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High resolution-liquid chromatograph mass spectrometer characterization of bioactive compounds in pineapple wastes: Valorization of antioxidant and enzymatic activity

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ABSTRACT

An high resolution liquid chromatograph-mass spectrometer (HR-LCMS) Orbitrap system was employed to detect bioactive compounds within pineapple fruit and peel waste. The primary objective was to analyze flavonoids, polyphenols, and the antioxidant capacity present in these waste materials. Methanol extracts from both pineapple fruit and skin trimmings exhibited a higher concentration of phenolic components compared to aqueous and hexane extracts. HR-LCMS analysis identified seventeen diverse polyphenols, including derivatives of benzoic acid, hydroxyl-cinnamic acid, and hydroxyl-benzoic acid groups. Regarding total phenols and flavonoids, the pineapple peel demonstrated a higher concentration of these compounds than the pineapple pulp. In addition, the methanol extract demonstrated superior antioxidant capacity compared to the aqueous and hexane extracts. The study also found that the methanol extracts from pineapple fruit and peel residues were effective in inhibiting the DPPH radical in a concentration-dependent manner. High-temperature SDS-Tris-HCl buffered protein extraction and digestion resulted in maximum protein production. The bromelain's proteolytic activity revealed that the fruit waste had a higher pH than the peel waste. HR-LCMS analysis identified 302 proteins in fruit waste and 77 proteins in peel waste. *In silico* analysis characterized 154 differentially expressed proteins, of which 110 were commonly expressed, and 38 remained uncharacterized. These proteins were categorized into seven functional groups. Going forward, the research will focus on separating and purifying proteins and phenolic compounds from the waste materials. The biological potential of these compounds will be assessed for various applications, including pharmaceuticals, nutraceuticals, and the food industry. This study aims to harness the valuable resources present in pineapple fruit and peel waste for practical utilization in diverse fields.

ARTICLE HIGHLIGHTS

- HRLC-MS characterization: Comprehensive analysis using high-resolution liquid chromatography-mass spectrometry (HRLC-MS).
- Bioactive compounds in pineapple wastes: Identification and quantification of beneficial compounds from discarded pineapple parts.
- Valorization of antioxidant activity: Demonstrating the potential for repurposing pineapple waste into valuable sources of antioxidants.
- Enzymatic activity exploration: Investigating the enzymatic potential of pineapple waste for potential industrial applications.

1. INTRODUCTION

An important food plant of the Bromeliad family, the pineapple (*Ananas comosus* (L.) Merr.) is grown on more than a million hectares of land in tropical regions of the world [1]. According to Yabor *et al.* [2], large-scale pineapple farming produces a $9 billion yearly contribution to the global economy. It is commercially produced as fruit jams, jams, juices, and concentrates, and its neutral color and taste make it an ideal base for other fruit concentrates. As one of the world's most traded fruits, he has great economic importance in international markets. The growing production and consumption of processed pineapple goods has, however, resulted in the production of significant amounts of trash in the form of by-products such skins, fruits, and stems, which frequently end up in landfills and cause environmental issues [3].

Polyphenols, flavonoids, and other important biomolecules are abundant in pineapple waste, which is made up of skin and fruit remnants [4]. Polyphenols are secondary metabolites with diverse biological potential, with anticancer, antibacterial, antipyretic, antioxidant, and wound-healing effects [5]. Indicating antioxidant capability, the presence of phenolic compounds might be employed for early screening [6]. Phenolic acids are classified into hydroxyl-cinnamic acid, hydroxyl-benzoic acid, and benzoic acid derivatives, each of which has specific activities such as antibacterial and antioxidant properties [7]. Known for their antioxidant properties, flavonoids act

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as plant pigments and are thought to be involved in the antioxidant function of membrane lipids [8]. Pineapple's high nutritional content, including minerals such as calcium, iron, phosphorus and Vitamin C, further enhances its antioxidant capacity [9]. Bromelain, a proteolytic enzyme found in pineapple, belongs to the cysteine group and has many commercial uses, including anticoagulation, beer clarification, breakdown of gluten proteins during baking, and meat tenderization [10]. Its anticancer properties and its role in pharmaceuticals, cosmetics and dietary supplements have attracted attention [6]. Pineapple by-products contain phenols, flavonoids, and proteins and are of global interest for their potential antioxidant and proteolytic activity. Identification and quantification of these molecules are important for elucidating the bioactivity of proteins and phenolic compounds [11]. For identifying the variety of phenolic chemicals in plants, chromatographic approaches, particularly high-resolution liquid chromatography mass spectrometer (HRLC-MS) combined with mass spectrometry, are crucial [12]. To maximize the antioxidant and proteolytic activity of pineapple fillets and skins, this study aimed to assess, identify, and quantify the total phenolic compounds, flavonoids, and proteins present.

2. MATERIALS AND METHODS

2.1. Sample Collection

Pineapples from various varieties commonly available in Indian marketplaces were considered for the study. The Giant Kew/Kew and Queen varieties, prevalent in North Eastern India, particularly in Odisha, were chosen for the experiment. Large-sized pineapples of these varieties were procured from the local market in Odisha.

2.2. Soxhlet Extraction

200 g of diced pineapple skins were crushed, exposed to Soxhlet heat extraction, and air dried in the shade at room temperature. For the 8-h extraction procedure, 100 mL each of water, methanol, and hexane were employed as the solvents. A rotary evaporator operating at 50°C was used to filter and concentrate the extract after that. The extract's yield was then determined.

2.3. Determination of Total Phenol

The Folin-Ciocalteu colorimetric technique [13] was applied to extracts to assess their total phenol contents, and external calibration was carried out using standard gallic acid solutions. 0.2 ml of each of the reagents — Folin-Ciocalteu and the extract — were combined. After 4 min, 1 mL of 15% Na_2CO_3 was added, and the mixture was allowed to sit at room temperature for 2 h. The total phenolic content was determined in milligram of gallic acid equivalent based on the gallic acid calibration curve after the absorbance at 765 nm was measured using a Perkin Elmer spectrophotometer. For phenolic chemicals in the fractions, a three-estimate average was employed.

