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Impact of *Vateria copallifera* Extract on Quality, Antioxidant Properties and Antimicrobial Activity in Palmyrah (*Borassus flabellifer* L.) Fruit Pulp During Preservation

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ABSTRACT

Purpose: *Palmyrah fruit is nutritious yet seasonal; hence, it needs to be preserved naturally for year-round availability in its authentic characteristics. This study explores the effect of Vateria copallifera (Vc) bark extract, which showed greater antimicrobial activity than Acorus calamus (Ac) root extract and Myristica fragrans (Mf) seed extract, on the quality, antioxidant potential, and antimicrobial activity of Palmyrah Fruit Pulp (PFP) during preservation.*

Research Method: *Treatments of PFP preservation were designed by varying the pH (3.5, 4, and 4.5) and concentration of Vc extract (1, 3, and 5 g/L). Treated PFP samples were evaluated for quality characteristics over 182 days. Antioxidant activity, total phenolic content, and heavy metals were determined in treated and untreated PFP.*

Findings: *Total Soluble Solids, acidity, and microbial count of the PFP (1 g/L of Vc extract, pH 3.5) were within the acceptable limit. The antioxidant activity of treated PFP (IC_{50} = 40.59 ± 2.00 mg/mL) was higher compared to untreated PFP (IC_{50} = 69.72 ± 2.00 mg/mL). Total phenolic content was higher in treated PFP (0.60 ± 0.01 mg GAE/g) than untreated PFP (0.29 ± 0.01 mg GAE/g). These findings suggest that Vc extract is a suitable natural preservative for PFP, enhancing its shelf life and nutritional value, whilst preserving its organoleptic characteristics.*

Research Limitation: *Collection and storage of Palmyrah fruit are feasible only during the season. It is prone to insect attack and mechanical damage, and gets spoiled quickly.*

Originality: *In this study, the inherent characteristics of PFP are preserved by natural means without chemical-induced discoloration and loss of aroma, eliminating food safety concerns.*

Keywords: *Natural Preservation, Palmyrah Fruit Pulp, Vateria copallifera Extract*

INTRODUCTION

Palmyrah fruit (*Borassus flabellifer* L.) is a highly valued fruit in South Asia, particularly in Sri Lanka, India, and Bangladesh. The fruit can be consumed fresh or processed into a variety of products, such as ready-to-serve beverages, cordials, jams, sauces, and sweets. It is a highly nutritious food source, rich in vitamins, minerals, and dietary fiber (Vengaiah *et al.*, 2021). Palmyrah fruit pulp (PFP) is a seasonal product that is only available from August to October. However, its shelf life is short and it is susceptible to spoilage due to various reasons, including the presence of microbes, enzymatic reactions, improper storage conditions, and contamination during processing. The spoilage of PFP can lead to changes in its physicochemical properties such as color, texture, flavor, and nutritional content, affecting its quality and safety for consumption; hence, the PFP is usually preserved using chemical preservatives such as sodium metabisulfite (SMS) and benzoic acid (Robika *et al.*, 2016).

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Although chemical preservatives extend shelf life and prevent spoilage, they may also pose health risks, such as allergic reactions, and reduce the nutritional value and natural quality of the pulp by interacting with essential nutrients. SMS causes a significant loss in vitamins such as thiamin (Ribeiro *et al.*, 2011), and benzoic acid can react with vitamin C (Walczak-Nowicka and Mariola, 2022). Therefore, there is a need for natural preservation methods that can extend the shelf life of PFP without compromising its quality and nutritional value. In this regard, the use of plant extracts as natural preservatives has gained attention in recent years due to their antimicrobial and antioxidant properties.

In this study, preservation of PFP was focused on using aqueous extracts of *Acorus calamus* root, *Vateria copallifera* bark, and *Myristica fragrans* seed. Selection of these plants is primarily driven by their antimicrobial properties, and they align with the preference for eco-friendly and non-toxic preservation methods as well as their historical use in food preservation. In addition, the easy accessibility of these plants is another factor influencing their selection for the preservation of PFP.

Acorus calamus is known for its preservative properties, and the presence of active compounds such as beta-asarone and eugenol has been found to have antimicrobial and antioxidant properties, making it an effective natural preservative (Bai *et al.*, 2022). *Vateria copallifera* contains a variety of bioactive compounds, such as alkaloids and flavonoids, which are believed to be responsible for its therapeutic effects (Samaradivakara *et al.*, 2017). In recent years, there has been growing interest in exploring the potential of *V. copallifera* as a natural preservative in the food and beverage industries due to its antimicrobial properties (Marikkar and Muneeb M, 2022). *Myristica fragrans* is valued for its antimicrobial and antioxidant properties, making it a popular choice for food preservation to prevent spoilage and extend shelf life (Matulyte *et al.*, 2021).

There are limited studies on the microorganisms that cause spoilage of PFP and the natural preservation of PFP. This study aims at the molecular level identification of the microorganisms causing spoilage of PFP using a DNA sequencing method and focuses on plant extracts with antimicrobial potential that can inhibit the growth of the microorganisms accountable for the spoilage of PFP, to identify a viable, natural, and safe preservation method for this nutritious fruit. Addition of plant extracts may lead to an increase in total phenolic content, which would consequently lead to a slight increase in antioxidant activities. Increased antioxidant activities lead to longer shelf life. Therefore, this research aims to investigate the antioxidant activity of the selected plant extracts together with the antimicrobial effect, to determine the preservation effect of these extracts, including prolonged shelf life and the effect on the quality characteristics of PFP, such as acidity. Here, the quality and antioxidant potential of the treated pulp, fresh pulp, and chemically preserved pulp are determined and compared. The overall objective of this research study is to determine the feasibility of a natural preservation technique that effectively preserves the PFP, whilst maintaining its authentic nutritional and quality characteristics and prolonging its shelf life.

MATERIALS AND METHODS

Identification of Microbes Present in Spoiled PFP Using DNA Sequencing Method

Bacterial and fungal cultures of spoiled PFP were prepared by the streak plate method using Nutrient Agar and Potato Dextrose Agar media, respectively (Sue Katz *et al.*, 2016).

