QUALITY EVALUATION OF TISANE BREWED FROM SOURSOP LEAVES AND PREKESE FRUIT

BY

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CERTIFICATION

This is to certify that this project was carried out by **NWANKWO, Divine Favour** with Matriculation Number **19/10AQ111** of the Department of Home Economics and Food Science, University of Ilorin, Ilorin, Nigeria for the Award of B.Sc. degree in Food Science during the 2024/2025 Academic Session.

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DEDICATION

This project is dedicated first to GOD who saw me throughout the period of this program and also to my mum Mrs. Nwankwo for her immense support and love throughout the course of this program.

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ABSTRACT

Tisane refers to beverages that are not brewed from the cured leaves of the tea plant; *camellia sinensis*. This study investigated the phytochemical content (total flavonoids, phenolics, and tannins), antioxidant activities, and physicochemical properties of tisanes prepared from varying ratios of soursop leaf powder and Prekese fruit powder. Soursop leaves powder was combined with prekese fruits powder in ratios of 100:0, 90:10, 80:20, 70:30, 60:40, 0:100. Sample S70P30 (70% soursop leaf powder and 30% prekese fruit powder) has the highest flavonoid content (0.21 mg CE/g) and phenolic content (0.23 mg GAE/g), suggesting a synergistic enhancement of these bioactive compounds. Tannin content (0.07 mg CE/g) remained consistent across all blends. Antioxidant activity, assessed using DPPH, FRAP, ABTS, phosphomolybdate (PMA), and metal chelating agent (MCA) assays, generally showed high scavenging and reducing power, with Sample S70P30 exhibiting the highest DPPH scavenging activity of 78.77% and MCA activity of 20.42 mg EDTA equivalent/g, while 100% soursop leaf (Sample S100P0) showed the highest ABTS activity of 90.98 mg Trolox equivalent. Physicochemical analysis revealed acidic pH values for all samples, with increasing Prekese fruit proportion correlating with higher pH and, generally, total titratable acidity (TTA). Total dissolved solids (TDS) were highest in the 80:20 blend (21.60µmg/l) (80% soursop leaf powder, 20% prekese fruit powder). Sensory evaluation indicated the 80:20 blend was most preferred for color and after taste, overall acceptability was also highest for this blend. These findings highlight the potential of combining soursop leaves and Prekese fruit to create a tisane with enhanced phytochemical and antioxidant properties, with the 80:20 blend demonstrating favorable sensory attributes and 70:30 demonstrating high antioxidant properties.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Tisane refers to any beverage that is not brewed from the cured leaves of the tea plant; *Camellia sinensis.* Instead, tisanes are prepared from other plant materials, such as leaves, flowers, fruits, seeds, or roots, making them distinct from true teas (Chen *et al.*, 2015). Therefore, in this literature the term tisane would be used interchangeably with herbal tea. Tisane or herbal tea, is a caffeine-free beverage with nutritional benefits made from infusing or decocting herbs, spices, or plant materials in hot water. Common herbs used include mint, ginger, chamomile, and lemon balm. Tisanes are categorized by plant part: leaves (lemon balm, mint, soursop), flowers (rose, chamomile), bark
(cinnamon, slippery elm), roots (ginger, echinacea), and fruits/berries (raspberry, blueberry, Prekese), they offer antioxidant properties, therapeutic applications, and stimulant, relaxant, or sedative properties, promoting overall health and wellness (Bennet *et al.*, 2016).

Prekese (*Tetrapleura tetraptera*), commonly known as Aidan in Western Nigeria and Prekese as its general name is a tropical deciduous forest tree characterized by distinctive four-winged fruits consisting of a woody shell, a fleshy pulp, and small, brownish-black seeds. It is used in traditional African medicine and as a spice to flavor soups, notably the traditional pepper soup, which is delicacy consumed especially by new mothers to prevent post-partum contractions and aid lactation. Efforts are underway to promote the utilization of Prekese fruit, with innovative applications including its incorporation into chocolate products, transformation into jam, and use as a flavor enhancer for biscuits and some selected alcoholic beverages (Gyamfi *et al*., 2016).

Soursop (*Annona muricata*) is a small, evergreen tree native to the Americas, specifically Mexico, Central America, and South America. It has since spread to tropical and subtropical regions worldwide, often becoming invasive. The tree grows up to 5-6 meters tall and belongs to the Annonaceae family. Various parts of the soursop tree, including its leaves, bark, fruits, and roots, are utilized in making tisanes (Nhi *et al*., 2020). In Nigeria, soursop leaves are called efinrin oso in Yoruba and Akpu Okpo in Igbo language. The use of the leaves has been identified in tropical regions to treat various diseases and has been proven in vitro, such as anti-inflammatory, antioxidant, and cytotoxic activities against tumor cells (Coria-Téllez *et al*., 2018). These properties are attributed to the presence of the more than 200 chemical compounds identified and isolated from the leaf, some of which are; alkaloids, phenols, acetogenins, and other compounds like vitamins, carotenoids, amides, and cyclopeptides (llango *et al*., 2022). The leaves also contain essential nutrients like calcium, potassium, iron, and vitamins A, B, and C (Ukachi *et al* 2019).

This study therefore aims at utilizing these plant parts (soursop leaves and prekese fruit) as a tisane blend.

1.2 Justification of study

Nutraceutics and functional foods offer a promising alternative for addressing various diet-related disorders. Consuming herbal beverages or tisanes as part of a balanced diet can enhance antioxidant levels, reduce oxidative stress in humans, and provide a caffeine-free alternative to traditional coffee and tea. This is why global demands for herbal teas and infusions are on the rise, driven by their appealing flavors and numerous health benefits, as well as their status as caffeine free alternatives (Lui *et al*., 2023).

Prekese *(Tetrapleura tetraptera*) is an underutilized fruit with significant potential. Although efforts are underway to promote its utilization, with innovative applications such as its incorporation into chocolate products, transformation into jam, and use as a flavor enhancer for biscuits and some selected alcoholic beverages, it still remains underutilized (Gyamfi *et al*., 2016). The African Network for Agriculture, Agroforestry and Natural Resources Education (ANAFE) included soursop (Annona muricata) as one of the underutilized trees in Africa (Suzana, 2018).

Given the potential health benefits of herbal beverages, their high global demands and the underutilization of prekese and soursop, it is important to explore new ingredient combinations to expand the range of available flavors and potential health benefits of tisanes. Therefore, this study aims to develop and characterize a novel herbal beverage incorporating prekese fruits and soursop leaves, this would promote the utilization of prekese fruit and soursop leaves as a new beverage option and also contribute to a more sustainable and healthy food system.

1.3 Objectives of the research

General objective: The general objective of this research is to produce soursop-prekese (Tisane) tea bags.

Specific objectives: To determine the

i. Physicochemical properties of the tisane

ii. Phytochemicals present in the tisane.

iii. Anti-oxidant activities of the tisane

iv. Organoleptic properties of the soursop-prekese tisane

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tisanes

For centuries, tisanes or herbal beverages have been a staple of social gatherings and traditional medicine, offering a natural remedy for various health issues. In today's society, they are increasingly recognized for their potential to reduce the risk of chronic diseases, including type 2 diabetes, high blood pressure, abnormal cholesterol levels, and certain types of cancer (Chandrasekara *et al.,* 2018). Despite extensive research, medicinal plants remain underutilized, with the World Health Organization (WHO) estimating that over 80% of the global population relies on alternative medicine for primary healthcare needs (Ogungbenle, 2022). For many lowincome families, medicinal plants like moringa, papaya, tea, soursop, prekese and lemon have become essential for treating diseases such as cancer, rheumatism, and urinary tract infections (Huang *et al.,* 2020). Research suggests that oxidative stress is a primary contributor to chronic and degenerative diseases. Fortunately, tisanes derived from these medicinal plants have demonstrated robust anti-oxidative activity, attributed to their bioactive compounds, which shield the body from free radical-induced oxidative diseases and other metabolic diseases, those natural bioactive compounds found in tisanes includes carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins and terpenoids, among others (Chandrasekara *et al.,* 2018).

Tisanes can be brewed from a single plant part or a combination of various parts, including those from the same plant or different plants (Pohl *et al*., 2018). According to Caleja *et al*. (2019), blending various tisane plant parts can create a synergistic effect, amplifying their nutritional benefits and flavor profiles. This is evident from recent studies on the formulation and nutritional evaluation of herbal tea blends made from pineapple peels, ginger, and lemon grass In the ratios of

(2 : 0.1 : 0.1), (2.0 : 0.25 : 0.25), and (2.0 : 0.5 : 0.5) respectively (Samoh *et al.,* 2023). The study revealed that the nutrient optimal blend ratio of 2.0 : 0.1 : 0.1 (pineapple peels: ginger: lemon grass) was the blend mixture having the highest antioxidant activity. For those monitoring their sugar intake, the 2.0 : 0.5 : 0.5 ratio was recommended while for those not restricting their sugar intake, the 2.0:0.1:0.1 ratio was ideal. Notably, both blends demonstrated comparable antioxidant activity, making them suitable options for overall well-being.