2.4. Identification of Phenolic Compounds by HPLC

The technique reported by Campos *et al.* was used to analyze phenolic chemicals in pineapple fruit and peel waste extracts using a Waters e2695 separation module system connected to a photodiode array ultraviolet/visible (UV/Vis) detector. The technique mentioned. A chromatographic separation utilizing particular mobile phases (water, methanol, and formic acid) was carried out on a C18 reversed-phase column to more effectively separate the phenolic pineapple components. The phenolic compounds were chromatographically separated in 50–55 min, with mobile phase A returning to 100% in 4 min (up to 59 min). The diode array detector captured wavelengths between 200 and 400 nm at intervals of 2 nm, with an injection volume of 20 µl. To detect the catechins or procyanidins (280 nm), phenolic compounds (320 nm), and antioxidants (330 nm) in comparison to pure standards, retention durations and spectra were studied.

2.5. Phenolic Chemical Identification using Mass Fragmentation

Phenolic substances in pineapple fruit and peel waste extracts were analyzed according to Monforte *et al.* using his HRLC-TOF-MS [14]. Samples were characterized using a Thermo Fisher Scientific UltiMate 300 Dionex UPLC and a Bruker Daltonics Impact II Qq time-of-flight mass spectrometer with an extremely high resolution (FSR) of 50,000. The separation was performed on a PepMap RSLC C18 column with an injection volume of 1 L. With a flow rate of 0.25 mL/min, mobile phases of acetonitrile and formic acid (99.9:0.1% v/v) and water (99.9:0.1% v/v) were utilized. Starting at 5% mobile phase B, the gradient rose to 95% in 7 min, maintained for 2 min, and then dropped down to 5% after 1 min. Spectra in the m/z 20–1000 range were collected using positive ionization mode. Using precise mass and isotope ratio calculations called mSigma (Bruker Daltonics, USA), the elemental makeup of the compounds was confirmed. The presence of phenolic chemicals in the fractions was confirmed within 5 mDa range of accurate mass measurements with mSigma values of 20. Purified standards (LC-MS grade) in methanol were used for identification, and accurate amounts of each phenolic component allowed accurate identification.

2.6. Total Flavonoid Content Measurement

After making a few minor changes, the aluminum chloride colorimetric technique published by Willet [15] was used to estimate the total flavonoid content of pineapple. 0.5 ml of methanol extract, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 4.3 ml of distilled water were combined after 30 min of room temperature incubation. Using a LAMBDA 365 UV/Vis Spectrophotometer, absorbance at 430 nm was recorded and a standard curve with quercetin was used for quantification. An average of three estimates of total flavonoids in the extract was calculated.

2.7. The Phosphomolybdenum Technique is used to Assess Antioxidant Capability

To assess the overall antioxidant capacity of pineapple extracts in ethyl acetate, methanol, and water, the method of Prieto *et al.* [16] was utilized. Sample solution (100 g/ml), reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and a boiling water bath were heated at 95°C. for 90 min. Each sample's absorbance at 695 nm was assessed against a blank after cooling to room temperature. The same conditions as the sample were used to incubate a typical blank solution, which included sample solvent and reagent solution. For samples with an unknown composition, the amount of water-soluble antioxidant activity was reported in equivalents of ascorbic acid (mol/g extract).

2.8. Test for 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was used to assess the extract's capacity to scavenge free radicals. Different concentrations of extract and ascorbic acid were prepared (10, 25, 50, 100, 250, 500, 750, and 1000 µL, corresponding to 10, 25, 50, 100, 250, 500, 750, and 1000 µg). To make the total capacity 1000 l, methanol

(MeOH) was poured to each tube. After that, each tube received 5 ml of DPPH in 0.1 mM methanol, and the mixture was rapidly agitated. The tube was thereafter maintained at 27°C for 20 min. For baseline correction, MeOH was employed, and extract-free control samples were created. The samples' variations in absorbance were quantified at 517 nm. The following formula was used to compute the percentage of inhibition, or scavenger activity:

Scavenger activity percentage is ([control OD – sample OD]/control OD) × 100

2.9. Protein Extraction and in-gel Protein Digestion

Protein extraction was performed according to Jefferies *et al.* [17]. The method described was performed. 10–12 ml of 2% SDS extraction buffer with a pH of 8.5 were subjected to 2 g of each pineapple fruit and peel waste sample. The samples were heated for 8 min at 95°C and then centrifuged for 15 min at 8000 rpm at 4°C. Proteins were precipitated by adding three volumes of acetone containing 10% TCA and 20 mM DTT, incubating for 45 min at −20°C, and then centrifuging for 10 min at −7°C at 18,000 rpm. The resultant pellet was centrifuged at 20,000 rpm for 10 min with the supernatant being discarded after being washed with 20 mM DTT and ice-cold acetone. After air-drying for 5 min, the protein pellet was dissolved in rehydration buffer.

2.10. Protein Assay

Protein concentrations were determined using the Lowry method. In this method, divalent copper ions are reduced to monovalent ions through complex formation with peptide bonds in an alkaline environment. Folin reagents react with copper ions, tyrosine, tryptophan, and cysteine radical groups to form unstable compounds that are ultimately reduced to molybdenum/tungsten blue. Reagent A (Na-K-Na tartrate, sodium carbonate, NaOH), reagent B ($CuSO_4$, H_2O, 1N NaOH), and reagent C (Folin-Ciocalteu reagent) were used for testing and measure the optical density at 650 nm using LAMBDA 365 UV/Vis Spectrophotometer.

2.11. Bromelain Enzyme Proteolytic Activity

The resulting crude bromelain enzyme was further purified using an ethanol extraction technique. Ethanol was used for extraction. The crude extract was mixed with 60% pure ethanol and refrigerated at 4°C overnight. After decanting the clear solution, a small residue remained at the bottom of the beaker, which was centrifuged at 8000 g, 4°C for 10 min. The precipitate was lyophilized and dissolved in an aliquot of pH 7.4 phosphate buffer. Casein (1.5% w/v) was used as a substrate to test the protease activity of bromelain extract while cysteine and EDTA were also present. The reaction was run for 10 min at 37°C and pH = 7.0. 3.0 ml of 5% (w/v) TCA was added to terminate the reaction exactly 10 min after it started. By centrifuging the protein precipitates at 14,000 g for 10 min, absorbance of the cleaned supernatant was determined at 280 nm. The quantity of enzyme that produced a product corresponding to 1 mole of tyrosine/ml/min under the test circumstances was used to define one unit of protease activity.

2.12. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS PAGE)

Proteins are separated using the SDS-PAGE method according to the molecular weight of the proteins. This technique uses SDS to denature proteins, which are then separated on a polyacrylamide gel. Separated proteins can be visualized using silver staining or other staining methods. In this study, 10% SDS-PAGE gels were used for protein separation and protein spots were identified by silver staining. Protein molecular weights were determined using Bio-Rad's "Quantity One" software.

