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Isolation and Characterization of Different Compounds Present in Pineapple: Ananas Comosus

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Abstract

Pineapple is a sub-tropical fruit grown in India, Bangladesh, Malaysia, Sri Lanka, Maldives and many more. It has a juicy taste and flavour and has been used from the historical ages and used for different purposes like cardiovascular effects, antianginal, wound healing, anticancer, osteoarthritis, antidiarrheal, and anti-aging properties. There are different important phytoconstituents available in different parts of pineapple like root, peel, core, and leaves like flavonoids, terpenoids, glycosides, carbohydrates, amino acids, and many more. Most important is Bromelain which is a very good enzyme having proteolytic activity, burn wound healing activity, and good digestive properties. Due to enormous uses in versatile pharmacological profiles, there are numerous formulations that have also been discovered. These formulations are overcoming the side effects of synthetic drugs and playing a crucial role in herbal drug technology. Bromelain is a complex enzyme and different amino acids are present in it. Many researchers are trying to isolate and are trying to establish unknown pharmacological activity. The pineapple is a magic fruit and many more are yet to be discovered.

Keyword: Pineapple, tropical fruit, different pharmacological activity, bromelain, phytoconstituents, isolation.

Introduction

The word pineapple (*Ananas comosus*) is derived from the Spanish word pina meaning pine cone that was used in 1398**.** Native to Central and South America, Pineapple belonging to the family *Bromeliaceae* can be found in the Philippines, Brazil, Costa Rica, Thailand, Hawaii, India, Malaysia, Indonesia, Kenya, and China. However Brazil and Thailand are holding the first two positions in the production of the fruit worldwide[1]. India is the fifth largest producer of pineapple with an annual output of about 1.2 MT. The pineapple plant is considered an herbaceous, tropical, and monocot perennial. The size of the plant is ranged from approximately height of 75-150 cm with a spread of 90-120 cm tall and wide which means It is short in size, have a stout stump with slender, fibrous and spinose leaves that develops to a cone-shaped juicy and fleshy fruit with crown at the top[2]. There are varieties of pineapple plants that are cultivated all over the world. The most common variety found in India is Giant Kew. Still there are some other varieties that can be found such as Charlotte Rothchild Kew, Maurities, Desi, Lakhat, Queen Jaldhup etc[3]. This fruit consists of a sweet and sour flavour in it which makes it one of the most consuming

fruits to the consumers all over the world[4]. This commonly used fresh fruit can be consumed raw, it can also be canned, processed juice or as a topping ingredient on other dishes. Some of the important bioactive substances like vitamin C, β-carotene, phenolic compounds and flavonoids, polyphenolic[5], chemical, enzyme, volatile compounds are present in the pineapple leaves and waste such as peel, stem, crown, core for cure, prevention and treatment of disease .

Characterization of Pineapple roots

Diazotrophic bacteria

Diazotrophic bacteria that are generally found in pineapple roots are phylogenetically diverse and include organisms with vastly different physiological properties[6].

Materials and Methods

Multiple duplicate samples of roots of pineapple were collected[7].

Isolation of diazotrophic bacteria

According to Döbereiner et al., 1995 the diazotrophic bacteria is enumerated and isolation from plant material by washing samples of roots in sterilized water and 1g of fresh material was macerated and diluted up to 10−5 in test tubes containing 9 ml of 1/4 salt solution of malate NFb medium with pH adjusted to 7.0 where separation of 0.1 ml were inoculated into vials containing 5 ml of semi-specific semi-solid, N-free medium to test their nitrogen fixation ability and incubated for 5 days at approximately 30 ◦C, the vials shows a veil-like pellicle at the near surface of the media that were considered positive and usefull to estimate the amount of diazotrophs present in the sample by the MPN count technique. The cultures from the vials that were positive are subjected to further purification steps by streaking them onto a specific agar plate which contains 20 to 30 mg.l−1 of yeast extract [7]. The distinct colonies which grew on these media were randomly picked up and thereafter transferred to a fresh semi-solid N-free medium for further purification [7].