A previous research study investigated the effects of soursop and moringa leaves, both individually (100% soursop, 100% moringa) and in various combinations (50:50, 60:40, 40:60 soursop to moringa ratios), the research revealed that the 50:50 soursop-moringa tea blend had an optimal pH range (7.28-7.81), desirable limits of total solids(3.47-3.82 mg/l) and the highest antioxidant capacity using DPPH and FRAP method (89.04% and 531.44 µM/L, respectively) (Ukachi *et al*., 2019) . The synergistic effects observed in the 50:50 blend resulted in enhanced antioxidant properties compared to the single-ingredient teas (100% soursop and 100% moringa).

In light of these research results, it is clear that utilizing multiple plant parts for tisane preparation can yield a synergistic effect, resulting in a more nutritionally beneficial beverage, and as such this research aims at utilizing soursop leaves and prekese fruits into a soursop-prekese tisane to investigate if their combination yields enhanced synergistic health benefits.

The table below shows the various plant parts that are used for tisanes and some examples;

Tab 2.1: plant parts used for making tisanes

Plant parts.	Examples.	Source
Bark	Black cherry bark *(Prunus serotina).*	Wink, (2015)
Flowers	Chamomile (*Matricaria recutita)*,	(Srivastava *et al.,* 2016)
	Hibiscus (*Hibiscus sabdariffa*)	(Zhang *et al.,* 2018)
Fruits	Apple (*Malus domestica).*	(Kumar *et al* 2018) .
	blueberry (*Vaccinium corymbosum)*	(Srivastava *et al.*, 2020)
Leaf	Peppermint *(Mentha piperita)*,	(McKay *et al*. 2016)
	Rooibos *(Aspalathus linearis)*	*(*Joubert *et al.* 2017)
Root	Echinacea (Echinacea purpurea),	(Schapowal *et al.* 2015)
	Ginger (Zingiber officinale)	(Ali *et al* . 2017)

2.2 Bioactive compounds in herbal teas (tisanes)

Bioactive compounds are specialized molecules produced by living organisms, such as plants, that exhibit health-promoting properties. These compounds can be found in various forms, including lipids, polysaccharides, carotenoids, vitamins, phenolics, and phycobiliproteins (Nwoye *et al.,* 2023)

Tisanes, have been identified as a rich source of diverse bioactive compounds. These compounds have been found to have potential health benefits and may contribute to a reduced risk of developing various non-communicable diseases (NCDs) (Vasic *et al.,* 2023). These bioactive compounds present in tisanes include:

(I) Polyphenols

Polyphenols are a class of bioactive compounds that are abundant in plant-based foods and beverages, including tisanes. These compounds have been extensively studied for their potential health benefits, particularly in relation to chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. While a lack of polyphenols in the diet does not cause specific health disorders, consuming sufficient amounts of polyphenols may provide numerous health advantages, particularly in reducing the risk of chronic diseases (Fraga *et al.,* 2019).

(ii) Phenolic acids

Phenolic acids are compounds that have been shown to have anti-inflammatory and antioxidant effects. Plants contain two primary classes of phenolic acids: hydroxybenzoic acids and hydroxycinnamic acids. Hydroxybenzoic acids, characterized by a C6-C1 structure, encompass a range of compounds, including gallic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, and protocatechuic acid. Hydroxycinnamic acids, also referred to as phenylpropanoids (C6-C3), comprise p-coumaric acid, caffeic acid and ferulic acid. Notably, herbal beverages have been shown to be a rich source of various phenolic acids (Shahidi *et al.,* 2018).

(iii) Flavonoids

Flavonoids are produced through the condensation of phenylpropanoid compounds with malonyl coenzyme A, facilitated by the enzyme chalcone synthase. This process yields chalcones, which are then converted into flavonoids under acidic conditions. The flavonoid family comprises various subclasses, which includes, Flavones (e.g., luteolin, apigenin), Flavonols (e.g., quercetin, kaempferol), Flavanols (e.g., catechin, epigallocatechin gallate), Flavanones (e.g., narigenin, hesperitin), Anthocyanidins (e.g., cyanidin, malvidin), Isoflavones (e.g., genistein, daidzein). These subclasses differ in their molecular structure, particularly in the presence or absence of hydroxyl groups and double bonds. Flavonoids are commonly found in various plant-based foods and beverages, contributing to their nutritional value and potential health benefits. A class of compounds that have been found to have antioxidant, anti-inflammatory, and anti-cancer properties (Chandrasekara *et al.,* 2018).

(IV) Tannin

Tannins are water soluble polyphenols that give foods and beverages their astringent taste and can bind to iron thereby reducing iron absorption. Tannins bind to iron, forming insoluble complexes that are less available for absorption in the gut. This can be particularly relevant for individuals with iron deficiency or those at risk of developing iron deficiency anemia. Tannin decreases palatability due to their bitter or astringent taste, emphasizing the need for balanced tannin levels in food products (Akintola *et al.,*2015).

2.3 Potential Health Benefits of Tisanes

The consumption of tisanes has been associated with a reduced risk of developing various NCDs (Nwoye *et al.,* 2023) such as:

1. Cardiovascular diseases (CVD): Tisanes may help to reduce the risk of heart disease by lowering blood pressure and cholesterol levels.

2. Type 2 diabetes mellitus (T2DM):: Tisanes may help to reduce the risk of developing type 2 diabetes by improving insulin sensitivity and glucose metabolism.

3. Certain types of cancer: Tisanes may help to reduce the risk of developing certain types of cancer, such as breast, prostate, and colon cancer.

4. Arthritis: Tisanes may help to reduce the risk of developing arthritis by reducing inflammation and improving joint health.

5. Autoimmune disorders: Tisanes may help to reduce the risk of developing autoimmune disorders, such as rheumatoid arthritis and lupus.

6. Neurodegenerative disorders: Tisanes may help to reduce the risk of developing neurodegenerative disorders, such as Alzheimer's and Parkinson's disease.

The established understanding of tisanes as rich sources of bioactive compounds with proven antioxidative activity and their potential to reduce the risk of various chronic diseases provides a strong foundation for further exploration. Despite extensive research into the benefits of individual medicinal plants, a significant portion of the global population still relies on traditional remedies, highlighting the underutilization of these natural resources in mainstream healthcare

2.4 Soursop leaves and its nutritional composition

Soursop *(Annona muricata)* is a plant belonging to the Annonaceae family that has been widely used globally as a traditional medicine for many diseases (Mutakin *et al.,* 2022). It also is a rich source of essential and non-essential nutrients that support various metabolic functions in the body. Essential macronutrients found in the leaves includes carbohydrates which constitutes 27.81% of the leaves nutrient composition followed by fat (2.19%) which is closely followed by its moisture content (21.14%). They also contain moderate protein content (12.69%) and lower levels of ash (8.67%) and crude fiber (8.50%), of greater importance is the essential minerals attributed to the leaves and bioactive compounds; these essential minerals, includes calcium (72.52 ppm), potassium (49.92 ppm), sodium (30.33 ppm), iron (25.8 ppm), magnesium (18.52 ppm), phosphorus (10.87 ppm), and zinc (2.63 ppm) and bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids and saponin. (Olude *et al.,* 2020).

Tab 2.3: nutritional composition of soursop leaves

Nutrient	Amount per 100g (approximate)
Carbohydrates	27.81g
Protein	12.69g
Fat	21.19g
Fiber	8.50g
Ash	8.67g
Moisture	21.15g
Calcium	1.18mg
Iron	24.26mg
Magnesium	42.26mg
Potassium	23.35mg
Sodium	20.15mg
Zinc	0.87mg
Phosphorus	29.36mg

Source: Olude *et al.* (2020); Akosua et al. (2020).

2.4.1 Bioactive compounds in soursop leaves

1. Acetogenins

Acetogenin specifically Annonacin,is a bioactive compound present in soursop leaves and has been found to exhibit potent anti-cancer properties (Moghadamtousi *et al*., 2015)

2. Alkaloids

Reticuline, an alkaloid found in soursop leaves, has been linked to anti-inflammatory effects (Chatterjee *et al.,* 2016).

3. Phenolic compounds and Flavonoids

The high content of phenolic compounds, such as flavonoids, in soursop leaf extracts is largely responsible for their potent antioxidant and anti-inflammatory effects, as reported by Jiménez *et al.* (2014) and Coria-Tellez *et al.* (2018).

4. Terpenoids

Limonene, a terpenoid compound found in soursop leaves, plays a role in the antimicrobial properties exhibited by soursop leaf extracts (Chatterjee *et al*., 2016).

2.4.2 Health benefits of soursop leaves

(I) Anti-cancer: Soursop leaf extracts may inhibit the growth of certain cancer cells.