2.13. HR-LCMS Analysis with in-gel Digestion

The samples' proteins were identified and examined using in-gel digestion. After being washed with 25 mM ammonium bicarbonate, the gel slices were dried with acetonitrile (ACN). The di-sulfide bonds in the gel slices were then reduced by rehydrating them with 50 mM di-thiothreitol and incubating them for an hour at 56°C. After that, gel slices were exposed to 100 mM iodo-acetamide in the dark to alkylate cysteine residues. The gel slices were treated with trypsin, and digestion was carried out overnight at 37°C. With different ratios of his ACN solution, the resultant peptides were extracted, and the extracted peptides were then subjected to mass spectrometry analysis using his ZIPTip.

3. RESULTS AND DISCUSSION

3.1. The Total Phenolic Content

From pineapple fruit residue, extracts of water, methanol, and hexane were found to produce 4.8%, 23.4%, and 18.6% of the total yields, respectively. The yields from extracting pineapple peel residue in water, methanol, and hexane were very similar, coming in at 3.2%, 16.4%, and 10.3%, respectively. The Folin-Ciocalteu technique, which uses gallic acid as the standard, was used to evaluate the concentrations of total phenolic components in different solvent extracts of pineapple fruit and peel waste. The total phenolic content of the extract, expressed in mg/g based on dry weight per gram of sample, was determined by obtaining absorbance values at various doses of gallic acid and using those values to create a standard curve. Among the three pineapple fruit and skin trimming extracts, the fruit methanol extract (42.7 ± 0.72) and skin trimming (54.8 ± 0.62) had total phenolic component concentrations that are greater than the aqueous extract (14.4 ± 0.3; 16.59 ± 0.48) and hexane extract (15.01 ± 0.24; 22.7 ± 0.3) [4] from pineapple fruit and skin waste [Table 1].

These results indicate that the methanol extraction method is more effective in extracting phenolic compounds from both pineapple fruit and skin trimmings. Of note, pineapple skins contained more total phenolic compounds compared to pineapple fruit trimmings. Similar observations were reported in a study on pomegranate peel and seeds, in which methanol extract had the highest yield of extractable chemicals compared to other solvents such as hexane, ethyl acetate, and water [18]. Methanol and aqueous methanol solutions are commonly used to extract phenolic components from fruits [19]. It is critical to remember that the total amount of phenolic compounds measured by this method represents the chemical reduction capacity of phenolic compounds toward gallic acid. The phenolic antioxidant index proposed by Elliot [20] considers both the quantity and quality of antioxidants in vegetables. Variations in the phenol compounds found in the different extracts, either in terms of kind or quantity of pineapple fruit

Table 1: Total phenolic components in Pineapple fruit and peel wastes using gallic acid equivalent.

Sample	Total phenolic contents in different solvent extracts (mg/g)		
	Aqueous	Methanolic	Hexane
Pineapple fruit waste	14.4±0.3	42.7±0.72	16.59±0.48
Pineapple peel	15.01±0.24	54.8±0.62	22.7±0.34

and skin cuttings may contribute to the different responses in this test.

3.2. Total Polyphenol Content

HRLC-MS analysis was used in accordance with the particular procedures stated above to characterize the total polyphenols in pineapple fruit and skin liquid extracts. Phenolic chemicals were identified by comparing their UV spectra and retention periods to established standards, and quantification was carried out using the wavelength that produced the strongest reaction. The liquid-based extracts of pineapple fruit and skin samples were analyzed for the presence of polyphenols, and their masses were quantified [Table 2]. In the pineapple peel, a total of 17 polyphenols were identified and quantified between 200 and 330 nm using HRLC-MS [Table 2 and Figure 1a].

The identified polyphenols in the pineapple peel included pyrocatechol at 207 nm, quercetin at 240 nm, gentisic acid at 242 nm, myricetin at 254 nm, apigenin at 269 nm, cinnamic acid at 270 nm, three phenolic compounds at 280 nm (gallic acid, hydroxytyrosol, and ellagic acid), trans-4-coumaric acid at 286 nm, kaempferol at 290 nm, cryptochlorogenic acid at 303 nm, caffeic acid at 304 nm, syringic acid at 310 nm, ferulic acid at 325 nm, chlorogenic acid at 330 nm, and p-hydroxybenzoic acid at 330 nm. On the other hand, only 12 polyphenols were identified in pineapple fruit waste (PFW), and no specific many phenolic substances, including hydroxytyrosol, caffeic acid, ellagic acid, myricetin, and quercetin, were found [Figure 1b].

Chromatograms and retention times (rT) of standard mixes of ferulic acid, gallic acids, catechol derivatives, hydroxytyrosol, chlorogenic acids, caffeine, syringic acid, trans-4-coumaric acid, ellagic acid, myricetin, and cinnamic acid p-hydroxybenzoic acid, quercetin, cryptochlorogenic acid, gentisic acid, kaempferol, and polyphenols apigenin were used for identification and quantification [Table 2].

The results indicated that the polyphenols identified in the pineapple skin and fruit waste extracts belonged to the hydroxyl-benzoic acid and hydroxyl-cinnamic acid groups. The presence of polyphenols in pineapple components confirms the results of previous studies by Difonzo *et al.* [21]. In methanol extracts of pineapple peels, gallic acid as well as catechin, epicatechin, and ferulic acid substances have been found, according to other investigations on pineapple waste [22]. No specific flavanol peaks were detected, which may be attributed to more effective extraction with organic solvents such as methanol. However, the peak values found were consistent with those in this study. The examination of polyphenols extracted from pineapple skin by Hossain and Rahman [23] using several solvents, including methanol and water, is consistent with our findings. According to their research, pineapple samples did not respond well to water as a solvent, and the methanol extract had the highest concentration of antioxidants. They also described several pineapple components and located the hydroxyl-cinnamic acid and hydroxyl-benzoic acid groups in phenolic substances such as gallic acid, gentisic acid, syringic acid, vanillic acids, sulfuric acid, sinapinic acid, and isoferulic acid.

According to this and earlier investigations, pineapple peel and fruit trimmings had different polyphenol compositions, which is likely due to environmental factors. In addition, a prior investigation by Larrauri [24] on the distinction between free and bound polyphenols in pineapple waste revealed that a sizeable amount of polyphenols. The samples' total polyphenol content and antioxidant potential rose because they included more pineapple fibers.