Isolation of DNA from Bacterial Culture of Spoiled PFP: A small colony of bacteria was taken from the spoiled PFP and grown in nutrient broth overnight at 28 °C with shaking. An amount of 1.5 mL of the overnight culture was taken into a 2 mL sterile Eppendorf tube. The bacterial cells were pelleted by centrifugation at 5000 rpm, and the supernatant was removed. The bacterial cells were re-suspended in 200 µL of GET solution (50 mM glucose, 70 mM EDTA, 50 mM Tris-HCl, pH 8.0). The contents were divided into two Eppendorf tubes (100 µL each). An amount of 1.4 mL of lysis buffer (1% SDS, 50 mM Tris-HCl, 50 mM EDTA, pH 8.0) was added. About 30 µg of proteinase K per mL was added, and the mixture was incubated at 37C with end-over rotation for 2–18 hours. The volume in each tube was divided again into two Eppendorf tubes. A volume of 250 µL of saturated ammonium acetate was added to each tube, followed by vigorous vortexing to precipitate polysaccharide and protein contaminants in the lysate. The mixture was kept in a refrigerator for one hour. After precipitation, centrifugation was performed at 17,000 g for 10 minutes. Ethanol precipitation was carried out using 2.5× volume of absolute alcohol. The pellet was washed with 70% ethanol and dried. Each pellet was dissolved in 50 µL of TE buffer and treated with RNase (20 µg/mL). The extracted DNA was stored

at −20 °C (Kapilan, 2015).

Analysis of Bacterial Culture of Spoiled PFP: Five (05) µL of DNA isolated from the bacterial culture of the spoiled PFP was subjected to Polymerase Chain Reaction (PCR) using 27F/1492R primers (Forward Primer: Sequence: 5'-AGAGTTTGATCMTGGCTCAG-3'; Reverse Primers: Sequence: 5'-GGTTACCTTGTTACGACTT-3'). Amplified DNA was sequenced using the same primers, and the obtained DNA sequences were compared with existing sequences in GenBank (http://www.ncbi.nlm.nih.gov/blast).

Analysis of Fungal Culture of Spoiled PFP: Total DNA was isolated from 15 mg of the fungal sample based on the CTAB method (Kapilan, 2015). Five µL of the extracted DNA was subjected to PCR using primers ITS1 and ITS4 (Huang *et al.*, 2018). Amplified DNA was sequenced, and the sequences were compared with existing DNA sequences in GenBank (http://www.ncbi.nlm.nih.gov/blast).

Preparation of Plant Extracts

The three plant extracts were prepared from seeds of *Myristica fragrans* (Mf), root of *Acorus calamus* (Ac), and bark of *Vateria copallifera* (Vc). Each plant material was dried at 40 °C in the oven until constant weight was obtained. They were ground well and sieved using a 20-mesh sieve. Dried powder of each plant was mixed with distilled water in the ratio of 1 g: 10 mL in a conical flask. The mixture was shaken using ultrasonicator for 2 hours at ambient temperature (29 C). The mixture was kept for 72 h in the dark and was filtered through Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator at 40 °C. The extract was stored in the refrigerator until further use.

Selection of Best Plant Extract for the Preservation of PFP

Total phenolic content: Accurately, 0.1 g of ground powder of plant was added into 5 mL distilled water. Mixture was homogenized and centrifuged at 3000 rpm for 15 minutes. An amount of 1 mL of extracted sample was mixed with 2.5 mL of 10% v/v Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3(aq) and was stored for 30 min at 25 °C. After 30 minutes, absorbance was measured using UV-Visible spectrophotometer at a wavelength of 765 nm. TPC was determined and expressed as gallic acid equivalent (GAE).

Screening for anti-microbial activity Aqueous extracts of each plant were assayed for antimicrobial activity against three bacterial isolates, such as *Acetobacter aceti*, *Staphylococcus aureus*, and *Escherichia coli*, as well as a fungal isolate, such as *Saccharomyces cerevisiae*. The microbial isolates were maintained on corresponding agar slopes (Nutrient Agar for bacteria and Potato Dextrose Agar).

McFarland turbidity method was used to prepare a standard inoculum (Souza *et al.*, 2020). Initially, test suspension was prepared by inoculating a broth tube with 1-3 like those that colonies harvested from culture plate. It was placed in the incubator for 1-2 hours. The 0.5 McFarland standard and test suspension were mixed well. Test suspension was compared visually to the McFarland standard, which can be facilitated with the use of a Wickerham card or scale.

Screening for anti-microbial activity of aqueous extracts of plant materials was performed using the cut well agar diffusion method. Each agar plate was inoculated with 100 µL of the liquid test suspension, which is equivalent to 0.5 McFarland standard. The culture was spread well using sterile cotton bud. Wells, 8 mm in diameter, were bored in the agar plates using a sterile cork borer. The well was filled with the plant extracts in concentrations of 100, 50, and 10 mg/mL. After few minutes, agar plates were incubated at 37 C for 24 hours and at 25 ºC for 72 hours to inspect the bacterial and fungal growth, respectively. Chloramphenicol and sterile water were used as the positive and negative controls, respectively. The plates were examined for inhibition of growth around the well, and diameters of the zone of inhibition (ZOI) were measured.

Different Treatment on PFP for Its Preservation

Different treatments on PFP were done by varying the Vc extract (in g/L) and pH levels (Table 01). The initial pH of the PFP was measured, and then it was adjusted using citric acid and sodium bicarbonate to reach pH levels of 3.5, 4.0, and 4.5 separately. The pH-adjusted PFP was pasteurized at 80 °C for 30 minutes, allowed to cool, and stored for a period of six months. PFP without any preservatives (control) is denoted as T1, and PFP with sodium metabisulphite (SMS) added is denoted as T2.

Table 1: Different treatments on PFP for its preservation.

Treatments	Concentration of Vc extract (g/L)	pH
T1	0.0	4.4
T2	0.0	3.8
T3	1.0	3.5
T4	1.0	4.0
T5	1.0	4.5
T6	3.0	3.5
T7	3.0	4.0
T8	3.0	4.5
T9	5.0	3.5
T10	5.0	4.0
T11	5.0	4.5

Table 2: Analytical methods for the quality parameters of PFP.

Quality parameters	Method
Total soluble solids (TSS)	Refractometric method
pH	Potentiometric method (pH meter)
Titrable acidity	Titrimetric method (AOAC Method 942.15, 2019)
Reducing and non-reducing sugar	Lane and Eynon Method (AOAC method 923.09, 2019)
Total plate count	Pour plate method (SLS 516: Part 1, Section 1:2013)
Yeast and Mold	Swab plate method (SLS 516: Part 2, Section 1:2013)

Effect of Factors and Optimization of Best Treatment for the Preservation of PFP based on its Quality Characteristics

Quality characteristics were evaluated with storage period of 182 days to analyze the effect of pH and concentration of Vc extract for the preservation of PFP. Quality parameters were determined and compared for PFP samples with Vc extract, control (T1) and SMS added PFP (T2) using national and international standard methods as shown in (Table 02.).