(Moghadamtousi *et al.*, 2015; Chatterjee *et al.,* 2016)

(ii) Anti-inflammatory: These extracts can help reduce inflammation within the body. (Chatterjee *et al.,* 2016; Coria-Tellez *et al.,* 2018)

(iii) Antimicrobial: Soursop leaf extracts may be effective against certain bacteria, fungi, and parasites. (Chatterjee *et al*., 2016)

(iv) Antioxidant: Due to their rich content of bioactive compounds, soursop leaf extracts can protect cells from damage caused by free radicals. (Coria-Tellez *et al.,* 2018)

Fig 2.1: Soursop leaves and its fruit

2.5 Prekese and its nutritional composition

Prekese (*Tetrapleura tetraptera*) also called *Aidan* in *Yoruba* has many economic and medicinal uses. The fruits are used to make seasoning spices, pomades, and soaps due to their pleasant aroma.

In some regions, the fruit is also used as a vitamin supplement and in soups for nursing mothers. An infusion of the whole fruit is used for bathing to relieve fever, constipation, and as an emetic. The plant parts contain sugars, tannins, trace amounts of saponin, and amino acids. Traditionally, the plant is used to manage convulsions, leprosy, inflammation, and rheumatic pains. An infusion of the whole fruit can also be taken as a tonic. Prekese fruit may offer potential health benefits, including antitumor activity, reduced inflammation, and the inhibition of HIV replication (Akintola *et al., 2015*).

The therapeutic properties of the fruit is attributed to the secondary metabolites or phytochemicals present in the fruit which includes flavonoids, tannins, cardiac glycosides, saponins, steroids, terpenoids, reducing sugars, and alkaloids. These secondary metabolites are known for their potent biological activities (Dongmo *et al*., 2022)

Prekese fruit is also a good source of essential nutrients; the high carbohydrate content suggests the fruit's potential to stabilize blood sugar levels and prevent excessive protein breakdown for energy. The ash content in the fruit indicates a rich mineral composition, crucial for various bodily functions such as water balance, bone health, and overall metabolism. The fruit also contains dietary fiber which is an essential nutrient and plays a vital role in promoting digestive health and may contribute to the prevention of conditions like constipation, high blood pressure, diabetes, cardiovascular disease, and cancer. The low moisture content of the fruit likely contributes to its natural resistance to microbial degradation, thus enhancing its shelf-life. The moderate fat content in the fruit enhances its palatability. Fats serve as an energy source, provide insulation, and play a crucial role in various bodily functions (Akintola *et al*., 2015)

Tab 2.4 Nutritional composition of prekese fruit

Proximate	Content (%)
Moisture	5.06–8.22
Ash	2.65–4.02
Protein	5.61–6.69
Carbohydrate	58.48–63.86
Fat	11.19–24.71
Fiber	3.14 - 4.11

Source: Mensah *et al*. 2024; Adu-Gyamfi et al., 2024)

Tab 2.5 Mineral composition of prekese fruit

Mineral	Range (mg/kg dry weight)
Iron (Fe)	29.69 - 65.06
Zinc (Zn)	5.35 - 25.16 mg/kg
Copper (Cu)	4.00 - 12.54
Magnesium (Mg)	392.35 - 2951.28
Manganese (Mn)	16.23 - 178.91
Sodium (Na)	119.48 - 2364.93
Calcium (Ca)	1348.63 - 13839.86
Potassium (K)	8631.09 - 14881.00
Boron (B)	1.14 - 6.23

Source: Akin-Idowu *et al.,* (2011)

2.5.1 Bioactive compounds in Prekese fruit

1. Flavonoids:

Flavonoids are a prominent class of polyphenolic compounds known for their strong antioxidant, anti-inflammatory, and antidiabetic properties. Studies consistently report the presence of flavonoids in Tetrapleura tetraptera fruit (Ogunlakin and Sonibare, 2024; Dike et al., 2024; Abarikwu et al., 2019). Specific flavonoids identified include apigenin, quercetin, rutin, epicatechin, luteolin, and catechin (Abarikwu et al., 2019). These compounds contribute significantly to the fruit's ability to scavenge free radicals and protect against oxidative damage (Dike et al., 2024).

2. Phenolic Acids and other Phenolic Compounds:

Beyond general phenolics, specific phenolic acids are also significant constituents of Prekese. Research indicates the presence of gallic acid, caffeic acid, ellagic acid, and chlorogenic acid (Abarikwu et al., 2019). These compounds, along with other phenolic constituents, contribute to the fruit's antioxidant potential and its ability to inhibit enzymes involved in oxidative stress (Ogunlakin and Sonibare, 2024; Abarikwu et al., 2019). Total phenolic content has been quantified in various extracts, demonstrating its abundance (Dike et al., 2024; Ezekiel et al., 2024).

3. Saponins:

Saponins are a group of plant glycosides that have been linked to various health benefits, including hypolipidemic (cholesterol-lowering), antioxidant, and anti-inflammatory properties (Ogunlakin and Sonibare, 2024; Ezekiel et al., 2024). Several studies have confirmed the presence of saponins in Tetrapleura tetraptera fruit (Ogunlakin and Sonibare, 2024; Dike et al., 2024; Ezekiel et al., 2024).

4. Tannins:

Tannins are astringent, bitter polyphenolic compounds that have shown antitumor, antiviral, and antibacterial activities (Okolie et al., 2023). Their presence in Prekese fruit has been consistently reported (Ogunlakin and Sonibare, 2024; Dike et al., 2024; Ezekiel et al., 2024).

5. Alkaloids:

Alkaloids are a diverse group of nitrogen-containing organic compounds with various pharmacological effects. Studies have detected alkaloids in Tetrapleura tetraptera fruit extracts (Ogunlakin and Sonibare, 2024; Dike et al., 2024; Ezekiel et al., 2024).

6. Terpenoids and Steroids:

Terpenoids and steroids are also present in Prekese fruit (Ogunlakin and Sonibare, 2024; Ezekiel et al., 2024). These compounds can possess a range of biological activities, including antiinflammatory and antimicrobial properties.

7. Glycosides:

Glycosides, which are compounds containing a sugar molecule bound to a non-sugar component, have been identified in Tetrapleura tetraptera (Ogunlakin and Sonibare, 2024; Ezekiel et al., 2024).

8. Fatty Acids:

The fruit is also reported to be rich in unsaturated fatty acids, which are important for cardiovascular health and inflammation reduction (Akinmoladun et al., 2019).

2.5.2 Health benefits of prekese fruit

(i) Antimicrobial properties: Studies have shown that prekese extracts may have antibacterial and antifungal activities (Akin *et al.* (2021) and Korang *et al.* (2019)

(ii) Antioxidant properties: Prekese is rich in antioxidants, which can help protect the body from oxidative stress and damage (Lin *et al*., 2019)

(iii) Anti-inflammatory properties: Some research suggests that prekese may have antiinflammatory properties (Mbaveng *et al.*, 2021)

(iv) Blood sugar control: Animal studies have shown that prekese may help regulate blood sugar levels (Adusei *et al.*, 2021)

Other potential benefits of prekese leaves have also been traditionally used to treat various ailments, including fever, malaria, and respiratory infections (Dongmo *et al.,* 2022)

This targeted research seeks to unlock the full potential of these two powerful plants when combined, offering a promising avenue for accessible and nutritionally beneficial health solutions.

Fig 2.2: Prekese fruits

CHAPTER THREE

3.0 Materials and methodology

Materials

Sour sop leaves, prekese fruits, tea bags, electric oven, electric blender.

3.1.1 Preparation of soursop leaves powder.

Fresh soursop leaves were plucked from university of Ilorin research farm, washed with distilled water, and oven dried at 60°c for 30 minutes, grounded into fine powder for 42 seconds with an electric blender, and then sieved using a 100-mesh sieve (Akintola *et al*., 2015; Murray, 2024).

3.1.2 Preparation of prekese fruit powder.

Dried prekese fruit were gotten from ipata market, ilorin Kwara state, washed with distilled water, oven dried at 60°c for 30 minutes, grounded into fine powder for 42 seconds with an electric blender and sieved using a 100-mesh sieve (Gyeeke *et al*., 2024).

3.1.3 Preparation of soursop- Prekese fruit tisane

Soursop leaf powder and prekese powder were combined respectively at the ratios of 60:40, 70:30, 80:20, 90:10 and 100:0 as the control. The blends were measured in a tea bag, and stored at room temperature prior to analysis. Formulation of the tisane samples are shown below

Table 3: Formulation table of the tisane samples

Samples	Soursop (%)	Prekese (%)
S90P10	90	10
S80P20	80	20
S70P30	70	30
S60P40	60	40
S0P100	0	100
S100P0	100	0

Keys: OO = 100% soursop (control), O=100% Prekese (control), A= 90% soursop and 10% prekese, B= 80% soursop and 20% prekese, C= 70% soursop and 30% prekese, D= 60% Soursop and 40% prekese.

Soursop leaves

↓

Washing

↓

Draining

↓

Drying (60°c for 30mins)

↓

Milling

↓

Sieving and cooling (+_ 24°c)

↓

Soursop leave powder

Figure 3.1: Production of soursop leave powder

Source: Akintola *et al*., (2015); Murray, (2024).