3.3. Total Flavonoid Content

Using the aluminum chloride colorimetric technique created by Willet [15] using quercetin as a reference, the total flavonoid content in different solvent extracts of pineapple fruit and peel waste was ascertained. To determine the total flavonoid content of the extract in milligram of quercetin equivalent per gram of dry weight (mg/g), a standard curve was created by acquiring absorbance readings at different quercetin concentrations. The examination of pineapple fruit and peel trimming extracts for total flavonoid concentration is presented in Table 3. There was 26.9–32.8 mg of quercetin/g of weight

Table 2: HRLC-MS analysis of methanolic extracts of pineapple peel and fruit waste for the profiling and quantification of phenolic compounds and metabolites.

S. No.	Phenolic substances	Retention period (tR min)	UV absorption at its greatest (max in nm)	Molecular weight (g/mol)	Conceptual mass (m/z)	Complied mass (m/z)	Peel waste (mg/L)	Fruit waste (mg/L)
1	Gallic acid	9.324	280	170.120	169.014	169.013	0.18±0.02	0.23±0.04
2	Pyrocatechol	13.473	207	110.100	109.621	109.620	0.24±0.01	0.32±0.02
3	Hydroxytyrosol	21.415	280	154.160	153.042	153.041	0.32±0.02	--
4	Chlorogenic acid	23.823	325	354.310	353.087	353.086	2.58±0.04	1.31±0.04
5	Caffeic acid	25.248	304	180.160	179.035	179.034	13.08±0.01	--
6	Syringic acid	26.289	310	198.170	197.045	197.044	0.38±0.03	1.22±0.06
7	trans-4-Coumaric Acid	27.924	286	164.047	163.040	163.039	2.48±0.06	2.02±0.01
8	Ferulic acid	28.682	318	194.180	193.050	193.049	1.69±0.02	0.52±0.04
9	Elagic Acid	31.165	280	302.197	301.012	301.011	0.16±0.01	--
10	Myricetin	31.587	254	318.235	317.120	317.119	0.17±0.02	--
11	Cinnamic Acid	33.408	270	148.158	147.045	147.044	18.26±0.04	14.25±0.06
12	p-hydroxybenzoic acid	33.872	330	138.120	137.024	137.023	10.82±0.02	0.82±0.04
13	Quercetin	34.238	240	302.236	301.035	301.032	0.14±0.02	--
14	Cryptochlorogenic acid	34.702	303	354.310	353.087	353.086	0.14±0.01	0.18±0.01
15	Gentisic acid	35.382	242	154.120	153.082	153.081	0.34±0.01	0.23±0.03
16	Kaempferol	37.892	290	286.230	285.040	285.039	0.33±0.02	0.33±0.01
17	Apigenin	38.438	269	270.240	269.136	269.135	0.28±0.03	0.24±0.02

HRLC-MS: High resolution-liquid chromatography mass spectrometer

of total flavonoid concentration in several PFW extracts. On the other hand, pineapple peel extracts in various solvents had a total flavonoid concentration that ranged from 35.3 to 41.06 mg quercetin/g weight. Among the three extracts of pineapple fruit and peel waste, the fruit methanol extract (41.58 ± 0.5) and peel waste (49.7 ± 0.3) exhibited significantly higher total flavonoid contents than the aqueous pineapple fruit extract (26.9 ± 0.9; 35.3 ± 0.3) and hexane extract (32.8 ± 0.7; 41.06 ± 0.3). This variation in flavonoid content may result from specific environmental conditions that may affect the composition of plant constituents.

The results also showed that pineapple peel contained higher amounts of total flavonoid compounds compared to pineapple pulp. Significant levels of flavonoids are present in methanol extracts, which is consistent with other research showing how well methanol solvents used for extraction of flavonoids from diverse plant sources [18]. In addition, it is common to use methanol or aqueous methanol to extract flavonoids from fruits [19]. Flavonoids are widely recognized for their antioxidant capacities and associated health advantages, and the high concentration of flavonoids in the methanol extract indicates their potential value as an antioxidant source. The importance of high flavonoid content in pineapple skin is noteworthy, as flavonoids are known to have various health-promoting properties. According to earlier research, pineapple peel contains flavonoids such apigenin, kaempferol, myricetin, and quercetin as well as other phenolic compounds [25].

The abundance of these flavonoids in the pericarp ensures a higher overall flavonoid content compared to the pulp. In addition, the variation in flavonoid content between fruit and skin waste extracts may also be impacted by elements such as fruit development stage and environmental circumstances during growth. Overall, measuring the total flavonoid content in pineapple fruit and skin trimming extracts provides valuable insight into their potential health benefits and antioxidant properties. The high flavonoid contents in the methanol extract and the predominance of flavonoids in the pineapple skin warrant further research, emphasizing the significance of using different portions of the pineapple for potential nutritional and medical applications.

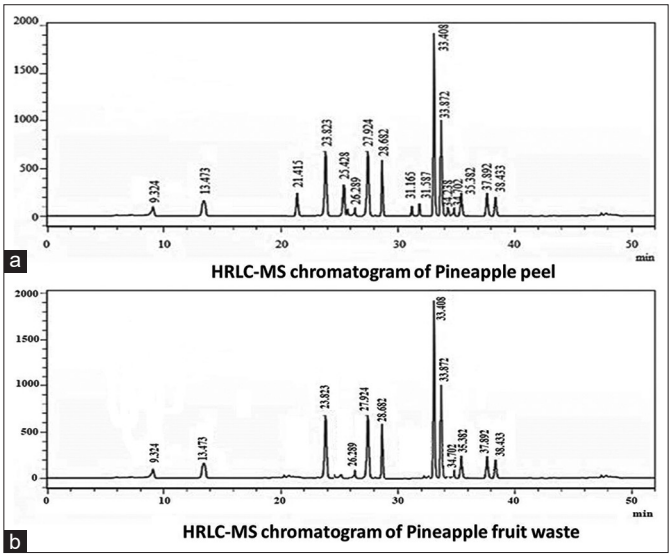

Figure 1: High resolution-liquid chromatogram of methanolic extracts from pineapple peel (a) and fruit waste (b) revealing retention times of various polyphenols.