Analysis of Phenolic Compounds using HPLC

Sample preparation. An appropriate amount of Vc bark was ground with a motor and pestle, and then transferred to a centrifuge tube with 10 mL of aqueous methanol (80:20 = methanol: 1% HCl). The mixture was centrifuged twice at 5000 rpm for 20 minutes to complete the extraction process. The combined supernatant was filtered through a 0.45 µm sized membrane filter before injection. The same procedure was repeated for the un-treated PFP and treated PFP (best treatment: 1g/L, pH 3.5).

HPLC instrumentation and chromatographic conditions. Chromatogrnalyses were carried out on a Dionex Ultimate 3000 ultra-high performance liquid chromatography (Thermo Fisher Scientific, Waltham, MA, USA), coupled to a Dionex Ultimate 3000 diode array detector as described in (Víctor *et al.*, 2014). Briefly, the separation was achieved with a Waters C18 column (150 × 3.0 mm i.d., 4 μm particle size) operated at 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and methanol (10/90, v/v; eluent B). The gradient used for the separation of phenolics was 0% B to 35% B (35 min), 35% B to 75% B (20 min), 75% B to 100% B (2 min), 100% B isocratic (5 min), 100% B to 0% B (2 min), and the running time was 60 minutes. A flow rate was 0.4 mL/min, the injection volume was 10 L, and detection was accomplished at 280 nm and 320 nm. Quantification was based on linear calibration curve of p-coumaric acid at 308 nm. The standards used were tannic acid, catechin, chlorogenic acid, syringic acid, vanillin, coumaric acid, and quercetin. The standards (HPLC grade; Sigma Aldrich) were purchased from Analytical Instruments (Pvt) Ltd, Sri Lanka. The concentration of 500 ppm of each standard stock solution of phenolic compounds was prepared by dissolving 25 mg of each standard in 50 mL HPLC-grade methanol. This was sonicated and passed through a Whatman nylon membrane filter (0.45 μm & 47 mm diameter) before injecting it into the column.

$$\text{Inhibition } \% = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100$$

Determination of Anti-oxidant Activity

Appropriate amount of each sample (treated PFP (best treatment: 1g/L, pH 3.5), untreated PFP, and extract of bark of Vc) was dissolved in methanol to make a stock solution. Aliquots from this stock solution were filtered and diluted with methanol as per the concentrations required. Accurately, 1.00 mL of each diluted sample was combined with 2.00 mL of the DPPH solution (0.006 % w/v) and kept them into the dark place at room temperature. The absorbance of each sample was measured after 30 min. IC_{50} was obtained from the graph of percentage inhibition against the concentration of the sample. An absorbance of control was obtained using methanol instead of the sample. The ability to scavenge DPPH radical was determined by the above equation.

Heavy Metal Analysis

Heavy metals such as Arsenic, Lead, Cadmium, Lead and Mercury were analyzed in treated PFP (best treatment: 1g/L, pH 3.5), untreated PFP, and extract of bark of Vc using pressure digestion and inductively coupled plasma mass-spectrometry (ICP-MS) (AOAC official method 2013.06). 1.0 g of each sample, accurately weighed and digested in accordance with Analar grade nitric acid, hydrogen peroxide (30 %) and concentrated Hydrochloric acid were used for the digestion. The digested samples were diluted with HNO_3 and analyzed by ICP-MS.

RESULTS AND DISCUSSION

Identification of Microbes Present in the Spoiled PFP using DNA Sequencing Method

The GenBank search revealed that there was a 98 - 100 % match between *Bacillus ocenisediminis* and the DNA sequence of the bacterial culture of spoiled pulp (Fig. 01.a.). As *B. oceanisediminis* contains phosphotransferase systems, they are used to obtain different carbon sources include glucose, mannitol, sucrose, maltose, and fructose (Lee *et al.*, 2012). Closest GenBank matches with *S. aureus* with 92 - 96 % similarity, while *Acetobacter* species with 81 - 92 % similarity. *B. ocenisediminis*, *S. aureus*, and *Acetobacter* species have the ability to grow in PFP, and these are known to produce acids that can lower the pH and increase the acidity of the PFP, leading to spoilage.

The GenBank search revealed that there is 89 - 100 % similarity with *Pichia manshurica* and DNA sequence of fungal culture of spoiled pulp (Fig. 01.b). *P. manshurica* is a common yeast found in fermented foods such as grapes, citrus, fermented maguey juice, wines, and silages (Toyotome and Yamamoto, 2019). *Pichia* yeast is associated with food and feed production and has the potential to grow in preserved food due to their low pH, high osmotic pressure and low oxygen content (Kadhim *et al.*, 2019). Closest GenBank matches with *S. cerevisiae* with 89 - 91 % similarity. Yeast species such as *P. manshurica* and *S. cerevisiae* species have the ability to grow in PFP.

Total Phenolic Content of Selected Plant Material

V. copallifera extracts showed the highest TPC compared to other extracts (Fig. 02). As TPC positively correlates with the antimicrobial potential (Ispiryan *et al.*, 2024), *V. copallifera* extract was selected as the best choice as a natural preservative, among the extracts tested.

Anti-microbial Activity of Selected Herbal Plant Extracts

Results of Zone of Inhibition (ZOI) of plant extracts against the four strains of microorganisms are presented in Table 03. The Vc extracts showed the highest inhibitory activity against all four strains, among the herbal extracts tested. As the concentration of Vc extracts decreased, the ZOI also decreased which is due to the decreasing amount of TPC of Vc (Table 03).

Effect of Factors for the Preservation of PFP on Quality Characteristics

Both untreated and treated samples showed a significant ($p<0.05$) increment in acidity and decrement in pH over 182 days of storage (Fig. 03.a and 03.b.). Acidity of fruit rises while they are in storage, and this causes a drop in pH due to the breakdown of

CTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAAC
TCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAA
GCAACGCGAAGAACCTTACCAGGTCTTGACATCTCCTGACAACCCTAGAGATAGG
GCGTTCCCCTTCGGGGGACAGGATGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC
AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAAT
GGATGGTACAAAGGGCTGCGAG

(a)

TCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAGCGCAGCGAAATGCGATA
CCTAGTGTGAATTGCAGCCATCGTGAATCATCGAGTTCTTGAACGCACATTGCGCC
CGTCGGTATTCCGGCGGGCATGCCTGTCTGAGCGTCGTTTCCTTCTTGAAGCTTTTT
TTTTTTTAAAAAAAGATTCAGAATTGGCCGTGCCACTGGCCCGGCCGAAAAGAAA
CGTTGCGGAC

(b)

Figure 1: DNA sequence of (a) bacterial and (b) fungal culture of spoiled Palmyrah Fruit Pulp (PFP)