Dried Prekese fruit

Washing

Draining

Drying (60°c for 30mins)

Milling

Sieving and cooling (+_24°c)

Prekese powder

Figure 3.2: Production of prekese powder

Source: Akintola *et al*., (2015); Murray, (2024).

3.2 Analytical procedures

3.2.1 Physicochemical analysis of soursop-prekese fruit tisane

i. Determination of total dissolve solids

The tea infusion was prepared by weighing 2-3 grams of tea bags containing soursop leaves and prekese fruit powders into a clean, dry tea infuser or a heat-resistant glass cup. Hot distilled water was poured over the tea bag and allowed to steep for 3-5 minutes. The tea infusion was strained into a clean container using a filter paper. The tea infusion was poured into a pre-weighed, heat-resistant glass dish or crucible. The water was evaporated from the tea infusion in a drying oven set at 105°C ± 2°C until a constant weight was achieved. The dish was removed from the oven and allowed to cool to room temperature in a desiccator. The dish and the dried tea solids were weighed using an analytical balance (AOAC 2012). The total dissolved solids (TDS) content was calculated using the equation:

$$\text{TDS (mg/L)} = \frac{\text{Weight of dried tea solids}}{\text{Volume of tea infusion}} \times 1000 \quad \text{........ Equation 3.1}$$

ii. pH determination

For pH determination, the tisane was prepared by steeping the tea bag in hot water (100ml at 80°c) for 30 minutes, it was allowed to cool slightly before measuring the pH. The pH meter was calibrated according to the manufacturer's instructions using standard buffer solutions. The pH meter was turned on and allowed to stabilize. The pH electrode was rinsed with distilled water and gently blot to dry with a clean tissue. The electrode was emersed in the tisane and allowed to read.

The result was displayed and recorded (Ukachi *et al*., 2019).

iii. Total titratable acidity

Titratable acidity, expressed as percent lactic acid, was determined using the AACC (2000) method. This involved titrating a 10 ml sample with 0.1 N NaOH until a light pink color appeared, signaling the endpoint. Phenolphthalein (1%) was used as an indicator (2-3 drops). Each sample underwent triplicate determination.

The titratable acidity was calculated using the following equation: Titratable Acidity

(%) = $\frac{V \times N \times 9.08}{W \times 10}$ …….. equation 3.2

Where:

V = titre value

N = normality of the titrant (NaOH)

W = sample weight

9.08 = equivalent weight of lactic acid (the predominant acid)

3.2.2 Determination of Phytochemicals presnt in soursop-prekese tisane.

The distilled tisane extract was used for qualitative screening of phytochemicals, following standard biochemical procedures. The crude extract was diluted with distilled water to a concentration of 6 mg/ml. The quantitative phytochemical analysis of the soursop leaf and prekese fruit tea extract was conducted to determine the presence of flavonoids, phenolic acid, and tannin (Njinga *et al.,* 2024).

i. Determination of total phenolic content (TPC) of soursop-prekese tisane.

The TPC was determined using the Folin-Ciocalteu reagent method. The distilled tisane extract (0.75 mL) was combined with 0.75 mL of 10% Folin-Ciocalteu reagent and 0.6 mL of 2% sodium bicarbonate solution. Gallic acid standards (0.1-0.7 mg/mL) were prepared similarly. After 30 minutes of incubation at room temperature, absorbance was measured at 765 nm. A standard curve was generated from gallic acid absorbance versus concentration, and TPC was expressed as milligrams of gallic acid equivalent per gram of extracted compound (mg GAE/g extract). All measurements were performed in triplicate.

ii. Determination of total flavonoid content (TFC) of soursop-prekese tisane.

The TFC was measured using a colorimetric aluminum chloride method. Each 50 µL extract was mixed with 1.45 mL of distilled water and 1.5 mL of 10% aluminum chloride. After standing for 30 mins at room temperature, the absorbance was read at 420 nm. Quercetin standards (0.06-0.18 mg/mL) were prepared identically to the extracts. TFC was calculated as milligrams of quercetin equivalent per milliliter (mg QE/mL), with extract samples evaluated at a final concentration of 0.1 mg/mL. All tests were conducted in triplicate.

iii. Determination of total tannin content (TTC) of soursop-prekese tisane.

The TTC was determined using a modified vanillin-HCl method 55 µL was mixed with 245µL of distilled water 3 mL of reagent (4% vanillin and 8% concentrated HCl in a 1:1 methanol ratio). The mixture was incubated at room temperature for 20 minutes, and absorbance was read at 500 nm. Results were expressed as milligrams of catechin equivalent per gram of extract (mg CE/g extract).

3.2.3 Antioxidant scavenging activities of soursop-prekese tisane

The antioxidant scavenging assays were caried out on five different assays; DPPH assay, ABTS assay, FRAP assay, Phosphomolybdate assay, and metal chelating agent assay using standard procedures by Njinga *et al.,* (2024).

i. DPPH Radical-Scavenging Activity of soursop-prekese tisane.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was assessed using 40 µL of each extract concentration mixed with 1.16 mL of distilled water, 1.2 mL of DPPH solution was added. The mixture was incubated in the dark at room temperature for 30 minutes. Vitamin C served as the standard control, and a mixture without extract was used as a blank. Absorbance was measured at 515 nm and converted to percentage antioxidant activity using the equation:

: $$\%\text{DPPH antioxidant} = \frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \times 100$$ …….. Equation 3.3

ii. Ferric Reducing Antioxidant Property (FRAP) assay of soursop-prekese tisane.

The FRAP assay involved preparing a FRAP reagent by mixing acetate buffer (pH 3.6), 10 µM TPTZ in 40 µM HCl, and 20 µM $FeCl_3$ in a 10:1:1 (v/v/v) ratio. 266µl of samples were mixed with 2 ml of distilled water (from a 6 mg/mL stock), 2 mML of the FRAP reagent were added, incubated at 37°C for 30 minutes, and absorbance was read at 573 nm. A standard curve was plotted using $FeSO_4·7H_2O$ (20–120 mg/mL), and results were expressed as milligrams of Fe (II) equivalent per gram of extract.

iii. Metal Chelating Activity (MCA) of soursop-prekese tisane.

The MCA was determined by adding 0.5 mL of 2M FeCl2 to 0.4mL of sample (6 mg/mL stock) or standard EDTA (20–120 µg/mL). The reaction was initiated by adding 0.1 mL of 5 mM ferrozine solution, followed by 0.9ml of distilled water to adjust the volume. The mixture was vigorously shaken and left at room temperature for 10 minutes. Absorbance was then measured at

562 nm. Na2-EDTA served as a positive control, and results were expressed as milligrams of EDTA equivalent per gram of extract (mg EE/g extract).

iv. ABTS Radical Scavenging Activity of soursop-prekese tisane.

The 2,2′-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization assay was performed. ABTS radical cations were generated by reacting ABTS solution with 2.45 mM potassium persulfate (1:1 ratio) and incubating in the dark for 12–16 hours. The ABTS solution was then diluted in methanol (1:89 ratio) to an absorbance of 0.7 (±0.02) at 734 nm. In a test tube, 2.5 mL of ABTS solution was mixed with 44 µmL of extract distilled with 82 µml (6 mg/mL stock) or standard, incubated at 30°C, and absorbance was measured at 730 nm exactly after 30 minutes, using distilled water as a blank. All solutions were prepared fresh daily. Results were expressed as milligrams of Trolox equivalent per gram of extract (mg TE/g extract).

V. Phosphomolybdate Assay (PMA) of soursop-prekese tisane.

The PMA, a total antioxidant capacity assay, involved mixing 55µL of sample (from a 6 mg/mL stock) or standard ascorbic acid (0.2–2.0 mg/mL) with phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) with the addition of 245µL of distilled water. The mixture was incubated in a water bath at 90°C for 90 minutes, cooled to room temperature, and absorbance was measured at 765 nm. Antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE/g extract).

3.2.4 Sensory Analysis of Soursop-prekese tisane

The sensory evaluation of tea samples was conducted to establish a preference rating for flavor, aroma, taste, appearance, and overall acceptability. A 9-point hedonic scale was used to measure consumer acceptability, suitable for both trained and untrained panelists.

Fifty untrained panelists from the Department of Food science University of Ilorin, evaluated the tea samples. 2g of blended tea was brewed using 100ml of freshly boiled water for 1 minutes. Samples were served in 100ml cups, coded with random alphabets, under warm conditions. The panel room was free from food/chemical odors, unnecessary sound, and daylight.

Each panelist received an evaluation card to record their opinions on sensory observations. They were provided with potable water for rinsing between samples. Verbal communication among panelists was prohibited. The following parameters were evaluated: color, aroma, taste, after taste mouth feel, and overall acceptability.

A 9-point hedonic rating test was used, with the following grading system:

9 - Like extremely

8 - Like very much

7 - Like

6 - Like slightly

5 - Neither like nor dislike

4 - Dislike slightly

3 - Dislike moderately

2 - Dislike

1 - Dislike extremely

3.5 Data Analysis

All results are presented as the mean ± standard deviation of at least three independent replicates. Statistical analysis was conducted using Analysis of Variance (ANOVA), and significant differences between sample means were determined using Duncan's multiple range test with SPSS software version 16. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physicochemical properties of soursop-Prekese tisane.