3.4. Antioxidant Power

The extract's antioxidant potential was determined using the phospho-molybdenum technique. The reduction of Mo(IV) to Mo(V) by analytes in the sample results in a green phosphate/Mo(V) complex with a maximum absorbance at 695 nm. Table 4 shows the antioxidant capacity results of various solvent extracts from pineapple fruit and skin waste. Methanol solvent extracts from both PFW (1912.2 ± 9.3) and peel (1804.1 ± 12.4) showed significantly higher antioxidant capacities compared to water extracts (596.3 ± 6.4; 534.1 ± 8.2) and hexane (1032.6 ± 9.6). 1004.4 ± 6.3) Extracts from pineapple fruit or peel waste.

The higher antioxidant capacity observed with methanol extracts is consistent with the previous studies demonstrating the efficacy of methanol as a solvent for extracting antioxidant compounds from various plant materials [18]. The methanol extract appears to be rich in antioxidant compounds that contribute to its superior antioxidant effect. The capacity of antioxidants to neutralize reactive oxygen species and protect cells from damage caused by oxidative stress is well established. The higher antioxidant capacity observed in the methanol extracts of both pineapple fruit and skin trimmings indicates their potential as valuable antioxidant sources. Antioxidants have a vital function in lowering the levels of oxidative stress, which is linked to a variety of chronic illnesses such as coronary artery disease and malignancies. The large difference in antioxidant capacity between different solvent extracts highlights the importance of solvent selection during the extraction process. Solvent polarity can affect the extraction of certain antioxidants. In this case, methanol appears to be more effective in extracting potent antioxidants from pineapple fruit and peel waste. Overall, the phospho-molybdenum technique of assessing antioxidant capability reveals the advantage of methanol extract in terms of antioxidant capacity in both pineapple fruit and skin waste. These results highlight the potential health benefits of using methanol extract from pineapple waste as a natural antioxidant source and encourage further research on its application in functional foods, dietary supplements, and pharmaceuticals.

3.5. *In Vitro* DPPH Assay

To examine the unconventional scavenging pastime of the diverse solvent extracts from pineapple fruit and peel waste, the DPPH technique become employed, and the consequences are illustrated in Figure 2. The essential position of antioxidants lies of their

Table 3: Total flavonoid components in pineapple fruit and peel wastes extracts.

Sample	Total flavonoid contents in different solvent extracts (mg quercetin/g)		
	Aqueous	Methanolic	Hexane
Pineapple fruit waste	26.9±0.9	41.58±0.5	32.8±0.7
Pineapple peel	35.3±0.3	49.7±0.3	41.06±0.3

Table 4: Antioxidant capacity of pineapple fruit and peel wastes using the phospho-molybdenum method.

Sample	Antioxidant capacity (%) as equivalent to ascorbic acid		
	Aqueous	Methanolic	Hexane
Pineapple fruit waste	596.3±6.4	1912.2±9.3	1032.6±9.6
Pineapple peel	534.1±8.2	1804.1±12.4	1004.4±6.3

interplay with oxidative unfastened radicals. The DPPH technique includes using solid unfastened radicals, particularly 1,1-diphenyl-2-picrylhydrazyl (DPPH), that is, darkish pink in shadeation. When antioxidants engage with DPPH, it undergoes discoloration, ensuing within side the formation of 1,1-diphenyl-2-picrylhydrazine. The diploma of shadeation extrade within side the response suggests the scavenging ability of antioxidants gift within side the sample.

In this study, all of the extracts from pineapple fruit and peel waste confirmed the cappotential to decolorize DPPH, indicating their unfastened radical scavenging ability. The order of scavenging ability become discovered to be methanol extract > hexane > aqueous extract. The previous research through way of means of Blois [26] said that the above-referred to extract samples may comprise diverse compounds together with cysteine, antioxidant glutathione, Vitamin C, Vitamin E, hydroquinone, pyrogallol, and aromatic amines (together with p-phenylenediamine, p-aminophenol, and others)., which own hydrogen-donating cap potential. These compounds gift with inside the extracts from pineapple fruit and peel waste are in all likelihood liable for the discovered DPPH radical scavenging pastime, as they are able to without difficulty donate hydrogen and act as antioxidants. The DPPH assay is a well-installed technique for assessing the antioxidant capacity of diverse samples, inclusive of plant extracts. The cap potential of the pineapple fruit and peel waste extracts to efficiently scavenge DPPH radicals shows that they own large antioxidant pastime. Antioxidants play a critical position in protective cells from oxidative harm resulting from unfastened radicals, which can be related to diverse illnesses and ageing processes.

Overall, the findings from the DPPH assay suggest the capacity of those pineapple waste extracts as a wealthy supply of antioxidants. The consequences similarly aid the perception that pineapple fruit and peel waste may be applied to achieve precious antioxidant compounds that can locate programs with inside the improvement of practical foods, nutritional supplements, and different fitness-selling products. Further studies into the precise antioxidant compounds found in those extracts and their capacity fitness blessings is warranted to completely discover their application and business viability.

3.6. Protein Extraction and SDS-PAGE Analysis

To achieve the entire protein content material and investigate the protein profiles of waste extracts from pineapple fruit (PFW) and peel (PPW), specific extraction methods, specifically phosphate buffer and warm SDS-Tris-HCl buffer methods, had been employed. The general quantity of protein extracted from pineapple fruit and pores and skin trimmings numerous relying at the extraction technique used. The protein content material of the extracted samples became predicted the use of a widespread bovine serum albumin (BSA) chart. For PFW, protocol A ended in a mean protein yield of 614.67 ± 12 µg/g, even as protocol B yielded 652.61 ± 8 µg/g of protein as shown in Table 5. As for pineapple peel waste (PPW), protocol A yielded a mean of 511.50 ± 8 µg/g protein, while protocol B ended in 581.49 ± 17.33 µg/g of protein. Among the 2 protocols, protocol B exhibited the very best performance in extracting protein from each pineapple fruit and peel waste samples. Following that, the isolated proteins were submitted to SDS-PAGE analysis to separate the proteins largely based entirely on their molecular masses. SDS-PAGE is a commonly used procedure for protein separation, permitting the estimation of molecular weights and the visualization of protein profiles. The protein profiles received from the SDS-PAGE evaluation of pineapple fruit and peel waste extracts are but to be presented. This evaluation will allow the identity and assessment of diverse protein bands gift with inside the samples, which can also additionally shed mild at the particular proteins that make a contribution to the capacity antioxidant and different useful residences of the pineapple waste extracts. The characterization of protein content material and profiles is critical for knowledge the capacity packages of those pineapple waste extracts in diverse industries, together with food, pharmaceuticals, and cosmetics. Identifying particular proteins of hobby can result in the improvement of novel merchandise or formulations with more suitable dietary or useful residences. Further evaluation and identity of those proteins could be critical to unraveling the whole capacity of using pineapple peel and fruit waste as a precious aid in diverse fields.