Physiological characteristics of diazotrophic bacterial isolates

According to Döbereiner et al., 1995, the isolated bacteria were Infused in liquid malate NFb medium containing 0.1% of ammonium chloride and incubated with agitation at 120 rpm for 2 days at 30 ◦C. Once their growth was observed, then a new aliquot was transferred to a fresh NFB liquid medium and grown at the same time and conditions above to repeat the procedure 10 times [7]. Then the aliquots of 10 µl were transferred into vials containing semi-solid N-free media to evaluate the formation of a veil-like pellicle near the surface of the semi-solid N-free media [7]. In addition, the cells from these cultures were examined under the light microscope (400×) to evaluate their shape and movement [7].

Characterization of Pineapple leaves

Bromelain

Bromelain which is found in pineapple is a proteolytic enzyme. It is promoted as a dietary supplement for reduction of pain and inflammation especially in nose and sinuses, gums and other body parts after surgery. It can also be applied topically for burns. It also has other benefits such as osteoarthritis, cancer, digestive problems and muscle soreness.

Materials and Methods

Extracted enzymes of pineapple leaves.

Chemicals - Azocasein(w/v), Ethanol(v/v), Phosphate buffer pH 7.0, Trichloroacetic acid (w / v)

Extraction of Enzyme

The enzyme extract was obtained from the leaves of pineapple (Ananas comosus) from local industries and were processed in a kitchen extractor and then centrifuged at 10,000 g for 20 min at 4 °C to remove insoluble material[8].

Determination of Enzyme Activity

According to Oliveira et al. (2006), the enzyme activity assayed by the azocasein method where azocasein 1.0% (w/v) was solubilized in 4% ethanol (v/v) and 0.1 M phosphate buffer pH 7.0, to be used as substrate[8]. The assay mixture, containing 125 µL of substrate and 125 µL of extract enzyme was incubated for 10 min at 37 °C and the reaction stopped by addition of 750 µL of 5 % trichloroacetic acid (w / v)[8]. The samples were centrifuged at 4000 g for 10 minutes at a temperature of 5°C and one unit of activity was defined as the amount of enzyme required for the production of the increase in optical density by one unit within 1 h[8].

Precipitation with Ethanol

As the methodology described by Englard and Seifter (1990) Bromelain precipitation was performed. Ethanol 98 % (w/w) cooled to 0 °C was added dropwise until concentrations of 30 and 70 % (w/w) were reached and Then the solution was then centrifuged at 10,000 for 20 min at 4 °C and the resulting pellet was suspended in 20 m M buffer phosphate pH 7.0[8].

Evaluation of pH, temperature and stability

Before and after ethanol precipitation, optimum pH, temperature and stability was evaluated[8]. Optimum pH assays were performed with exchanged buffers so the desired pH could be reached[8].Optimum temperature was accessed by changing the incubation temperature and bromelain stability was evaluated before and after purification for 180 min in all pH tested[8]. For all tests bromelain standard solutions were prepared for comparison[8].

Nanocellulose

Nanocellulose is a nanomaterial made from plant cellulose fibers with a width between 3 and 100 nanometers. It is nontoxic, non hazardous, biocompatible and environmentaly beneficial. It can be used as food stabilizer, dietary fiber, thickener, flavour carrier, in packaging, biomedical products such as adhesieve, optical materials, in cosmetics etc.

Materials and Methods

Chemicals

2 % w/v NaOH solution, 10 % aqueous dilution of sodium hypochlorite, 17.5 % w/v sodium hydroxide.

Pineapple leaf fibre characterization

The raw biomass material of the pineapple leaf fibre extracted[9].

Preparation of Test Samples

The pineapple leaf fibres were chopped into particle sizes of 2 - 6 mm, milled and then stored in clean bags before compositional analysis[9].