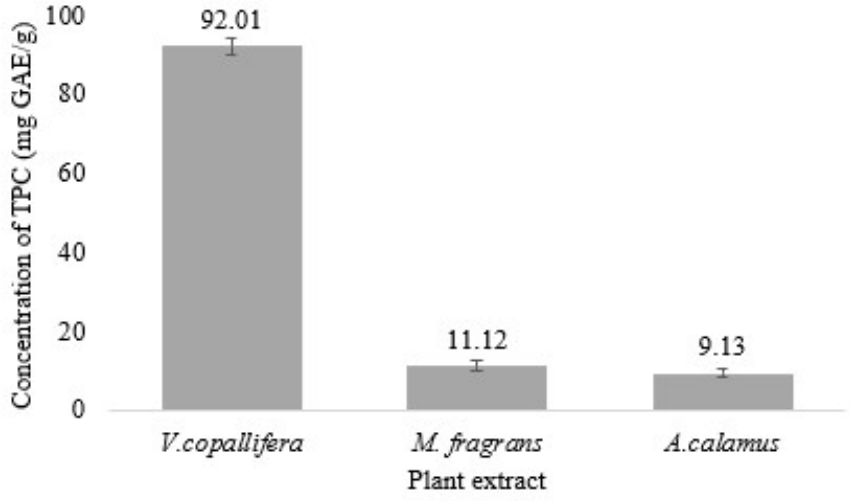

Figure 2: Total Phenolic Content (TPC) of aqueous plant extract.

Table 3: Zone of Inhibition (ZOI) for different concentrations of plant extracts against selected microbes (mm).

Plant material	Concentration (g/L)	A. aceti	S. aureus	E. coli	S. cerevisiae
V. copallifera	10	20	16	21	19
	5	17	13	18	13
	1	15	_	14	_
M. fragrans	10	12	_	_	_
	5	_	_	_	_
	1	_	_	_	_
A. calamus	10	_	_	_	_
	5	_	_	_	_
	1	_	_	_	_

the polysaccharides and pectic materials to produce organic acids. Oxidation of reducing sugars may also play a role in the increase in acidity. The basics of oxidation, reduction mechanisms, microbial fermentation, and acid production are well-established principles in biochemistry and microbiology (Rashmi and Negi, 2022). Among all samples, T1 sample showed highest acidity increment (138%) and pH decrement (54.5%) values from the initial values, as T1 is the untreated sample, which indicates spoilage of the sample over 182 days of storage. Meanwhile, T3 sample showed lowest acidity increment (22.3%) and pH decrement (1.7%) values from the initial values compared to other samples. Hence, the combined effect of treatment and storage significantly ($p \leq 0.05$) affected the acidity and pH of PFP.

As shown in Fig. 03.c., TSS of PFP decreased with increase in storage time in untreated samples. All

treatments increased the TSS of fruit pulp during the storage period of 182 days, which may be due to solubilization of fruit constituents during storage, hydrolysis of polysaccharides, application of heat (during pasteurization) results in disruption of cell structure and solubilization of cell constituents, and evaporation of water. It was observed that final TSS of PFP was highest in sample T4 that was about 7.8% increment compared to the initial value.

During the storage period, reducing sugar gradually increased for treated samples as shown in Fig. 03.d. It may be due to the progress of ripening and degradation of starch into glucose and fructose during storage. The minimum increment (37.42%) for reducing sugar was observed in T3 sample. Among all samples, T9 sample showed highest increment (160.07%) in TSS during storage, which may be due to the increased hydrolysis of cellular polysaccharides under acidic conditions. Non-reducing sugar content decreased in all samples significantly ($p \leq 0.05$) during 182 days of storage (Fig. 03.e.), which may be due to the conversion of non-reducing sugar to reducing sugar, and such conversion is accelerated by acidic conditions. The highest decrement (44.97%) in non-reducing sugar was observed in sample T6.

Total plate count of all the samples on day 1 was less than 1 $\log_{10}$ cfu/gm of sample (See Table 04.). T1 sample started to spoil after two weeks of storage time, as evident by the increase in total plate count and the growth of Yeast and Mold, while T2 sample started to spoil after 3 months of storage time (See Table 05.). Here, the pH level of food can play a significant role in preserving it. However, it may not be sufficient to preserve the food for an extended period, especially many months, as the food is rich in nutrients that can promote the growth of microorganisms easily. Hence, it is mandatory to consider other factors, including the use of chemical or natural preservatives and the pasteurization time and temperature. In this work, suitable pH together with preservatives were used to treat the PFP to increase its shelf life. Optimized factors of pasteurization temperature (80 °C) and time (30 minutes) as well as pH 4.2, as documented in the study of Robika *et al.*, (2016), were employed in this study. Microbial analysis of the samples treated with Vc extract had less microbial attack compared to the chemically preserved sample (T2). This may be due to the antimicrobial properties of the Vc extract and the pH of the sample. The T3 sample also had a longer shelf life of 6 months, which is longer than the other treated samples. Hence, T3 sample was selected as the best treatment (extract concentration 1 g/L, pH = 3.5) for the preservation of PFP. The anti-oxidant activity, presence of heavy metals, and abundance of phenolic compounds of T3 sample were determined.

Microbial Characteristics of Fresh, Treated, and Chemically Preserved PFP

In Table 06, the microbial characteristics of fresh PFP were compared with chemically preserved PFP and treated PFP. The Total Plate Count (TPC) for chemically preserved PFP was 1.48 ± 0.03 $\log_{10}$ cfu/g, while for treated PFP it was 1.56 ± 0.05 $\log_{10}$ cfu/g. The difference in counts between the two was relatively small, suggesting that the microbial load in both samples is comparable. Similarly, the presence of Yeast and Mould in chemically preserved PFP was 1.32 ± 0.05 $\log_{10}$ cfu/g, while in treated PFP it was 1.39 ± 0.06 $\log_{10}$ cfu/g. Again, the difference in counts was minor, indicating that the levels of yeast and mould were comparable between the two samples. Importantly, in all cases, the microbial counts did not exceed the acceptable threshold of 4 $\log_{10}$ cfu/g.

HPLC Analysis of Phenolic Compounds in the Extract of Bark of V. copallifera, Untreated PFP, and Treated PFP

Seven phenolic compounds were identified in the extract of *V. copallifera* bark, untreated PFP, and treated PFP samples. Symmetrical, sharp, and well-resolved peaks were observed for the seven polyphenolic standards. The elution order and the retention times for tannic acid, catechin, chlorogenic acid, syringic acid, vanillin, coumaric acid, and quercetin standards were 5.99, 7.54, 11.77, 12.13, 14.96, 17.89, and 19.92 minutes, respectively.