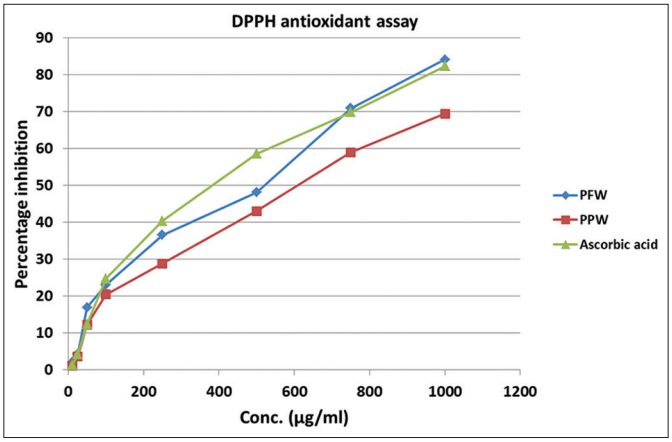

Figure 2: Percent inhibition of DPPH radical scavenging activity in relation to concentration in the methanolic extracts of pineapple fruit waste and peel waste.

The protein samples extracted from PFW and peel waste (PPW) the usage of the 2 protocols had been subjected to SDS-PAGE evaluation. The SDS-PAGE gel becomes categorized with lane 1 because the molecular weight marker, lane 2 as PFW pattern, and lane three as PPW pattern as shown in Figure 3. The marker protein bands spanned more than a few molecular weights from 10 kDa to excessive molecular weight proteins of 250 kDa, presenting reference factors for figuring out the molecular weights of the proteins with inside the samples as proven in discern three.

Due to the constrained quantity of protein received from Protocol A, most effective 10 µg of protein become loaded onto the SDS-PAGE gel for each PPW and PFW samples, and silver staining become used for visualization. In contrast, Protocol B yielded better protein concentrations, taking into account higher visualization of protein bands the usage of Coomassie Brilliant Blue (CBB) staining in each PFW and PPW extracts. The SDS-PAGE evaluation discovered a few variations among the 2 protocols. In Protocol A, the protein bands received had been of bad first-class and less in wide variety as compared to Protocol B. Moreover, many peptides with inside the molecular weight variety of 20-a hundred and fifty kDa had been absent from the PPW pattern in Protocol A.

Table 5: Protein yield from pineapple fruit waste and peel waste samples using phosphate buffer and SDS- Tris-HCl buffer methods.

Test sample	Protein yield (µg/g fresh weight)	
	Protocol – A	Protocol – B
Pineapple fruit waste	614.67±12.66	652.61±8.11
Peel waste	511.50±8.3	581.49±17.33

On the opposite hand, Protocol B produced higher outcomes, with better protein attention bands seen in each PFW and PPW samples. However, bands beneath 10 kDa had been now no longer seen, indicating the presence of low molecular weight peptides that could not be detected the usage of SDS-PAGE. These low molecular weight peptides are of hobby as they will show off huge antimicrobial hobby, as mentioned with the aid of using Raghavan and Kristinsson [27].

The presence of low molecular weight proteins/peptides in pineapple fruit and peel waste extracts highlights their ability as a precious useful resource for antimicrobial dealers or different bioactive compounds. Further characterization and identity of those low molecular weight peptides could be important to discover their precise bioactive houses and ability programs in diverse fields, consisting of prescription drugs and meals preservation.

In conclusion, SDS-PAGE evaluation furnished precious insights into the protein profiles of pineapple fruit and peel waste extracts. The outcomes indicated variations in protein content material and first-class among the 2 extraction protocols, and the presence of low molecular weight peptides with ability antimicrobial hobby provides to the importance of making use of pineapple waste for diverse useful programs.

The proteolytic pastime of bromelain became studied in fundamental waste merchandise generated for the duration of pineapple processing: the fruit (crown) and the peel (pericarp). Raw bromelain became analyzed to decide its properties, which include pH, general protein content material, and general pastime. Interestingly, the most pH cost became determined with inside the fruit (crown) location, indicating a better alkaline surroundings as compared to the pericarp location. This distinction in pH should have implications for the capability and pastime of bromelain in those waste merchandise.

To examine the whole protein content material of the samples, the Lowry approach became hired the use of BSA as a reference. The effects discovered that the fruit (crown) location exhibited the very best general protein content material, with a cost of 652.61 ± 8 µg/g, as compared to the peel location with a complete protein content material of 581.49 ± 17.33 µg/g as proven in Table 6. This shows that the fruit waste consists of a better attention of proteins, which can probably impact the proteolytic pastime of bromelain.

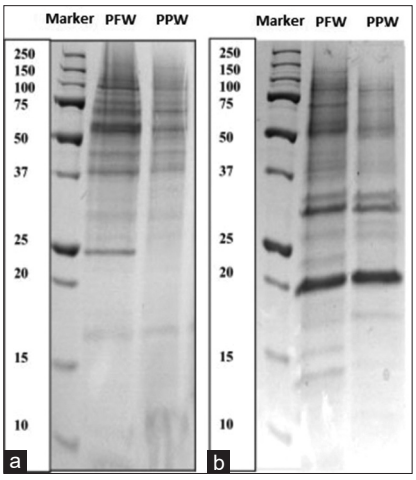

Figure 3: Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis evaluation of protein samples from Pineapple fruit waste and peel waste extracted with two different protocols (a) Phosphate buffer (b) SDS – Tris-HCl buffer.

Moreover, the pastime of bromelain became determined, and again, the fruit (crown) location displayed better bromelain pastime, with a cost of 188.86 ± 2.15 µmol/min/ml, as compared to the peel location with a pastime of 167.03 ± 2.25 µmol/min/ml. These effects advocate that the fruit waste possesses a more proteolytic capacity because of its better bromelain pastime.

The findings of this look at align with preceding studies on bromelain in one-of-a-kind elements of the pineapple. Manohar *et al.* [28] additionally mentioned the bromelain enzyme presence in various pineapple components and highlighted its softening impact on one-of-a-kind meats. Thangjam *et al.* [29] supplemented previous findings by analyzing unique pineapple components and their physicochemical properties, which have been discovered to correlate with bromelain pastime.