Compositional Analysis of the Pineapple Leaf Fibre (PALF)

The compositional analysis of pineapple leaf fibre was done using the gravimetric method and the chemical composition of PALF was measured as follows: extractives (Tappi Method T204 cm - 97, 1988; Tappi Method T207 cm-88, 1988), acid insoluble lignin (ASTM E1758 - 01, 2015), cellulose, hemicellulose (ASTM 1695 77, 2001) and ashes (UNE 57050:2003, 2003)., based on the procedure reported by Natalie et al., 2016[9].

Isolation of Nanoparticles from the PALF

Procedure

About 100 g of PALF was added into 2 % w/v NaOH solution and digested at 80 0C for 3 hours with the help of a thermostated hot plate to remove lignin in the form of soluble complexes[9]. Then the samples were washed thoroughly with distilled water and sieved with the help of a sieve of 40 mm mesh size[9]. The samples were decolorized with 10 % aqueous dilution of sodium hypochlorite for 30 minutes at 100 0C, washed and then again filtered[9]. The samples were further treated with 17.5 % w/v sodium hydroxide at 80 0C for 1 hour and the samples were washed thoroughly with water and subjected to a decolorizing process with 10 % sodium hypochlorite for 15 minutes at 80 0C[9]. Finally, they were washed with water until neutral and the dried samples obtained were milled until very fine particles were obtained[9].

Characterization of the Nano Cellulose PALF

Equipment

Carbon Evaporator (for carbon and gold coater) - Carbon Evaporator was used for preparation of the samples.

Scanning Electron Microscope (SEM) - Scanning Electron Microscope was used to carry out the morphological characterization of the PALF.

High Resolution Transmission Electron Microscope (HRTEM) - High Resolution Transmission Electron Microscope was used to carry out the characterization of the nanoparticles of the PALF.

Fourier Transform Infrared Spectroscopy of the Pineapple Leaf Fibre (PALF) -

FTIR was used for the analysis. It was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. Approximately 1.0 g weight of samples respectively was properly placed on a KBr pellet. During measurement, FTIR spectra were obtained at frequency regions of 4,000 – 600 cm-1 and co-added at 32 scans and at 4 cm-1 resolution[9].

Microcrystalline cellulose

Microcrystalline cellulose (MCC) is a cellulose derivative that is obtained by treating the alpha cellulose contained in fibrous plants using acid solutions[10]. It is used as a texturizer, anti-caking agent, fat substitute, emulsifier, extender and bulking agent as food production. Microcrystalline cellulose is widely used as excipients in pharmaceutical industries. It is also used in creams, lotions, gels, makeup and SPF enhancement.

Materials and Methods

Equipments

Analytical balance, Blender, Hot Plate, Fourier Transform Infrared Spectrophotometer, Oven, Cabinet Dryers, Filter Paper, Desiccator, pH meter, Water Bath, Universal Indicator and Other

Pineapple leaves (Ananas comosus L. Merr.), Nitric acid (HNO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium sulphite ($NaSO_3$), sodium hypochlorite (NaOCl), 37% hydrochloric acid (HCl), zinc chloride ($ZnCl_2$), potassium iodide (KI), iodine (I_2) and distilled water.

Sampling

Sampling of pineapple leaf is done purposely by the characteristics of dark green.

Preparation of reagents

Carbon Dioxide Free Distilled Water

Distilled water is boiled for up to 5 minutes and cooled. It shouldn't absorb carbon dioxide from the air.

Nitric Acid Solution 3.5% (v / v)

Concentrated nitric acid was measured at 75.3 mL and diluted with distilled water to 1.4 liters.

Sodium Sulfite Solution 2% (w / v)

Sodium sulfite weighed 20 grams and dissolved into distilled water up to 1 liter[10].

Sodium Hydroxide Solution 2% (w / v)

Sodium hydroxide weighed 20 grams and dissolved into carbon dioxide free distilled water up to 1 liter.

Sodium Hydroxide Solution 17.5% (w / v)

Sodium hydroxide weighed 119 grams and dissolved into carbon dioxide free distilled water up to 680 mL.

Hydrochloric Acid Solution 2.5 N

Concentrated hydrochloric acid was measured at 100 ml and then diluted with distilled water up to 480 mL.