The pH, total titratable acidity and total dissolved solids of the soursop Prekese tea was carried out. pH and titratable acidity were carried out to determine the acid concentration of the tisane.

4.1.1 pH (hydrogen ion concentration) of soursop-Prekese fruit

pH reflects the concentration of free hydrogen ions (active acidity). The pH values for all samples in this study are in the acidic range (below 7) which is expected for plant-based materials containing organic acids as observed by Giampeieri *et al.,* (2022). Samples S90P10 and S80P20 have the same pH (5.67), suggesting that the difference in the soursop leaf to Prekese fruit ratio

(90:10 and 80:20) does not significantly impact the overall hydrogen ion concentration. Sample S70P30 shows a slightly higher pH (5.72) compared to samples S90P10 and S80P20. Sample

S60P40 exhibits the highest pH (5.88) among the mixed samples, moving closer to the pH of 100% Prekese fruit. Sample S0P100 (100% Prekese fruit) has a pH of 5.82, which is higher than most of the mixtures and 100% soursop leaf sample while sample S100P0 (100% soursop leaf) has a pH of 5.72, the same as sample S70P30.

There is an increasing relationship between the pH values gotten in this study and the proportion of Prekese fruit added, the values show that as the proportion of Prekese fruit increases in the mixture, the pH tends to increase. This suggests that Prekese fruit had a different profile of acids that results in a slightly lower hydrogen ion concentration at higher proportions. This is similar to the findings reported by Gyeekye *et al*., (2024) on dawadawa and Prekese fruit bevearage in which the increase in Prekese fruit leads to an increase in the pH values. Samples S90P10 and S80P20 had the same pH even with its different sample proportions, this indicates the presence of a buffering systems in the mixture which resists changes in pH upon the addition of Prekese as observed in previous studies by price *et al.,* (2020).

4.1.2 Total titratable acidity (TTA)

Total titratable acidity measures the total concentration of titratable acidic compounds in the samples. It gives a broader picture of the potential acid content, including both strong and weak acids.

Samples S90P10, S80P20, S60P40 have the same TTA content (1.87 g/l), indicating a similar overall concentration of acidic compounds despite the varying ratios. This suggests that the contribution of titratable acids from soursop leaves and Prekese fruit would balance out at these proportions. Sample S70P30 has higher TTA value (2.00 g/l) compared to samples S90P10,

S80P20, S60P40. This indicates a greater overall concentration of titratable acidic in this specific 70:30 mixture. Sample S0P100 (100% Prekese fruit) has the highest TTA (2.67 g/l) implying it contains the highest concentration of titratable acidic compounds among all the samples. Sample S100P0 (100% soursop leaf) have a TTA of 2.27, which is also relatively high, suggesting a significant contribution of titratable acids from soursop leaves. Sample S0P100 (100% Prekese) has the highest TTA value due to its high pH content, these observations show that Prekese fruit has the tendency to increase the pH with increasing addition of Prekese, and also the TTA values. This is different in sample S60P40 in which TTA values does not increase with increase in prekese fruit this would suggest that certain compounds in soursop and prekese interact in that proportion in a way that leads to the precipitation or binding of acidic compounds as the ratio changes from 70:30 to 60:40 resulting in fewer free acidic compounds available for titration.

4.1.3 Total dissolved solids (TDS).

Total dissolved solids show the amount of organic and inorganic substances that are dissolved in water. Sample S80P20 has a total dissolve solid of 21.60 which is the highest TDS value which suggests that the combination of a high percentage of soursop leaves with some Prekese fruit results in the most dissolved solids when the sample is prepared. The Prekese fruit, even at 20%, seems to contribute significantly to the overall dissolved solids. Sample S0P100 has the secondhighest TDS of 16.00, indicating that Prekese fruit when used alone, releases a substantial number of dissolved substances. This suggests Prekese fruit is rich in soluble compounds. Sample S90P10 has a slightly lower TDS than Sample S0P100 (15.60), this suggests that while soursop leaves contribute to dissolved solids, the addition of even a small amount of Prekese fruit (10%) still increases the overall TDS noticeably. In sample S70P30 it was observed that the TDS continues to increase as the proportion of Prekese fruit goes up, this further support that Prekese fruit contributes significantly to the dissolved solids. Sample S100P0 has a relatively low TDS, indicating that soursop leaves when used alone, release fewer dissolved substances compared to Prekese fruit. Sample S60P40 surprisingly has the lowest TDS, even with the highest proportion of Prekese fruit among the mixed samples. This suggest a non-linear relationship where a certain ratio led to less overall dissolution due to an interaction between the compounds from the two plants at this specific ratio of 60:40. A similar decreasing ratio was observed by Olaoye et al., (2016) for kunun zaki bevearage.

Table 4.1: Physicochemical properties of soursop-prekese tisane

Samples	pH	TTA (g/l)	TDS(µmg/l)
S90P10	5.67^{a}±0.01	1.87^{a}±0.23	15.60^{d}±0.00
S80P20	5.67^{a}±0.01	1.87^{a}±0.23	21.60^{f}±0.00
S70P30	5.72^{b}±0.01	2.00^{b}±0.00	14.80^{c}±0.00
S60P40	5.88^{d}±0.01	1.87^{a}±0.23	12.00^{a}±0.00
S0P100	5.82^{c}±0.01	2.67^{d}±0.23	16.00^{e}±0.00
S100P0	5.72^{b}±0.01	2.27^{c}±0.46	13.00^{b}±0.00

Values are expressed as means of duplicate determination (n = 3) ± standard deviation. Means under the same column having different superscripts are significantly different ($p \leq 0.05$).

S80P10= 90% soursop leaf,10% Prekese fruit powder. S80P20= 80% soursop leaf,20% Prekese fruit powder. S70P30= 70% soursop leaf,30% Prekese fruit powder. S60P40= 60% soursop leaf,40% Prekese fruit powder. S0P100= 100% Prekese fruit powder. S100P0= 100% soursop leaf powder

4.2 Phytochemical analysis of soursop-prekese tisane.

The total flavonoid, phenolic content and tannin content of the tisane was measured.

4.2.1 Total flavonoid content

The total flavonoid content was estimated as mg/g catechin equivalent, which ranges from 0.10 mg QE/g to 0.21 mg QE/g (10- 21% when expressed as a percentage) with sample S70P30 having the highest value even higher than both individual sample (S0P100 and S100P0). This indicates a potential synergistic effect between soursop leaf powder and Prekese fruit powder, where the combination enhances the phytochemical content beyond what would be expected from each sample alone. Similar synergistic effects have been observed in herbal teas made from soursop leaf powder combined with other tisane plant parts, as reported by (Nhi *et al.,* 2020).

Flavonoids are a major type of polyphenol present in herbal teas; it exhibits strong antioxidant and antimicrobial properties. They have been shown to possess anti-cancer and anti-mutagenic effects, likely due to their ability to neutralize free radicals and reduce inflammation. (Ukachi *et al.,* 2019). The flavonoid content of the soursop- Prekese fruit tisane infusion obtained in this study (10-21%) is higher than those (3.61-7.34%) reported for soursop- moringa tisane infusion (Ukachi *et al.,* 2019). The higher flavonoid content observed in this study compared to the previous study mentioned is attributed to the addition of prekese fruit, which potentially contributes to the increased flavonoid content.

4.2.2 Total phenolic content

The total phenolic content was expressed as mg/g Gallic acid equivalent, which ranges from 0.08mg GAE/g to 0.23mg GAE/g with sample S70P30 also having the highest value which is also higher than both individual sample (S0P100 and S100P0). This trend in the phenolic content is also observed in the total flavonoid content as sample S70P30 has both the highest phenolic and flavonoid content. This underscores the established fact that there was a potential synergistic effect between soursop leaf powder and Prekese fruit powder, where the combination enhances the phytochemical content beyond what would be expected from each sample alone. Tab 4.1 shows the total phenolic content of all samples observed in this study.

4.2.3 Tannin content

The tannin content was estimated as mg/g catechin equivalent. In this study, it was observed that the tannin content was constant for all sample proportions. This suggests that the tannin content is not affected by the proportions of soursop leaf and Prekese fruit powders, the stability of tannin content across samples is attributed to its saturation due to tannin being a water-soluble polyphenol as reported by (Akintola *et al.,* 2015). Tannin can negatively impact nutritional value by reducing nutrient absorption particularly preventing iron absorption leading to anemia and decrease in growth rate and as such food high in tannin content is regarded as having low nutritional value. (Akintola *et al.,* 2015). The constant tannin content from this study is 0.07 mg CE/g which is very low (7% when expressed as percentage) this is lower than soursop- moringa tisane infusion (8.95% - 9.84%) reported by Ukachi *et al.,* 2019.