In conclusion, the proteolytic pastime of bromelain became tested in pineapple fruit (crown) and peel (pericarp) waste merchandise. The fruit waste confirmed better pH, general protein content material, and bromelain pastime as compared to the peel waste. These effects suggest that the fruit waste might be a precious supply of bromelain with capacity packages in diverse industries, which include meals processing and pharmaceuticals, as a result of its proteolytic properties.

The protein samples extracted from pineapple fruit and peel waste exhibited proteolytic activity. To investigate those protein samples in the same way, they were submitted to high-resolution liquid chromatography-mass spectrometry (HRLC-MS) analysis [Figure 4].

The outcomes of the HRLC-MS evaluation found out a complete of 302 proteins diagnosed in pineapple fruit trimmings and seventy-

Table 6: Proteolytic function of raw bromelain enzyme isolated from pineapple fruit and peel wastes.

Test sample	pH	Total protein (µg/g fresh wt.)	Total activity (µmol/min/ml)
Pineapple fruit waste	4.5±0.2	652.61±8.11	188.86±2.15
Peel waste	3.8±0.3	581.49±17.33	167.03±2.25

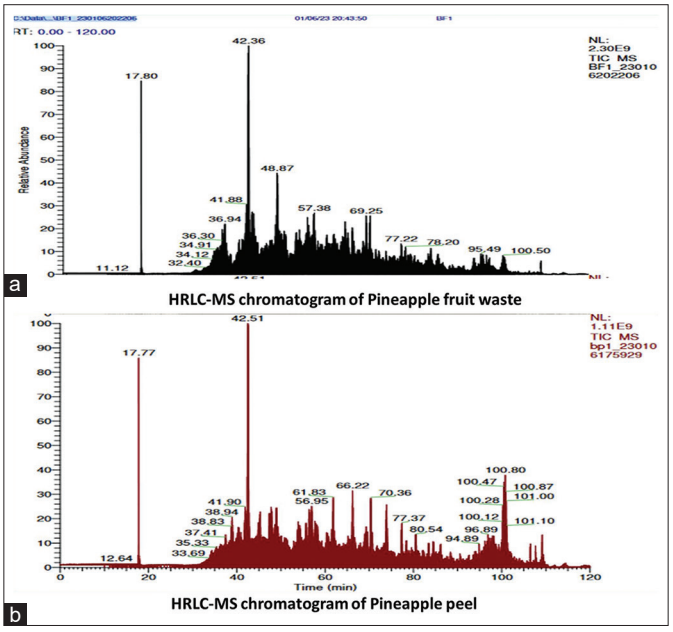

Figure 4: high-resolution liquid chromatography mass spectrometer chromatogram of pineapple fruit (a) and peel (b) waste protein samples.

seven proteins in pineapple pores and skin trimmings. This complete evaluation allowed for the identity and characterization of numerous proteins found in those waste merchandise.

For PFW, a comparative proteomic evaluation become conducted, main to the identity of 154 differentially expressed proteins as shown in Table 7. These proteins confirmed awesome degrees of expression as compared to different samples or conditions, suggesting capability roles in precise organic procedures or responses to environmental changes. In addition, one hundred ten proteins had been typically expressed throughout all samples, indicating their steady presence in PFW. Furthermore, 38 proteins remained uncharacterized, indicating the want for similarly research to decide their capabilities and capability significance. Similarly, for PPW, the comparative proteomic evaluation found out forty differentially expressed proteins as proven in desk 8. These proteins might also additionally play vital roles in numerous cell procedures precise to the peel waste. In addition, 32 proteins had been typically expressed in all samples, implying their steady presence in PPW. A small subset of five proteins remained uncharacterized, indicating the want for similarly studies to clarify their capabilities and importance.

The comparative proteomic evaluation of each pineapple fruit and peel waste samples gives treasured insights into the protein composition and capability functionalities of those waste merchandise. The differentially expressed proteins might also additionally keep good sized implications for information the particular organic procedures going on in those waste materials. The typically expressed proteins propose essential roles which might be always required in each pineapple fruit and peel waste. Moreover, the uncharacterized proteins found in each waste merchandise constitute capability goals for destiny studies and exploration.

3.7. Functional Differentiation of Proteins

The *in silico* analysis of the identified proteins from pineapple fruit and peel wastes provided valuable insights into their potential functional roles. Based on the predicted functions derived from the amino acid sequences, the proteins were categorized into seven different types, namely Electron transport, Defense, Development, Metabolism, Peptidase, Transcription, and Transport activities.

In PFWs, a total of 302 identified proteins were characterized. Among these, 89 proteins were found to be involved in Electron transport, indicating their potential roles in electron transfer processes within the cellular machinery. 64 proteins were associated with defense functions, suggesting their participation in the plant's defense mechanisms against various stresses and pathogens. 48 proteins were related to development, indicating their significance in regulating developmental processes in the fruit waste. 35 proteins were involved in metabolism, indicating their roles in various metabolic pathways and biochemical reactions. 21 proteins were identified as peptidases, highlighting their function in proteolytic processes. 27 proteins were linked to transcription, suggesting their involvement in gene regulation and expression. In addition, 18 proteins were responsible for transport activities, indicating their roles in transporting molecules across cellular membranes.

Similarly, in PPW, a total of 77 identified proteins were characterized. Among these, 22 proteins were associated with electron transport, indicating their involvement in electron transfer reactions. 16 proteins were related to defense functions, suggesting their potential role in protecting the peel waste from environmental stressors and pathogens. 4 proteins were involved in development, indicating their participation in developmental processes specific to the peel. 12 proteins were linked to metabolism, indicating their roles in various metabolic pathways within the peel waste. 8 proteins were identified as peptidases, indicating their involvement in proteolytic activities. 12 proteins were associated with transcription, suggesting their potential roles in gene regulation. Finally, three proteins were responsible for transport activities, indicating their function in transporting molecules across the cellular membranes in the peel waste.

The comparative analysis of functional differentiation of proteins in pineapple fruit and peel wastes revealed that all seven functional categories were observed in both samples as shown in Figure 5. However, it was noted that PFW exhibited a greater number of proteins in each functional category compared to PPW. This suggests that PFW may have a more diverse and complex proteomic profile compared to the peel waste.