Iodine Solution 0.05 N

weighed 0.2 grams of potassium iodide, dissolved it in a little amount of water and added 0.13 grams of iodine. Shake until dissolved and diluted with water to 10 mL[10].

Iodized Zinc Chloride Solution

Weighed 20 grams of zinc chloride and 6.5 grams of potassium iodide, dissolved both in 10.5 mL of water. Then add 0.5 gram of iodine and shake for 15 minutes. Filtered and stored in a place protected from light[10].

Sample Processing

Pineapple leaves were washed, drained, chopped and dried in the drying cabinet. The samples were dried to be powdered by using a blender and stored in a dry place. Weigh 100 grams of dry powder, mixed with 1.4 liters of 3.5% nitric acid containing 15 mg of sodium nitrite, put in a beaker glass, heated in a water bath at 90°C for 2 hours, filtered with filter paper. The residue was added 1 liter of a mixture of sodium hydroxide solution 2% and 1 liter of 2% sodium sulfite, heated at 50°C for 1 hour, filtered and bleached

with 1.2 liters of sodium hypochlorite 3.5% and 1.2 liter of water (1 : 1) boiled for 10 minutes, the mixture is washed and filtered to obtain alpha a cellulose[10].

Separation of Alpha Cellulose

Cellulose pulp that was obtained from pineapple leaf powder added with 680 ml of 17.5% sodium hydroxide and heated at 80°C for 30 minutes. The resulted product is then washed with water, filtered and the waste is combined with a mixture of 1.2 liters of sodium hypochlorite 3.5% and 1.2 liter of water (1: 1), heated at 100°C for 5 minutes, then washed with water until clear filtrate, filtered and squeezed, and then dried at 60°C in an oven to obtain alpha cellulose[10].

Production of Microcrystalline Cellulose

Weigh 10 grams of alpha cellulose to be added in a glass beaker and hydrolyzed with 480 ml HCl 2.5 N by boiling for 15 minutes, then poured in cold water while stirring vigorously with a spatula and let it stand for 24 hours. The microcrystalline cellulose produced from this process is washed with water to neutral pH, filtered and dried in an oven at 57°C - 60°C for 1 hour. The obtained microcrystalline cellulose were subsequently crushed. Final results are stored at room temperature in the desiccator[10].

Characterization of Pineapple Peels

Polyphenols are a class of compounds that are found in many plant foods which includes flavonoids, phenolic acids, lignans, stilbenes etc. It is beneficial to health by reducing blood sugar, increasing insulin sensitivity, decreasing cancer risk, reducing inflammation, improving digestion etc.

Materials and Methods

Material

Here the material that is used for the study was the peel of *Ananas comosus* fruit.

Chemical

Petroleum ether, Ethyl acetate, Ethanol

Hot Continuous Extraction in Soxhlet

250 g pineapple peels were finely chopped, air dried in shade at room temperature, powdered and successively extracted with 100 ml of petroleum ether, ethyl acetate, ethanol and water for eight hours using soxhlet hot continuous extraction method. The extracts were filtered and concentrated using a rotary evaporator at 50°C. The yields of extracts were calculated[11].

High Performance Liquid Chromatography (HPLC)

The petroleum ether, ethyl acetate, ethanol and water extracts of pineapple peel and 13 reference compounds (1 mg/mL) were filtered through 0.45µm PTFE filter, 20µL was injected into the HPLC. The mobile phase used here was, solvent A that is methanol- acetic acid – water (10:2:88, v/v) and solvent B that is methanol – acetic acid – water (90:2:8, v/v) with gradient program 0-15min 15% B, 16 -20min 50% B, 21 -35min 70% B, 36 -50min 100% B and finally the column was regenerated in 10min. The column was then maintained at room temperature and the eluted fractions were monitored at 280 nm. The sample peaks were identified by comparing with retention times of standard peaks.[11].