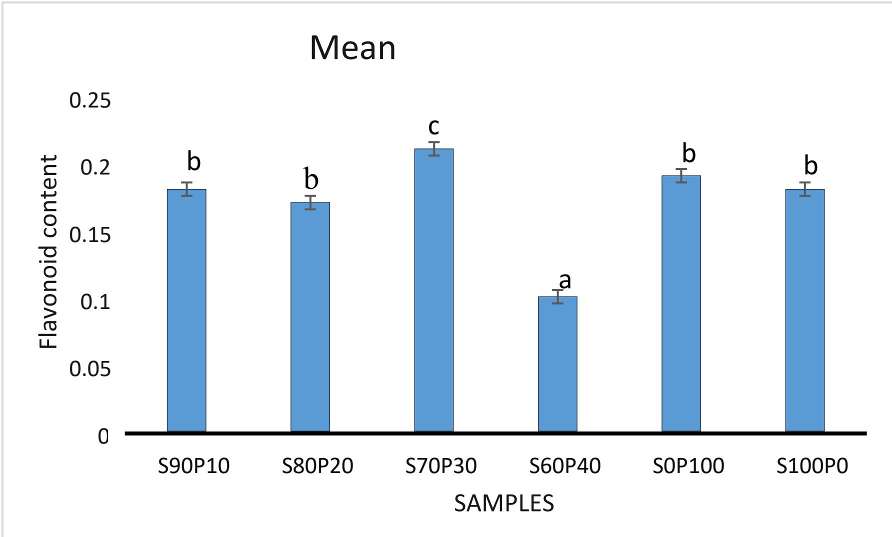

Fig 4.1: Flavonoid content

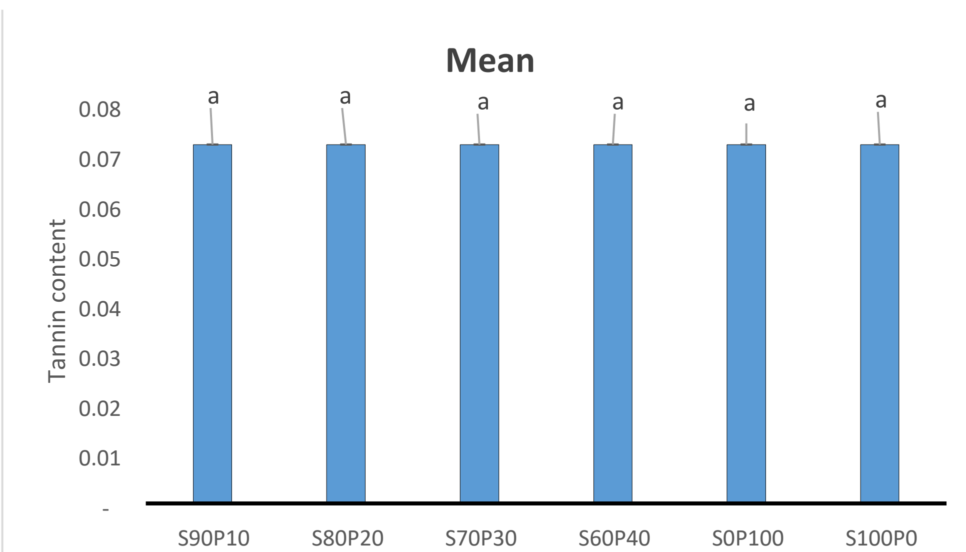

Fig 4.2: Tannin content

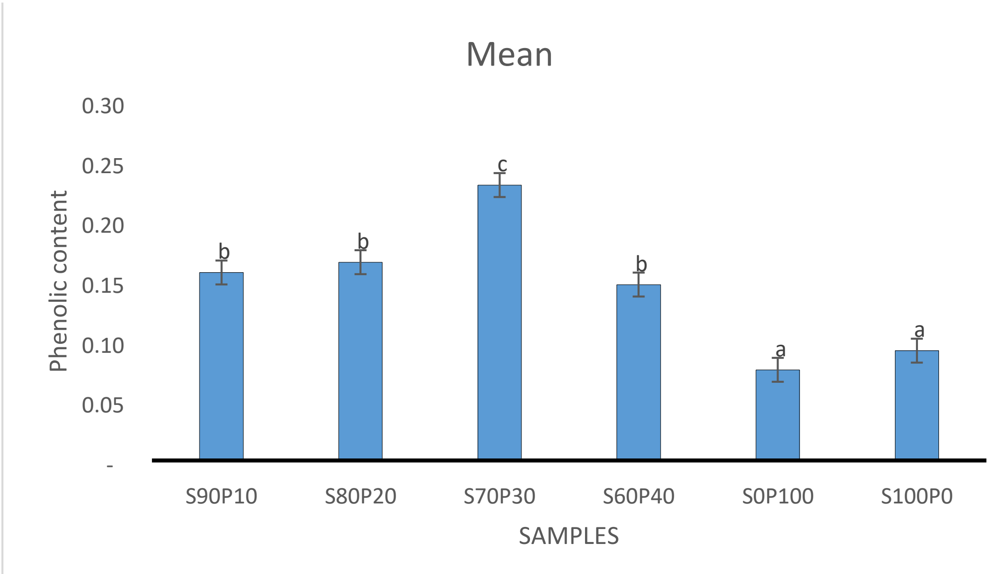

Fig 4.3: Phenolic content

4.3 Antioxidant activities of soursop leaf powder and Prekese fruit tisane.

The antioxidant scavenging activities of the SPT samples and control samples were analyzed using five different assays; DPPH assay, ABTS assay, FRAP assay, Phosphomolybdate assay, and metal chelating agent assay using standard procedures described by Arise *et al.*, (2021). These assays are known to measure the antioxidant power of fluids (Rumpf *et al.,* 2023).

4.3.1 DPPH Scavenging activity

DPPH assay measures the ability of antioxidants present in the samples to scavenge free radicals using the reaction between DPPH radical and the samples, these results were expressed in percentage. The antioxidant activity of the samples in this study ranges from 58.49% to 78.77% with sample S70P30 having the highest DPPH scavenging activity of 78.77%. This high antioxidant activity of sample S70P30 is attributed to the high flavonoid and high phenolic content found in this study. This result is higher than the control samples S0P100 (100% Prekese fruit tisane) and S100P0 (100% soursop leaf tisane) having dpph values of 58.49% and 66.14% respectively, showing a synergistic effect caused by the combination of both soursop leaf and Prekese fruit. Sample S70P30 dpph value is also higher than the drumstick leaf and guava leaf tisane (70.49%) reported by Irawan *et al.,* 2018.

4.3.2 FRAP assay.

FRAP assay measures the ability of antioxidants to reduce ferric ions (Fe3+) to ferrous ions (Fe2+), this reduction reaction was evidently seen in the color change which was monitored using a beam spectrophotometer. The antioxidant activity using FRAP assay ranges from 1.06-1.15 mg Fe (II) equivalent/g with sample S70P30 (70:30) and sample S100P0 (100% soursop) having the highest reducing power of 0.15±0.01 and 0.15±0.00 respectively while sample S0P100 (100% Prekese) has an antioxidant activity of 1.12±0.01 mg Fe (II). The difference in the values between sample S100P0 and S0P100 suggests that the addition of Prekese increased the antioxidant activity of sample S70P30 whose high value is also seen in its scavenging power using DPPH assay mentioned earlier. The high reducing power of sample S70P30 using FRAP assay is evidently attributed to its high flavonoid and high total phenolic content as observed in this study.

4.3.3 ABTS assay.

ABTS assay measures the ability of antioxidants to scavenge ABTS radicals. The antioxidant activity using ABTS assay ranges from 71.71-90.98mg Trolox equivalent/g with sample S100P0 exhibiting the highest ABTS activity and sample B having the lowest. The high antioxidant capacity of sample S100P0 measured by ABTS in this study correlate with the FRAP and PMA values of sample S100P0 as both assays likewise depicts the sample as having the highest antioxidant activity among all samples. This implies that sample S100P0 has high tendency of scavenging ABTS radicals and reducing ferric ions to ferrous ions. These values are higher than the water extract of *oldenlandia corymbosa* (diamond flower extract) (63.67±0.6 mg trolox equivalent/g) reported by Njinga *et al.,* 2024.

4.3.4 Phosphomolybdate assay (TAC assay)

PMA assay also measures the total antioxidant capacity of the samples to reduce molybdenum (VI) to molybdenum (V). From this assay, it was observed that sample S90P10, S80P20, S60P40 showed no ability to reduce molybdenum (VI) ions thus showing no reducing power which is in contrast with other reducing assays such as FRAP and ABTS where significant amount of reducing capacities for these samples were recorded. This implies that PMA assay cannot effectively detect the antioxidant capacities of these samples due to PMA specific mechanism of action and the sample having varying ingredient proportions. However, sample S70P30, S0P100 and S100P0 showed PMA reducing capacity of 0.04±0.02, 0.05±0.02 and 0.05±0.08 ascorbic acid equivalent/g respectively.