Both pineapple fruit and peel trimmings exhibited certain common functional features, such as cell cycle signaling, chromatin condensation, cell cycle regulation, and defense proteins. These shared functional aspects may indicate the presence of conserved biological processes and pathways in both waste materials. In addition, the identification of proteins involved in autophagy highlights their potential roles in cellular recycling and maintenance processes.

Our findings are consistent with and guide the impacts of previous studies on pineapple fruit and peel waste. Our study discovered that methanolic extracts of pineapple fruit and peel wastes had the highest levels of total phenolics, total flavonoids, and antioxidant capacity among the three extracts examined. This is consistent with the findings of Lubaina *et al.* [30], who said that both ethyl acetate and ethanol-derived extracts of PPW had a greater diversity of polyphenols than aqueous and petroleum ether extracts. Furthermore, the existence of specific phenolic substances such as apigenin and quercetin in PPW, as demonstrated by Nabavi *et al.* [31], may also contribute to the reduction of lipids and free fatty acids, undoubtedly providing cardiovascular health benefits. Furthermore, Ayoub *et al.* [32] identified caffeic acid, ferulic acid, and kaempferol as phenolic chemicals contained in pineapple peel and berry seeds that have been linked to antibacterial, anti-inflammatory, anticancer, and antioxidant effects.

The inexperienced chemistry method hired with the aid of using Debora *et al.* [33] in reading pineapple with the aid of using-merchandise aligns with our examine's awareness on exploring the capability bioactive compounds, overall polyphenols, phenolic compounds, and antioxidant capability found in pineapple fruit and peel wastes.

Furthermore, our findings guide the paintings of Banerjee *et al.* [11], who used superior strategies inclusive of HPLC-MS evaluation to perceive distinct proteins, such as bromelain, in pineapple stem and fruits. The presence of bromelain and different proteins in pineapple

Table 7: Comparative proteomic analysis of pineapple fruit and peel wastes.

S. No.	Sample	Differential expressed proteins	Commonly expressed proteins	Uncharacterized	Total no. of proteins identified
1.	Pineapple fruit waste	154	110	38	302
2.	Pineapple peel	40	32	5	77

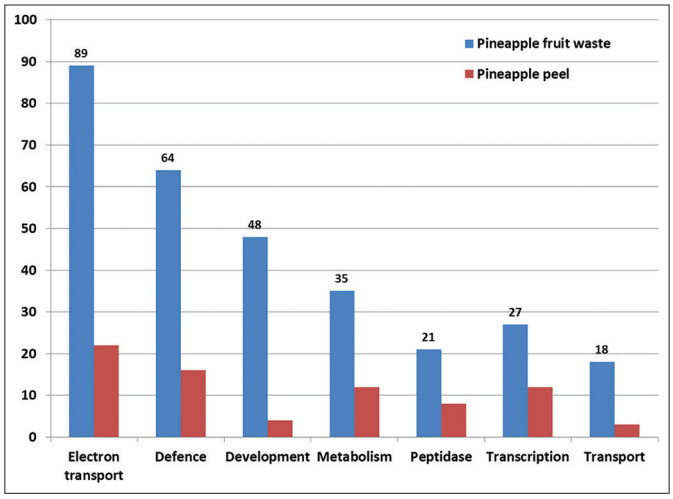

Figure 5: Bar-chat showing comparative functional differentiation in pineapple fruit and peel wastes.

waste substances highlights their capability roles in enzymatic activity, cell signaling, apoptosis, and anticancer activity.

Overall, the examine contributes to the developing frame of studies at the useful bioactive compounds and proteins found in pineapple waste substances, imparting insights into their capability packages in diverse industries and biomedical studies. The alignment of our effects with preceding findings strengthens the validity and importance of our examine's outcomes.

4. CONCLUSION

This examine explored the capability usage of pineapple fruit and peel wastes as a treasured supply of bioactive compounds and proteins. Various solvent extracts have been prepared, and their general phenolic content, general flavonoid content, and antioxidant capability have been determined. The methanolic extracts from each pineapple fruit and peel wastes exhibited the best degrees of general phenolics, general flavonoids, and antioxidant capability as compared to aqueous and hexane extracts. These findings propose that methanol-primarily based totally extraction is greater powerful in extracting bioactive compounds with capability fitness advantages from pineapple waste substances.

Through proteomic evaluation the usage of high-decision liquid chromatography-mass spectrometry (HRLC-MS), a numerous variety of proteins have been diagnosed in pineapple fruit and peel wastes. These proteins have been characterized primarily based totally on their expected functions, together with electron delivery, protection, improvement, metabolism, peptidase, transcription, and delivery activities. Both pineapple fruit and peel wastes exhibited proteins related to critical mobile processes, consisting of cell cycle signaling, chromatin condensation, mobile cycle regulation, and protection mechanisms, which might also additionally play essential roles in numerous organic pathways and autophagy.

The presence of numerous phenolic compounds in PPW, consisting of apigenin, quercetin, caffeic acid, ferulic acid, and kaempferol, indicates their capability fitness-selling properties, together with antimicrobial, anti-inflammatory, anticancer, and antioxidant activities. These findings align with preceding studies and emphasize the significance of exploring pineapple waste substances as treasured reasserts of bioactive compounds for capability use in practical foods, nutraceuticals, and pharmaceuticals.

Moreover, the identity of proteins, together with bromelain, in pineapple stem and culmination highlights their capability programs in enzymatic activity, cell signaling, apoptosis, and anticancer activity, similarly including to the cost of pineapple waste as a useful resource for biotechnological and biomedical programs.

Overall, this examine demonstrates the sizable capability of pineapple fruit and peel wastes as a wealthy supply of bioactive compounds and proteins with numerous practical properties. The consequences underscore the significance of thinking about sustainable waste control practices that capitalize at the cost of agricultural by-merchandise and decrease environmental impact. Utilizing pineapple waste for the extraction of treasured compounds now no longer handiest contributes to waste discount however additionally provides possibilities for the improvement of modern and sustainable merchandise with inside the food, pharmaceutical, and biomedical industries. More study into the separation, purification, and differentiation of certain biologically active substances and proteins from pineapple waste is required to fully exploit their possible benefits for human fitness and commercial applications.

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6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors requirements/ guidelines.

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The authors state that they have no known competing financial interests or personal ties that might have impacted the work disclosed in this research study.

9. ETHICAL APPROVALS

As this study does not involve human or animal subjects, ethical approval was not required.

10. DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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