Phenolic compounds are important plant constituents due to their scavenging ability, attributed to their hydroxyl groups(Moazzen *et al.*, 2022).

According to Fig 04.a, the presence of tannic acid, catechin, chlorogenic acid, syringic acid, and quercetin was confirmed in the *V. copallifera* extract. According to the Table 07., catechin was found in the highest concentration compared to other amounts, 293.747 mg/g in the bark extract of Vc. Both catechin and quercetin have demonstrated beneficial effects, such as antioxidant and anti-ageing potentials (Bjørklund *et al.*, 2018), while phenolic compounds like tannic acid, syringic acid, and chlorogenic acid also exhibit health benefits by mitigating oxidative stresses (Wang *et al.*, 2022).

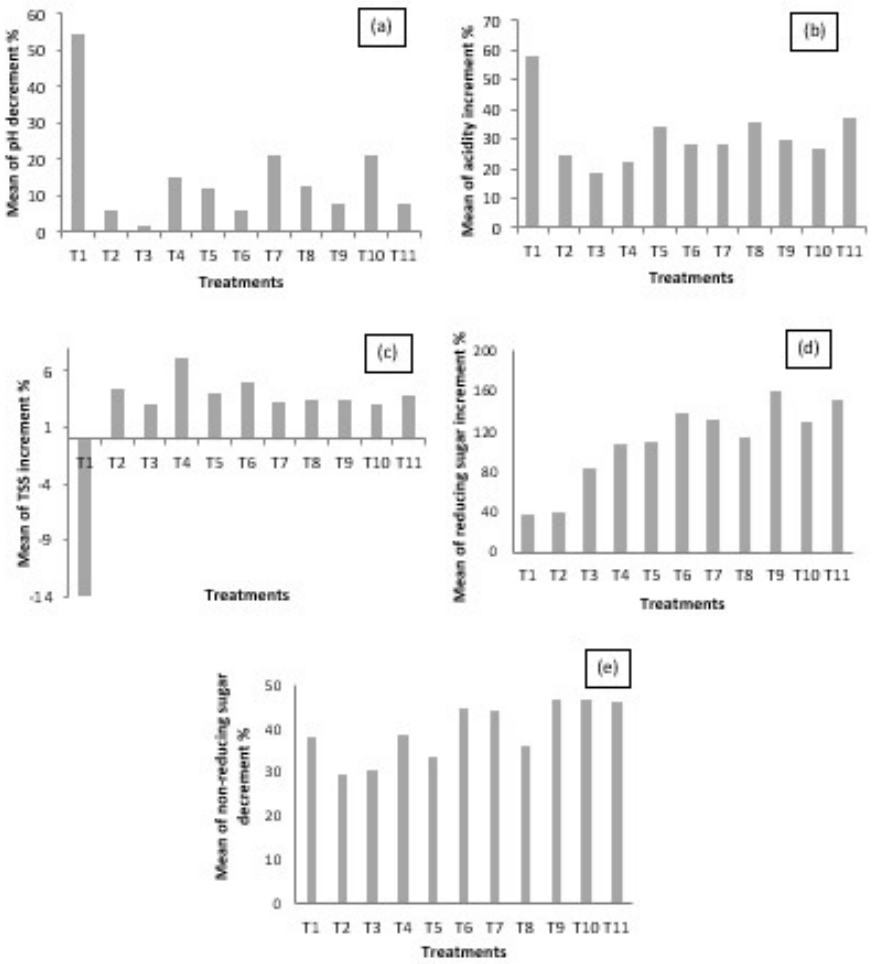

Figure 3: Changes of Palmyrah Fruit Pulp samples after storing for 182 days (a) pH decrement, (b) acidity increment, (c) TSS increment, (d) reducing sugar increment and (e) non-reducing sugar decrement. (T1: pH= 4.4,Vc = 0.0 ; T2: pH =3.8,Vc = 0.0 ; T3: pH=3.5,Vc = 1.0 ; T4: pH=4.0,Vc = 1.0 ; T5: pH= 4.5,Vc = 1.0 ; T6: pH= 3.5,Vc = 3.0 ; T7: pH= 4.0,Vc = 3.0 ; T8: pH= 4.5,Vc = 3.0 ; T9: pH= 3.5,Vc = 5.0 ; T10: pH= 4.0,Vc = 5.0 ;T11: pH= 4.5,Vc = 5.0) Vc::Concentration of *Vateria copallifera* Extract (g/L)

Table 04: Effect of storage period on the Total Plate Count (Log 10 cfu/g) of the different treatments of PFP

Sample	Days													
	1	14	28	42	56	70	84	98	112	126	140	154	168	182
T1	< 1 ± 0.04	2.4 ± 0.02	2.8 ± 0.05	3.1 ± 0.03	4.6 ± 0.03	5.4 ± 0.04	6.7 ± 0.06	7.9 ± 0.04	9.6 ± 0.05	>10 ± 0.02	> 10 ±0.04	> 10 ± 0.06	>10 ± 0.05	>10 ± 0.02
T2	< 1 ± 0.03	< 1 ± 0.05	< 1 ± 0.06	< 1 ± 0.03	< 1 ± 0.04	< 1 ± 0.05	1.01 ± 0.04	1.24 ± 0.04	1.45 ± 0.06	1.68 ±0.05	2.12±0.02	2.24 ± 0.06	2.47 ± 0.01	2.65±0.04
T3	< 1± 0.02	<1 ± 0.05	<1 ± 0.04	<1 ± 0.01	<1 ± 0.06	<1 ± 0.04	<1 ± 0.03	<1 ± 0.04	<1 ± 0.03	1.01 ±0.02	1.14±0.01	1.22 ± 0.05	1.31 ± 0.03	1.56±0.05
T4	<1 ± 0.02	<1 ± 0.04	<1 ± 0.06	<1 ± 0.05	<1 ± 0.03	<1 ± 0.01	1.03 ± 0.03	1.12 ± 0.04	1.26 ± 0.04	1.31 ±0.05	1.44±0.06	1.48 ± 0.04	1.51 ± 0.05	1.56±0.04
T5	<1 ± 0.04	<1 ± 0.03	<1 ± 0.04	<1 ± 0.02	<1 ± 0.05	<1 ± 0.04	<1 ± 0.05	1.08 ± 0.02	1.19 ± 0.04	1.21 ±0.06	1.24±0.05	1.38 ± 0.04	1.49 ± 0.05	1.57±0.04
T6	<1 ± 0.02	<1 ± 0.04	<1 ± 0.01	<1 ± 0.05	<1 ± 0.01	<1 ± 0.03	<1 ± 0.02	<1 ± 0.06	<1 ± 0.04	1.01 ±0.03	1.14±0.05	1.24 ± 0.06	1.38 ± 0.02	1.43±0.01
T7	<1 ± 0.05	<1 ± 0.02	<1 ± 0.06	<1 ± 0.01	<1 ± 0.05	<1 ± 0.03	1.02 ± 0.02	1.14 ± 0.04	1.19 ± 0.05	1.27 ±0.06	1.34±0.04	1.43 ± 0.03	1.52 ± 0.02	1.61±0.04
T8	<1 ± 0.04	<1 ± 0.02	<1 ± 0.03	<1 ± 0.01	<1 ± 0.05	1.09 ± 0.03	1.12 ± 0.01	1.23 ± 0.06	1.28 ± 0.05	1.31 ±0.02	1.47±0.01	1.51 ± 0.04	1.52 ± 0.05	1.57±0.03
T9	<1 ± 0.04	<1 ± 0.05	<1 ± 0.04	<1 ± 0.03	<1 ± 0.02	<1 ± 0.04	<1 ± 0.05	<1 ± 0.01	<1 ± 0.03	1.01 ±0.05	1.14±0.03	1.22 ± 0.02	1.31 ± 0.05	1.56±0.04
T10	<1 ± 0.05	<1 ± 0.04	<1 ± 0.03	<1 ± 0.04	<1 ± 0.02	<1 ± 0.01	1.02 ± 0.03	1.09 ± 0.01	1.19 ± 0.02	1.12 ±0.04	1.27±0.03	1.31 ± 0.04	1.42 ± 0.06	1.58±0.05
T11	<1 ± 0.02	<1 ± 0.04	<1 ± 0.05	<1 ± 0.04	<1 ± 0.02	<1 ± 0.03	<1 ± 0.04	1.03 ± 0.01	1.11 ± 0.02	1.23 ±0.02	1.33±0.03	1.45 ± 0.01	1.54 ± 0.01	1.61±0.04