4.3.5 Metal chelating agent (MCA assay).

MCA assay measures the ability of the samples to bind with or scavenge metal ions by forming stable complexes thereby preventing the harmful effect on humans. It was observed that the MCA values range from 16.13-20.68 mg EDTA equivalent/g with high values of 20.42±3.84 mg EDTA equivalent/g and 20.68±4.19 mg EDTA equivalent/g recorded for sample S70P30 and S60P40 respectively. This high EDTA is associated to the flavonoid and phenolic content of both sample combination. These values are higher than the water extract of *oldenlandia corymbosa* (diamond flower extract) (0.346±0.04 mg EDTA equivalent/g) reported by Njinga *et al.,* 2024.

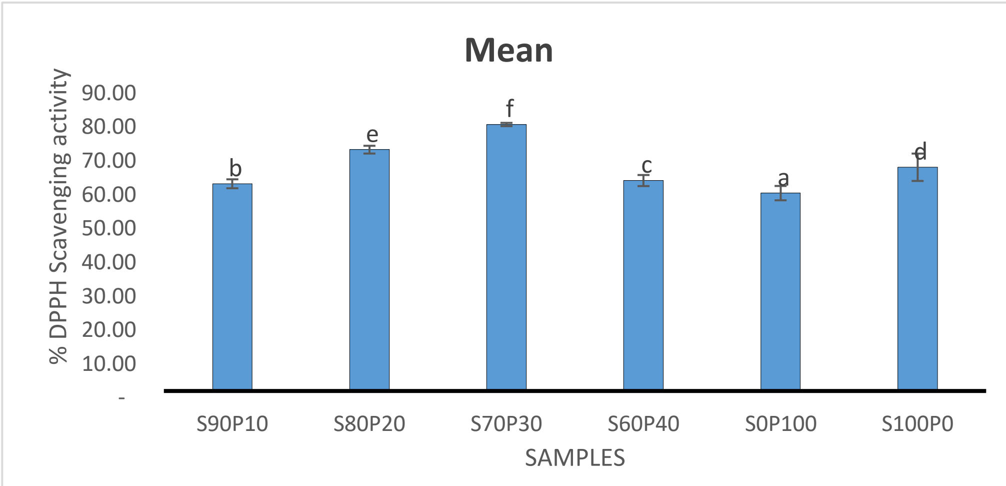

Fig 4.4: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

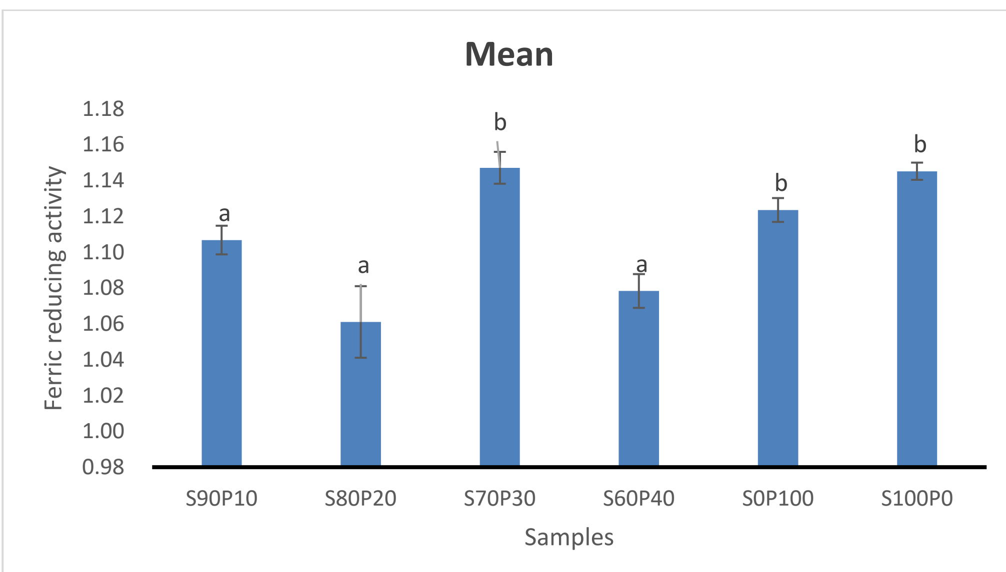

Fig 4.5: Ferric reducing antioxidant power assay

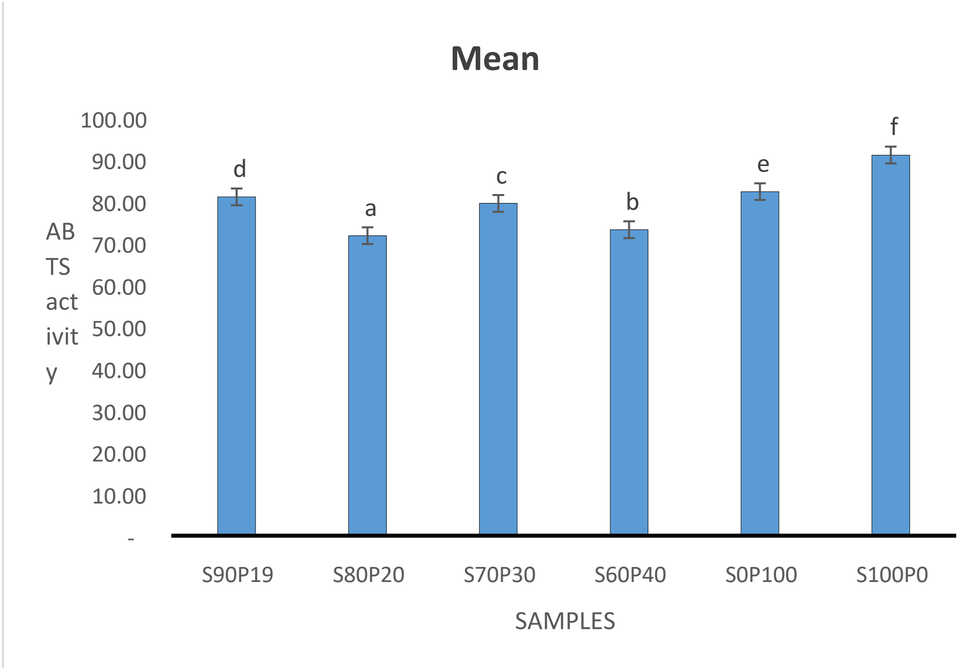

Fig 4.6: 2,20-azino-bis-(3-ethyl) benzothiazoline-6-sulfonic acid radical scavenging activity