Analysis

The polyphenol content in the four extracts of pineapple peels were analyzed by HPLC and thirteen

SL NO.	Polyphenols	Amount (µg/mg)
1	Gallic Acid	0.41048
2	Catechol	0.042269
3	Chlorogenic Acid	0.72014
4	Caffeic Acid	0.279394
5	Syringic Acid	1.162054
6	p-Coumaric Acid	0.01125
7	Ferulic Acid	1.0235
8	Elagic Acid	1.79662
9	Myricetin	20.31192
10	Cinnamic Acid	0.4919
11	Quercetin	3.27957
12	Kaempferol	4.43208
13	Apigenin	4.9092

standard polyphenolic compounds (1 mg/ml) were found that is shown in the table[11].

Titratable acidity (TA)

TA was evaluated to determine the citric acid in pineapple peel extract by reflecting the fruit quality and also it indicates the sourness of pineapple[12].

Materials

MD2-PPC with a maturity index of 2 and 25% of the peel base. It was packed into a 5 kg PP woven bag then brought to the laboratory, thoroughly washed under running water to remove dirt and stored overnight at −18 °C ± 5 °C until preparation of the sample[13]. Then the frozen peels and cores were weighed and melted in a chilled refrigerator (4 °C ± 5 °C) for 60 min in the beginning of the extraction and analysis[13].

Chemicals

Ethanol absolute, Petroleum ether, Sodium hydroxide, phenolphthalein indicator, butylated hydroxyanisole, Folin-Ciocalteu phenol reagent, Aluminium Chloride, Sodium carbonate, Sodium nitrite,

Gallic acid, quercetin and 2,2-diphenyl-1-picryl-hydrazyl-hydrate(DPPH).

Preparation of Liquid Extract

Phenolics were extracted from the MD2-PPC by using a solid–liquid extraction method. Then the liquid extract of 250 g was collected using a high-speed juice extractor. After that in 25 ml of extract 150 ml of distilled water was added and mixed using a digital mechanical plate stirrer at 30 °C ± 5 °C for 2 h. Then, the extract was filtered through whatman No. 4 (125 mm) filter paper and transferred into airtight bottles to be stored at -18 °C ± 5 °C[13].

Total Phenolic Content (TPC)

After transferring 200 L of extract into a test tube 0.2 mL of Folin–Ciocalteu solution was added and the tube was vortexed. After 4 min, 1 mL of 15% sodium carbonate (Na2CO3) solution was added to the mixture and it was left to stand in the dark for 2 hours at room temperature. The blank solution was prepared in the same manner except the extract wasn't added. The absorbance of mixtures was read at 765 nm against the blank using a UV–Vis Spectrophotometer[13]. Here gallic acid stock solutions were used to generate the standard calibration curve of different gallic acid concentrations (0, 25, 50, 100, 150, and 250 g/mL)[13]. The total phenolic content was measured as milligrams of gallic acid equivalents (GAE)/100 g of FW below :

$$TPC\ (mg\ GAE/100\ g) = C1 \times DF \times v/m$$

Where, C1 = concentration of gallic acid obtained from the standard curve in mg/mL,
DF = dilution factor,
v = volume of extract in mL,
m i= weight of the plant extract in grams

Total Flavonoid Content (TFC)

A solution mixture of 1 mL of extract, 4 mL of distilled water, and 0.3 mL of 10% sodium nitrite (NaNO2) was vortexed and allowed to stand for 5 min. Then, 0.3 mL of 10% aluminum chloride (AlCl3) was added, followed by the addition of 2 mL of 1 M sodium hydroxide (NaOH). The blank solution was prepared in the same manner except the extract wasn't added. The absorbance at 510 nm was immediately measured using a UV–Vis Spectrophotometer[13]. Here the quercetin stock solutions were used to generate the standard calibration curve of different quercetin concentrations (0, 25, 50, 100, 130, and 160 g/mL)[13]. The TFC was expressed in milligrams of quercetin equivalents (QE)/100 g of FW and calculated below :

$$TFC\ (mg\ QE/100\ g) = C1 \times DF \times v/m$$

Where, C1 = concentration of quercetin obtained from the standard curve in mg/mL,
DF= dilution factor,
v = volume of extract in mL,
m = weight of the plant extract in grams

SL. NO.	Composition	Amount
1	Moisture (g/100 g FW)	85.14 ± 1.37
2	Protein (g/100 g FW)	0.58 ± 0.00

3	Fat (g/100 g FW)	0.75 ± 0.20
4	Ash (g/100 g FW)	7.13 ± 0.54
5	Carbohydrate (g/100 g FW)	6.40 ± 1.82

Materials

The peel of ripe pineapple which is deep yellow collected.