The presence of tannic acid, catechin, syringic acid, vanillin and coumaric acid were confirmed in untreated PFP through the analysis of phenolic compounds. The results, shown in Table 07., indicate that tannic acid was found in the highest concentration among the phenolic compounds in untreated PFP (9.146 mg/100 g) followed by catechin, syringic acid and vanillin (2.708, 0.031, and 0.039 mg/100 g, respectively). When the PFP was treated with the bark of Vc extract, it was found that the levels of tannic acid, catechin, chlorogenic acid, syringic acid and vanillin increased significantly. However, quercetin acid was

Table 05: Effect of storage period on Yeast and Mould (Log 10 cfu/g) Count of the different treatments of PFP

Sample	Days													
	1	14	28	42	56	70	84	98	112	126	140	154	168	182
T1	< 1 ± 0.02	1.4 ± 0.01	3.26 ±0.04	4.14 ± 0.07	6.39 ± .05	8.48 ± 0.02	9.82 ± 0.01	> 10 ± 0.05	> 10 ± 0.03	> 10 ±0.06	> 10 ±0.01	> 10 ± 0.05	> 10 ± 0.04	> 10 ±0.02
T2	< 1 ± 0.02	< 1 ± 0.04	< 1 ± 0.03	< 1 ± 0.01	< 1 ± 0.05	< 1 ± 0.03	< 1 ± 0.02	1.15 ± 0.05	1.46 ± 0.01	1.54 ±0.04	1.87 ±0.02	2.06 ± 0.04	2.89 ± 0.02	2.98 ±0.01
T3	<1 ± 0.04	<1 ± 0.02	<1 ± 0.06	<1 ± 0.01	<1 ± 0.06	<1 ± 0.03	<1 ± 0.01	<1 ± 0.05	<1 ± 0.04	<1 ± 0.05	<1 ± 0.06	1.04 ± 0.02	1.23 ± 0.04	1.39 ±0.06
T4	<1 ± 0.04	<1 ± 0.06	<1 ± 0.02	<1 ± 0.04	<1 ± 0.01	<1 ± 0.06	<1 ± 0.04	<1 ± 0.05	1.23 ± 0.01	1.34 ±0.03	1.47 ±0.02	1.56 ± 0.04	1.63 ± 0.03	1.74 ±0.04
T5	<1 ± 0.01	<1 ± 0.05	<1 ± 0.06	<1 ± 0.04	<1 ± 0.02	<1 ± 0.06	<1 ± 0.04	<1 ± 0.03	1.01 ± 0.05	1.14 ±0.02	1.36 ±0.04	1.40 ± 0.05	1.59 ± 0.04	1.61 ±0.02
T6	<1 ± 0.03	<1 ± 0.04	<1 ± 0.04	<1 ± 0.01	<1 ± 0.05	<1 ± 0.02	<1 ± 0.04	<1 ± 0.06	1.06 ± 0.01	1.23 ±0.05	1.36 ±0.03	1.45 ± 0.04	1.50 ± 0.02	1.61 ±0.04
T7	<1 ± 0.03	<1 ± 0.04	<1 ± 0.01	<1 ± 0.05	<1 ± 0.04	<1 ± 0.03	<1 ± 0.01	1.03 ± 0.04	1.26 ± 0.01	1.43 ±0.02	1.51 ±0.04	1.55 ± 0.01	1.60 ± 0.03	1.65 ±0.02
T8	<1 ± 0.03	<1 ± 0.01	<1 ± 0.06	<1 ± 0.05	1.05 ±0.04	1.24 ± 0.05	1.28 ± 0.01	1.32 ± 0.03	1.44 ± 0.02	1.58 ±0.05	1.61 ±0.06	1.65 ± 0.03	1.74 ± 0.04	1.78 ±0.01
T9	<1 ± 0.03	<1 ± 0.05	<1 ± 0.06	<1 ± 0.01	<1 ± 0.03	<1 ± 0.05	<1 ± 0.02	1.11 ± 0.01	1.23 ± 0.05	1.30 ±0.03	1.46 ±0.03	1.52 ± 0.02	1.58 ± 0.02	1.63 ±0.01
T10	<1 ± 0.01	<1 ± 0.02	<1 ± 0.04	<1 ± 0.05	<1 ± 0.06	<1 ± 0.01	<1 ± 0.02	<1 ± 0.04	1.12 ± 0.03	1.28 ±0.05	1.34 ±0.03	1.49 ± 0.02	1.56 ± 0.05	1.63 ±0.02
T11	<1 ± 0.01	<1 ± 0.03	<1 ± 0.01	<1 ± 0.05	<1 ± 0.06	<1 ± 0.04	1.12 ± 0.02	1.23 ± 0.04	1.34 ± 0.05	1.40 ±0.06	1.53 ±0.04	1.58 ± 0.05	1.62 ± 0.04	1.68 ±0.04

Table 06 : Comparison of microbial parameters of fresh, chemically preserved, and treated Palmyrah Fruit Pulp (PFP)

Microbial Parameters	Fresh PFP	Chemically Preserved PFP	Treated PFP
Total Plate Count (Log_{10} cfu/g)	$< 1 \pm 0.02$	1.48 ± 0.03	1.56 ± 0.05
Yeast and Mould (Log_{10} cfu/g)	$< 1 \pm 0.04$	1.32 ± 0.05	1.39 ± 0.06

Table 07 : Contents of polyphenolic compounds in the PFP treated with the extract of bark of *V. copallifera* and the untreated PFP.

