while the kernel stably retained magnesium and potassium. Calcium content slightly increased and decreased in both pulp and kernel after 3 and 6 months of frozen storage respectively. Heavy metals were also detected in pulp and kernels. Lead and cobalt were detected after 3 months, and then vanished after 6 months of frozen storage. This suggests the use of freezing storage as a remediation measure for the reduction of toxic heavy metals such as lead (Pb). This study also revealed no significant change in fat content of the pulp, peels and the coats, however there was a significant reduction and a subsequent insignificant increase in fat content of the kernels after 3 and 6 months of frozen storage respectively. The ash content in coats and kernels did not change but showed significant decrease in both pulp and peels. However, the ash content in peels insignificantly increased after 6 months. Moisture content remained unchanged and the highest them all in the pulp sample, while a decrease was observed in peels and coats after 3 months of freezing. After 6 months the moisture content in coats increased and kept decreasing in peels. A fluctuating trend in moisture content was observed in kernels.

The antinutritional factor analysis revealed that all the wild plum fruit parts contained oxalates except the fruit pulp, tannins and alkaloids. An insignificant and significant decrease in alkaloid content was observed in the pulp and coats samples after 3 and 6 months of freezing respectively. However, the pulp sample further showed a significant decrease, but coats showed an increase. The peels showed a significant increase in alkaloid content after 3 months followed by an insignificant decrease after 6 months. Furthermore, tannin content decreased significantly across all fruit parts except in kernels where an increase was observed after 6 months.

Oxalates significantly decreased in peels, coats and kernels after 3 months of frozen storage. After a period of 6 months an increase was observed in peels and kernels. However, antinutrients (oxalates and

tannins) content values in kernels are below the permissible toxic levels in place, except tannins in the pulp according to the previous temporary limit, suggesting that the edible wild plum fruit parts consumption should be regulated to a certain extent. However, alkaloids do not have a general limit unless characterized. This study also reported changes in phytochemicals detected by GC-MS during long-term frozen storage. Variations in phytochemicals such as esters, fatty acids, tocopherols, aldehydes, phytosterols, amides, triterpenoids, poly-unsaturated hydrocarbons, alkane hydrocarbons were observed after harvest and after 3 and 6 months of frozen storage. Esters, fatty acids, amides, tocopherols and alkane hydrocarbons showed an appreciable retention after 3 and 6 months of frozen storage across all fruit parts. Long-term frozen storage had considerable effects on the nutritional value and phytochemical composition of the wild plum fruit and the rate of deterioration was more noticeable during frozen storage of which can make the fruit unmarketable. It is therefore concluded that the wild plum fruits should be frozen stored at -4°C and used within 6 months to protect their superior quality and make them healthier for human health. Further experiments on the rapid determination of antinutritional factors, microbiological quality properties, consumer acceptance evaluation, and the effect of preservation methods on total phenolic and flavonoid content of the wild plum fruits are however still needed to further determine the quality of this fruit.

This study has revealed that *Boscia foetida* roots are rich in vital mineral elements such as calcium, sodium, magnesium, and potassium. Their contents are sufficient to satisfy the recommended daily intakes, therefore, the roots of this plant can be considered as an alternative source of nutrients crucial in the prevention of malnutrition and deficiency ailments. However, the roots are contaminated with lead, cobalt, nickel, and zinc at concentrations higher than the permissible limits of the FAO/WHO. *B. foetida* roots also contain phytochemicals with different therapeutic and pharmaceutical properties which could be responsible for their folk medicinal use and could be useful in the pharmaceutical industry. The GC-MS non-polar root profile of *B. foetida* is reported for the first time. Further studies on the isolation, characterization, and antimicrobial activity of the compounds responsible for the ethnomedicinal uses of the roots and leaves of *B. foetida* are required.

Health risk assessment was performed based on the estimated daily intake of the wild plum pulp and kernels and *B. foetida* roots. The assessed heavy metals, however, did not present any non-carcinogenic health risks except in the pulp which showed a hazard quotient and index greater than 1 suggestive of the presence of non-carcinogenic health effects when ingested. The carcinogenic risk assessment results revealed that the edible roots (*B. foetida*), pulp and kernels of the wild plum (*X. americana*) were

contaminated with lead and nickel at values greater than the specific incremental lifetime cancer risk of 1×10^{-6} and 1×10^{-4} . Lead and nickel contamination assessment in these parts from different areas is required. In view of the study result, a continuous regulatory control is suggested to ensure the safety of the marketed plant products made from the two plants collected from the study area.

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