Chemicals

DPPH (2,2'-diphenyl-2-picrylhydrazyl radical), Standards of (+)–catechin (≥ 98%, 20 mg), (–)–epicatechin (≥ 98%, 20 mg), gallic acid (99.1%, 100 mg), ferulic acid (99.5%, 50 mg), ascorbic acid (100%, 100 mg), methanol and acetonitrile.

Extraction of Polyphenols from Pineapple Peels

The peels of pineapple were removed and rinsed thoroughly with water before being processed. Then dried in a ventilated oven at 60◦C For 48 hr and ground to a fine powder. The extraction of 25 g of pineapple peels were carried out by reflux, first with 150 ml of n-hexane which is to remove the non-polar compounds and then with 150 ml of methanol for 4hr at 60◦C. Then the supernatants were decanted and filtered through Whatman filter paper followed by concentration and drying in a vacuum rotary evaporator at 50 ± 10◦C, and the extracts were kept under nitrogen in a refrigerator for 1 h[14].

Identification and Quantification of Major Polyphenolic Compounds

The HPLC-MS analysis of PPE were performed on an HPLC system which is equipped with an autosampler, binary pump, degasser, and a UV-Vis diode array detection (DAD), connected directly to the mass detector with an Electrospray ionization (ESI) source. PPE (20 µL) was injected into a column. The conditions of polyphenols for the HPLC were as follows: System No 1: The mobile phase that consists of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B) using a gradient program as follows: from 10–24% B (20 min), from 24–30% B (20 min), from 30–55% B (20 min), from 55–100% B (15 min), 100% B isocratic (8 min), from 100–10% B (2 min). After that simultaneous monitoring was performed at 280 nm at a flow rate of 1.0 mL/min. System No 2: The mobile phase that consists of the same eluents as described for system no 1 using a gradient program as follows: from 10–15% B (10 min), 15% B isocratic (3 min), from 15–25% B (7 min), from 25–55% B (30 min), from 55–100% B (1 min), 100% B isocratic (5 min), from 100–10% B (0.1 min). Then again simultaneous monitoring was performed at 320 nm at a flow rate of 1.0 mL/min[14].

Mass spectra were obtained by electrospray-ionization in the negative mode where the ESI parameters were drying gas (N2) flow and temperature, 10 L/min and 350◦C, in respect with nebulizer pressure, 30 psi, and capillary voltage, 4000 V. Scan range of the mass spectrometry was m/z 50–1500[14].

Evaluation of Total Antioxidant Capacity (TAC)

To 0.1 ml of sample solution (1 mg/ml) and ascorbic acid were combined with 1 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were closed and incubated in a boiling water bath at 95◦C for 90 min. After the samples had cooled to room temperature,

the absorbance of the aqueous solution was measured at 695 nm against blank using a UV-Vis spectrophotometer where the blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent that was used for the sample and it was incubated under the same conditions. The higher value of antioxidant activity indicates that the total antioxidant activity which is higher[14].

Identification of polyphenolic compounds in pineapple peels by HPLC

Compound	Amount
Gallic acid	31.76 ± 2.28
Catechin	58.51 ± 3.59
Epicatechin	50.00 ± 4.39
Ferulic acid	19.50 ± 1.93

Characterization of Pineapple fruit

Materials

Pineapple fruit which was sanitized with 200 ppm chlorinated water for 3 min and dried at room temperature[15].