Phenolic Compounds	Bark of Vc Extract (mg/100 g dry weight)	Untreated PFP (mg/100 g wet weight)	Treated PFP (mg/100 g wet weight)
Tannic acid	92.881	9.146	13.626
Catechin	293.747	2.708	16.046
Chlorogenic acid	2.284	ND	0.406
Syringic acid	11.705	0.031	0.367
Vanillin	ND	0.039	0.145
Coumaric acid	ND	ND	0.067
Quercetin acid	163.768	ND	ND

not identified in the treated PFP, which suggests that it may have degraded during the pasteurization process. Interestingly, coumaric acid was identified in the treated PFP. This presence could be attributed to the thermal processing, such as pasteurization, which potentially facilitated the formation of coumaric acid (Arrieta-Baez *et al.*, 2012).

Antioxidant Activity by DPPH Radical Scavenging Assay

The presence of phenolic compounds are responsible for the antioxidant effects of plant extracts (Gulcin, 2020). The IC_{50} values of the samples were calculated from the graphs obtained by plotting the percentage scavenging against the concentrations used (Fig 5.a and 5.b.). According to the Fig 5.a., the scavenging effect of untreated PFP and treated PFP on the DPPH radical was found to be concentration dependent and was in the order of treated PFP > untreated PFP The results indicate that the antioxidant activity of treated PFP ($IC_{50} = 40.59 \pm 2.00$ mg/mL) was higher than that of untreated PFP ($IC_{50} = 69.72 \pm 2.00$ mg/mL). The IC_{50} value of the *V. copallifera* bark extract was determined to be 0.08 ± 0.01 mg/mL. When PFP was treated with the *V. copallifera* extract, its antioxidant activity increased by approximately 58% compared to the untreated PFP.

Analysis of Heavy Metals using ICP-MS Method

Heavy metal contamination during food processing can arise from various sources, such as water, soil, equipment, and additives. Heavy metals are toxic to human health and can cause a range of adverse effects if consumed in high concentrations. Therefore, it is essential to ensure that the levels of heavy metals in food products, such as fruit pulp, remain within safe limits to protect consumer health. Additionally, the presence of heavy metals can affect food quality, taste, and may cause discoloration and off-flavors. There are regulations in place that require food products

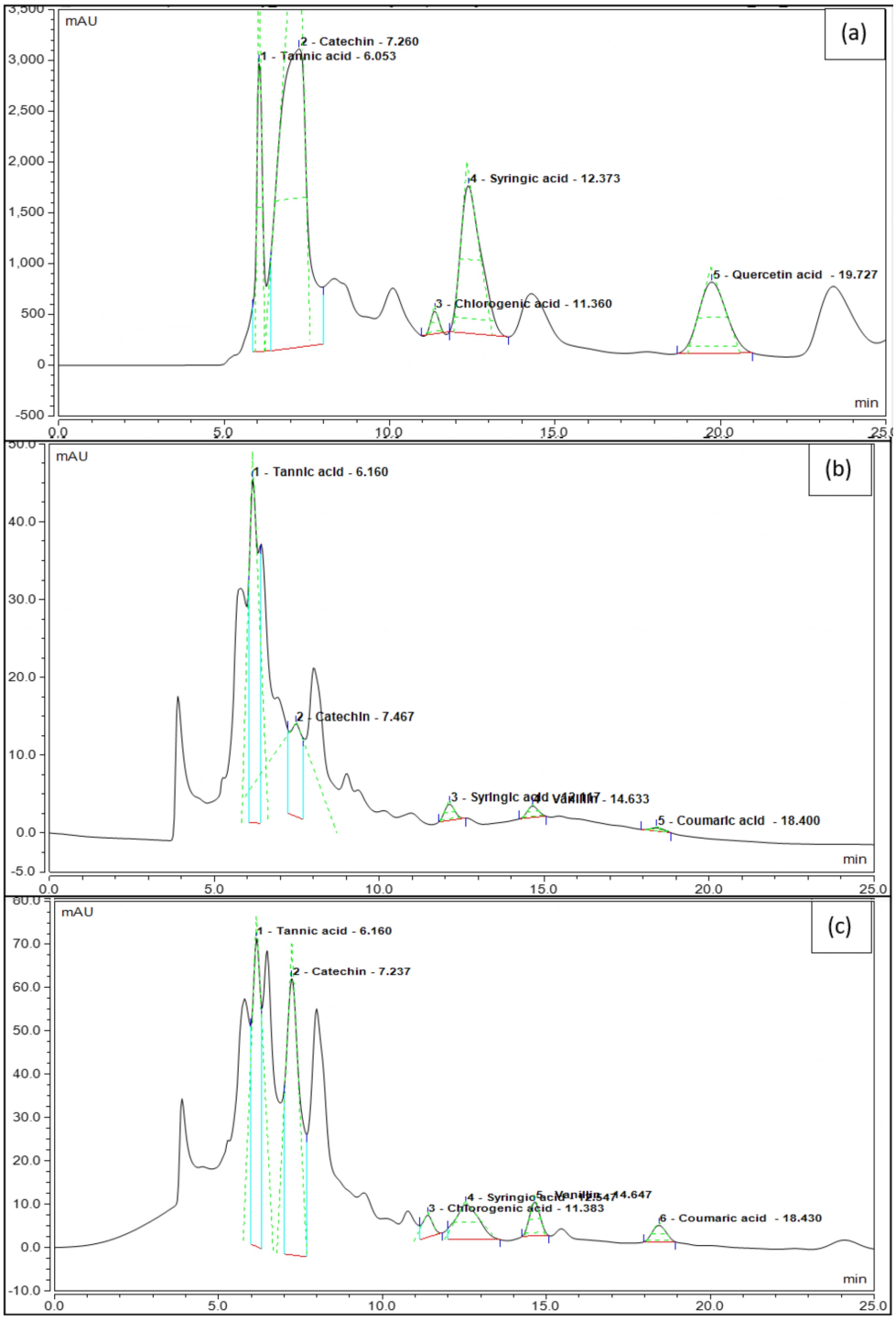

Figure 4: HPLC chromatogram of (a) Bark of V_c extract, (b) Un-treated PFP, and (c) PFP treated with V_c extract

Figure 4: HPLC chromatogram of (a) Bark of Vc extract, (b) Un-treated PFP, and (c) PFP treated with Vc extract.