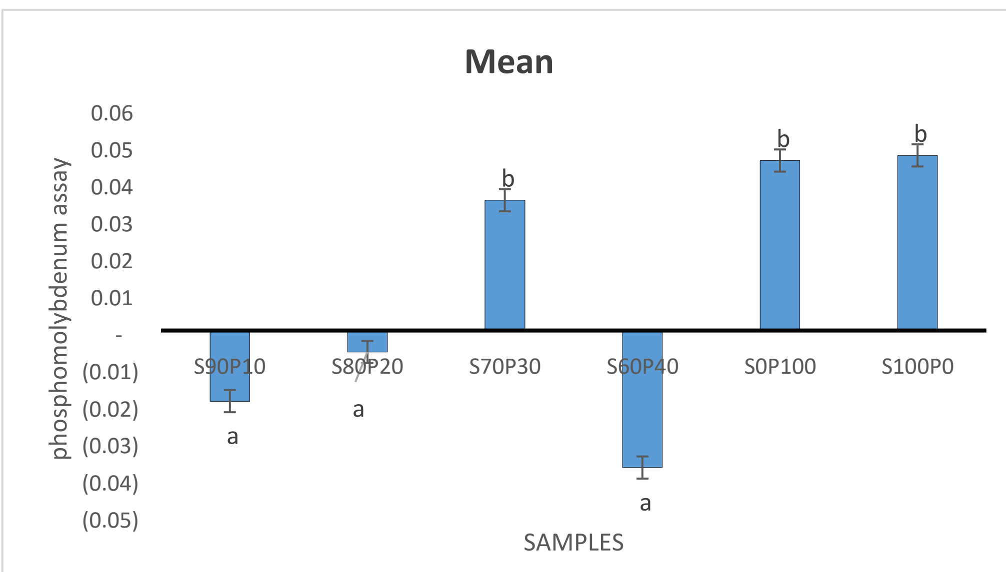

Fig 4.7: Phosphomolybdenum assay

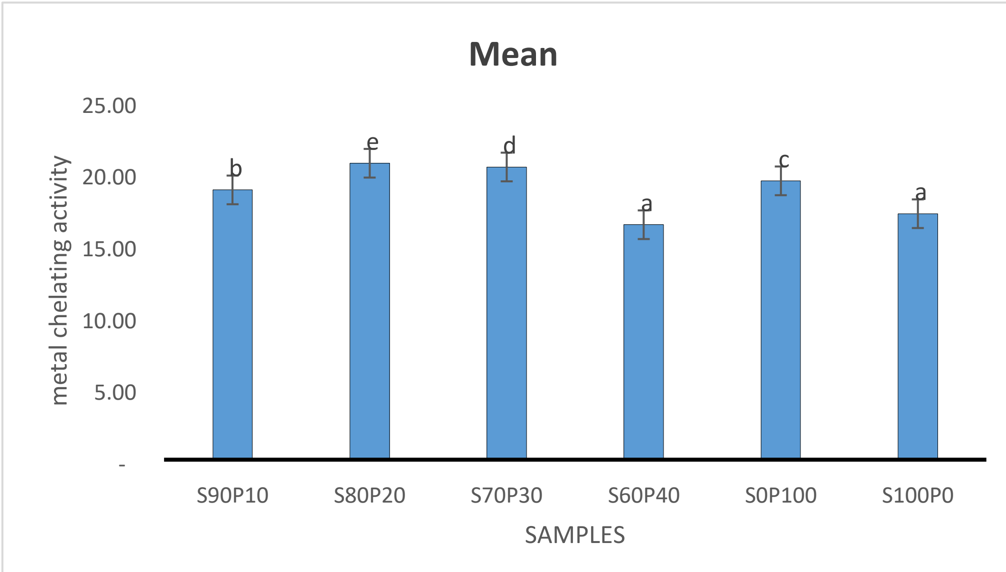

Fig 4.8: Metal chelating activity

4.4. Sensory evaluation of soursop-Prekese tisane

The sensory evaluation results, as presented in Table 4.4, offer valuable insights into the consumer acceptability of soursop-Prekese fruit tisane blends with varying ratios, as well as the individual tisanes made from 100% Prekese fruit and 100% soursop leaf.

The table presents the mean scores for five sensory attributes: color, flavor, taste, aftertaste, and overall acceptability, rated by 50 untrained panel of tasters. Higher scores generally indicate greater preference for that attribute. The letters (a, b, c, d, e, f) as superscripts indicate statistically significant differences ($p < 0.05$) between the samples for each attribute.

Among all the tested formulations, S80P20 (80% soursop leaves, 20% prekeses fruit) consistently emerged as the most preferred. It scored highest in overall acceptability (7.78 ± 1.18), indicating that the combination of 80% soursop and 20% prekese was the most appealing to the panelists. This sample also received the top score for its colour (7.50 ± 1.15), suggesting it had the most visually attractive appearance, and its flavour (7.48 ± 1.97), indicating a highly desirable aromatic profile. Furthermore, S80P20 was rated highest for its taste (7.50 ± 1.43), signifying a wellbalanced and pleasant oral sensation, and performed very well in aftertaste (7.18 ± 1.64), leaving a favourable lingering impression. These consistently high scores across all sensory attributes make S80P20 the standout formulation.

Other formulations showed varying degrees of acceptance. S60P40 (60% soursp leaves, 40% prekese fruit) demonstrated strong performance in overall acceptability (7.48 ± 1.69) and particularly excelled in aftertaste (7.22 ± 1.56), leaving the most pleasant lingering sensation. However, it received the lowest score for flavour (6.84 ± 1.75), suggesting that while its finish was good, the initial flavour was less preferred. D90P10 (90% soursp leaves, 10% prekese fruit) also showed good overall acceptability (7.34 ± 1.12) and had a relatively high flavour score (7.20 ± 1.41), but its taste (6.90 ± 1.15) and aftertaste (6.86 ± 1.67) were not as highly rated as S80P20 or S60P40.

Conversely, formulations with extreme ratios tended to be less acceptable. S100P0, representing 100% soursop with no prekese, received the lowest overall acceptability score (6.94 ± 1.85), indicating it was the least preferred by the panelists. It also scored low in colour (6.52 ± 1.45) and aftertaste (6.62 ± 1.78). Similarly, S0P100 which was 100% prekese, also had low scores in overall acceptability (7.12 ± 1.57), colour (6.46 ± 1.34), and aftertaste (6.60 ± 1.67). These results suggest that a blend of both soursop and prekese is crucial for an optimal sensory experience.

Finally, S70P30 (70% soursp leaves, 30% prekese fruit) presented moderate scores across all attributes, including overall acceptability (7.26 ± 1.70), placing it in an intermediate position among the formulations. Its flavour (7.10 ± 1.27) and taste (7.06 ± 1.63) were reasonably wellreceived, but its aftertaste (6.68 ± 1.90) was among the lower-scoring ones.

In summary, the sensory evaluation clearly indicates that a balanced blend, specifically the S80P20 ratio, yields a soursop-prekese tisane with superior sensory qualities, making it the most promising formulation from this project work. The presence of both soursop and prekese appears to be necessary for a well-rounded and acceptable product, as evidenced by the lower ratings of the single-ingredient samples. The highest preference for sample S80P20 is based on their familiarity with beverages from Prekese as reported by Gyeekye, (2024).

From this study, it is evident that the inclusion of Prekese fruit at a 20% ratio significantly enhanced the sensory properties. Thus, indicating that Prekese fruit contributes positively to the sensory profile, making the blend more appealing. The enhancing sensory attributes of prekese fruit was also observed in the research work on beverage made from locust fruit and prekese fruit by Gyeekye, (2024).

Table 4. Sensory properties of the soursop-prekese tisane

Samples	colour	flavour	taste	After taste	Overall acceptability
S90P10	$6.88^{e}\pm1.24$	$7.20^{e}\pm1.41$	$6.90^{a}\pm1.15$	$6.86^{c}\pm1.67$	$7.34^{d}\pm1.12$
S80P20	$7.50^{d}\pm1.15$	$7.48^{f}\pm1.97$	$7.50^{d}\pm1.43$	$7.18^{d}\pm1.64$	$7.78^{e}\pm1.18$
S70P30	$6.70^{d}\pm1.54$	$7.10^{c}\pm1.27$	$7.06^{c}\pm1.63$	$6.68^{b}\pm1.90$	$7.26^{c}\pm1.70$
S60P40	$6.64^{c}\pm1.56$	$6.84^{a}\pm1.75$	$7.06^{c}\pm1.74$	$7.22^{d}\pm1.56$	$7.48^{e}\pm1.69$
S0P100	$6.46^{a}\pm1.34$	$7.22^{d}\pm1.17$	$6.90^{a}\pm1.34$	$6.60^{a}\pm1.67$	$7.12^{b}\pm1.57$
S100P0	$6.52^{b}\pm1.45$	$7.02^{b}\pm1.71$	$6.98^{b}\pm1.59$	$6.62^{a}\pm1.78$	$6.94^{a}\pm1.85$

Values are expressed as means of duplicate determination (n=3) standard deviation. Means under the same column having different superscripts are significantly different ($p \leq 0.05$).

S90P10= 90% soursop leaf,10% Prekese fruit powder. S80P20= 80% soursop leaf,20% Prekese fruit powder. S70P30= 70% soursop leaf,30% Prekese fruit powder. S60P40= 60% soursop leaf,40% Prekese fruit powder. S0P100= 100% Prekese fruit powder. S100P0= 100% soursop leaf powder.

CHAPTER 5

5.2 Conclusion

Based on the findings of this study, blending soursop leaf powder and Prekese fruit powder in varying ratios significantly influences the phytochemical content and antioxidant activities of the resulting tisanes. The 70:30 soursop leaf to Prekese fruit blend (S70P30) exhibited the highest total flavonoid and phenolic content, along with notable DPPH scavenging, FRAP and metal chelating activities, suggesting a potential synergistic effect between the two plant materials in enhancing these bioactive compounds. The 80:20 blend (S80P20) offers a balance of desirable sensory attributes and good overall properties, suggesting it as a promising formulation for a palatable and potentially health-beneficial tisane.

5.3. Recommendations.

Based on the results of the study, the following were recommended:

1. The 70:30 blends of soursop and Prekese fruit tisane can be commercialized as a tea blend as growing trend is seen in functional foods. For this purpose, further research should also be carried out to improve the sensory properties of this blend ratio.

2. Research on *in-vivo* studies (animal studies) should be carried out to ascertain the effect of the tisane blend on the different organs and systems and how the tisane is metabolized and excreted by the body.

3. Further research should also be conducted to explore the specific synergistic interactions between the compounds and investigate the stability and long-term effects of these tisane blends.

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APPENDICES

SENSORY EVALUATION FORM

NAME: SEX: DATE:

INSTRUCTIONS: You are presented with six (6) samples of herbal tea. Please rate these samples for colour, flavour, taste and overall acceptability using the scale given below. Please, ensure you rinse your mouth with water after taking each sample.

SCALE: 1- Dislike extremely 2- Dislike very much 3- Dislike moderately 4- Dislike slightly 5- Neither like nor dislike 6- Like slightly 7- Like moderately 8- Like very much 9- Like extremely

Samples	Colour	Flavour	Taste	After Taste	Overall acceptability
S90P10					
S80P20					
S70P30					
S60P40					
S0P100					
S100P0					

FIG A1: Prekese fruit powder

FIG A2: Soursop leaves powder

FIG A3: Tea bags containing soursop-Prekese tisane powders.