Chemicals

Metaphosphoric acid, Acetic acid, Acetonitrile, Potassium dihydrogen phosphate(KH_2PO_4), butylated hydroxytoluene (BHT), Methanol, Tetrahydrofuran.

Ascorbic acid

Ascorbic acid was analyzed using an UV-VIS spectrophotometer. After Lyophilization the fruit tissue (0.2 g) was homogenized with a homogenizer for 1 min with 10 mL of an aqueous solution that contained 30 g/L of metaphosphoric acid and 80 mL/L of acetic acid. The filtered homogenate was centrifuged for 15 min at 9,400 g. Then the supernatant was filtered and analyzed by HPLC with a analytical column and a mobile phase of acetonitrile/KH_2PO_4 (75:25 v/v) with a flow rate of 1.5 mL/min which detects the presence of ascorbic acid at 268 nm[15].

β-carotene

After Lyophilization the fruit tissue (0.2 g) was homogenized for 1 min with 10 mL of tetrahydrofuran which contained 0.4% butylated hydroxytoluene (BHT). Then this filtered mixture was centrifuged for 15 min at 6,700 g and analyzed by HPLC in which mobile phase was acetonitrile/methanol/tetrahydrofuran (58:35:7 v/v/v) with a flow rate of 1.0 mL/min detects the presence of β-carotene at 460 nm[15].

Conclusion

So there are numerous methods to isolate and characterize the different constituents from pineapple extract. If we can establish the method we will get different active phytoconstituents.

Materials

Two type of pineapple cultivars "Phulae" and "Nanglae" (Ananas comosus L. Merr) were harvested at the commercial maturity stage which is 20-40% yellow colour of fruit peel. Then the fruit pulp was immediately frozen in liquid nitrogen and stored under - 30ºC until analysis of the total phenolic, β-carotene, vitamin C and antioxidant capacity[16].

Methods

Vitamin C content

According to Leong and Shui, Vitamin C content was analyzed by using liquid chromatography on an RP-Phase with UV detection. Standard solution of ascorbic acid was taken at 5, 10, 20, 40, 80, 100 and 200 µg/ml. Then two grams of frozen pulp were homogenized in 18 ml of cold 3% meta-phosphoric acid and centrifuged at 1500×g for 15 min at 4ºC. After that the sample was immediately filtered through a Millipore membrane (0.2µm) before injection. The separation was performed using 3mM KH2PO4 in 0.35% ortho-phosphoric acid as the mobile phase at a flow rate 1.0 ml / min at 30ºC oven temperature and the eluent was monitored at 248 nm[16].

Results and Discussion

The physical and physicochemical characteristics of "Phulae" and "Nanglae" pineapple are summarized in the Table. The fruit pulp colour, firmness, pH and moisture content are significantly different between both pineapple cultivars, but no significant difference was shown in fruit peel colour, TSS, TA and TSS/ TA ratios[16].

Table: Physical and physicochemical characteristics of "Phulae" and "Nanglae" pineapple

Characteristics	Phulae	Nanglae
Peel colour (hue)	77.95 ± 2.47 a	75.10±6.28a
Pulp colour (L)	61.03 ± 1.49b	70.99±0.64a
Firmness (N)	17.19 ± 0.01a	15.64±0.01b
TSS (oBrix)	14.45 ± 0.18a	13.10±0.43a
TA (%)	0.65 ± 0.03a	0.68 ± 0.08a
TSS/TA	22.60 ± 0.66 a	21.45±2.92a
pH	3.55± 0.01b	4.56 ±0.06a
Moisture content (%)	84.87±0.18b	86.79±0.52a

Materials

Isolation of starch

The pineapple plant stems were collected, washed with distilled water and mild acid to remove the soil and other dirts. Then the stem was ground in a mixer grinder with distilled water and filtered through a

double-layered cloth. These steps were repeated several times until the milkiness of the slurry disappeared or become minimal, then the slurry was centrifuged and the supernatant was discarded. Then the residues obtained were washed with 60 % alcohol, 0.1 N NaOH, and distilled water. The centrifuged residues were dried at 40 ºC, powdered and passed through a standard sieve (75 µm), collected and stored in desiccators[17].