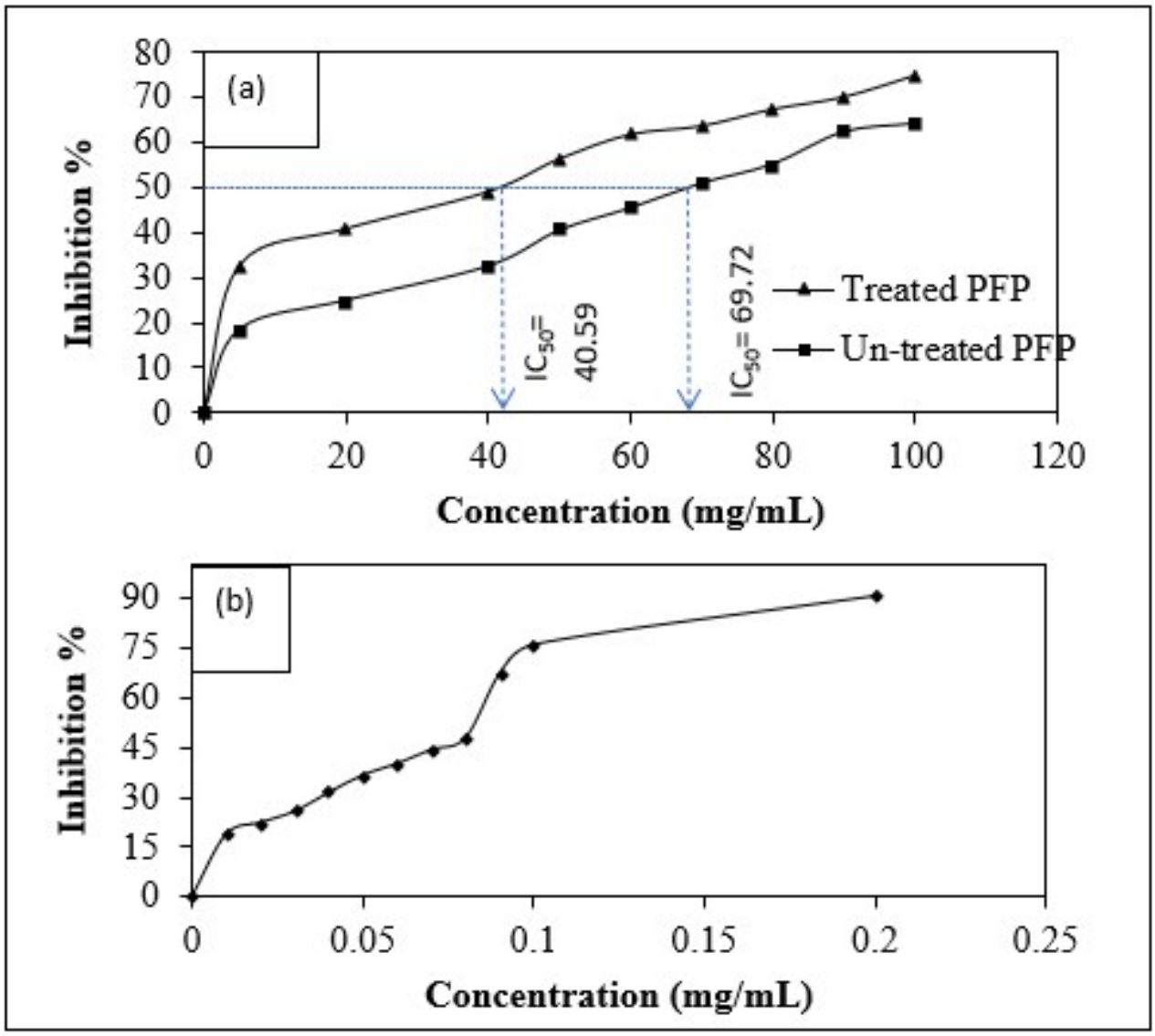

Figure 5: Percentage inhibition against concentration showing DPPH free radical scavenging activity of (a) untreated PFP and treated PFP and (b) Vc extract.

to be tested for heavy metal contamination and to be labeled accordingly. Therefore, it is important to conduct regular heavy metal analysis to ensure that the products meet the safety standards and regulations.

The ICP-MS analysis indicated that the concentrations of heavy metals in both untreated and treated fruit pulp samples were within permissible levels, as shown in Table 08. The concentration of heavy metals in the bark of Vc showed that Hg had the highest concentration, followed by Pb, As, and Cd. The concentration of Hg (0.08 mg/kg) in the bark of Vc exceeded the WHO/FAO standard (0.03 mg/kg), as it is in the dry weight. However, when the bark extract of Vc was added to the fruit pulp, the Hg concentration was found to be within permissible levels set by WHO/FAO standards (Table 08).

CONCLUSION

The use of natural preservation methods is essential to enhance the marketability of Palmyrah Fruit Pulp (PFP) as chemical preservation induces discoloration and the loss of aroma, whilst raising food safety concerns. Among the three plant extracts evaluated as potential natural preservatives in this study, Vc bark extract showed superior antimicrobial activity and a greater amount of TPC. The optimal preservation conditions were found to be 1 g/L of Vc extract at pH 3.5. This treatment was able to effectively inhibit microbial growth, maintain quality characteristics such as acidity, pH, TSS, sugars, and retain antioxidant activity, and phenolic compounds in PFP over a period of 182 days. As, Pb, Cd, and Hg concentrations in treated PFP were found to be within permissible levels set by WHO/FAO standards. The findings of this study suggest that Vc extract could be a promising natural preservative for PFP in the food industry.

Table 08 :Heavy metals in treated PFP, untreated PFP, and extract of bark of *V. copallifera*, along with their acceptable limits for fruits.

Heavy Metals	Samples (mg/kg)			Acceptable Limits (mg/kg)	
	Untreated PFP (wet weight)	Treated PFP (wet weight)	Vc Bark (dry weight)	SLSI	WHO/FAO
Arsenic (As)	< 0.01	< 0.01	0.07	0.1	0.5
Lead (Pb)	< 0.01	0.01	0.08	0.5	5.0
Cadmium (Cd)	< 0.01	< 0.01	0.02	1.0	0.2
Mercury (Hg)	< 0.01	< 0.01	0.08	–	0.03

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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