Nuclear magnetic resonance spectroscopic (NMR) study

The Solid-state 13C CP/MAS spectra were collected at x frequency of 100.5 MHz on a DELTA2_NMR spectrometer (JNM-ECX400II) operating at 25 ºC. 9.38976 [T] field strength was used and a spin set at 15 Hz, x 90 pulse width was 2.8 µs with a recycle time of 5 s. A contact time of 3500 µs was used for all samples; the filter width was 18 kHz. Total scans were 1028 with dimension 1[17].

FTIR spectra was recorded by using a FTIR 4100 JASCO model instrument (FT/IR-4100typeA and serial number-B076161016) and compared with corn starch. 5 mg of powdered sample was blended with potassium bromide (KBr) and made a pellet and used for this study. The resolution was 8 cm -1, and the range of wavenumber 4000-400 cm-1 was used [17].

Bioactive components present in pineapple waste

The parts that are known as waste of pineapple are Crown, Peel, stem, and Core. As example citric acid and ferulic acid can be found in pineapple leaves, bromelain can be found in leaves, stem, and peel, ascorbic acid in the core, etc[18].

Some polyphenols such as myricetin, salicylic acid, tannic acid, trans-cinnamic acid and p-coumaric acid are identified in a high dietary fiber powder from pineapple shell, a part of waste[18].

Table 1: Bioactive components present in pineapple

Bioactive compounds	Type	Part of pineapple used	Amount	Extraction method/Fermented by	Health benefits
Starch	Amylase	Stem	97.77% starch content (dry basis)/gm	Simple sample steeping method	Prevention of colonic cancer Hypoglycemic effects Resistant starch as a prebiotic Inhibition of fat accumulation
Bromelain	Sulfhydryl proteolytic enzymes	Stem, Core, Peel, Crown	23.33 µm/ml 24.13 µm/ml 13.158 µm/ml 113.79 µm/ml	Azocasein method	Anti-inflammatory Effects Immunomodulatory Effects Effects on Cancer Cells

Dietary fiber	Insoluble	Core	99.8% (dry basis)	Alcoholic extraction	
Citric acid		Peel	45-46% yield	Fermentation with *Aspergillus niger*	Prevent from liver injury Maintains tissue and cellular integrity
Ferulic acid		Core	19.50 ± 1.93 mg/100g	Fermentation HPLC–ESIMS	Prevention against atherosclerosis Cholesterol-lowering activity Antimicrobial activity

Phenolic contents

Components like p-Coumaric acid, Syringic acid, p-Hydroxybenzoic acid, Vanillin, Catechin, Epicatechin, Caffeic acid, Malic acid and Cinnamic acid are found in pineapple waste. Chromatographic methods play a crucial role in the identification of new and groundbreaking medicinal and biomedical compounds[18].

Table 2: Phenolic components present in pineapple waste

Phenolic contents	Source	Amount present	Extraction technique/Fermented by
p-Hydroxybenzoic acid	Stem	680.45±12.42 GAE mg/100 g	HPLC
Vanillin	Stem	141.00 mg L-1 of vanillin was produced from 5 g of pineapple cannery waste	Biotransformation of vanillic acid by Pycnoporus cinnabarinus
p-Coumaric acid	Crown	163.04 m/z	HPLC-DAD-ESI-MS
Syringic acid	Stem	788.21 ± 13.22 µg/g	GC-MS and HPLC
Catechin	Peel	58.51 mg/100 g dry extracts	HPLC–ESIMS
Epicatechin	Peel	50.00 mg/100 g	HPLC–ESIMS
Caffeic acid	Peel	179.03 m/z	HPLC-DAD-ESI-MS
Cinnamic acid	Stem	149.27 ± 11.11µg/g	GC-MS and HPLC
Malic acid	Liquid pineapple	0.016g/l	By using immobilized Lactobacillus delbrueckii

	waste		

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