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Pineapple (*Ananas comosus* [L.] Merr.) Cv. queen peel herbal tea with a variety of drying temperatures: bioactive compounds, antioxidant activity and antimicrobial activity

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Abstract

Pineapple peel is a by-product that is potentially developed to be herbal teas. The research objectives were to find out the proper drying temperature on total biochemical content, antioxidant and antimicrobial activity, and sensory properties of pineapple peel herbal tea. The pineapples peel herbal tea was prepared by drying peels in a cabinet dryer for 12 hours at 50, 60 and 70°C. Qualitatively, phytochemical screening was analyzed for the presence of flavonoids, alkaloids, tannins, phenols, and terpenoids. Quantitatively, total phenols, flavonoid, and alkaloid were determined by the colourimetric method. The DPPH methods applied to measure free radical scavenging activity, and the diffusion method was determined antimicrobial activity. The phytochemical screening showed the absence of terpenoid in pineapple peel teas dried at three different temperatures. The drying temperature at 60°C and 70°C produced total bioactive compounds (phenolic, flavonoid, and alkaloid) better than 50°C. Furthermore, the most significant antioxidant activity against DPPH was obtained from the product dried at 60°C with an IC_{50} of 448.31 ppm. All those herbal teas were suitable for the growth suppressing of *Bacillus subtilis* and *Candida albicans*. This study found that pineapple peel tea herbal with 60°C drying temperature was the most significant value based on bioactive components, antioxidant and antimicrobial activity, which are considered potential herbal tea products.

1. Introduction

A pineapple (*Ananas comosus* L.) is one popular fruit grown well in a tropical climate and is considered an exotic plant belonging to the Bromeliaceae family (Lawal, 2013). Bromeliaceae is named after the bromelain enzyme produced and has a proteolytic activity that can be utilized as a therapeutic agent (Bresolin *et al.*, 2013). In addition, several studies showed that bromelain has anti-inflammatory and anticancer properties, debridement of necrotic tissue and more (Houck *et al.*, 1983; Secor *et al.*, 2009; Pavan *et al.*, 2012).

According to (Food and Agriculture Organization (FAO), 2018), Indonesia was the 5th largest pineapple fruit producer in the world (1,805 M tons) after the Philippines(2,730 M tons) and Thailand (2,113 M tons) in the same region. The fruit is generally consumed as juice and jams. Each pineapple produces 9.12% of the core, 13.48% of peels, 14.49% of pulp, 14.87% of crown, and 48.04% of finished products (Ayala-Zavala *et al.*, 2010). The peel, crown, core and stem were discarded during processing. About 30 - 50% of the total fruit weight become waste during canning (Lun *et al.*, 2014). Thus, it can be estimated that Indonesia produced about 541.5 – 902.5 M tons of total waste or specifically 243.314 M tons of peels. This solid waste is generally reused as fertilizer and animal feed. However, as a high amount of waste products of the pineapple processing, its peel could be a potential source of beneficial bioactive compounds such as pineapple peel herbal tea.

In modern times, health issues become the most concerning sector due to bad lifestyle and diet habits. Researchers have been eager to solve the problem and improve health quality by studying and finding out the remedies and their new sources. It is known that every part of plants contains biochemical that could benefit human health. (Lawal, 2013) reported that the extract of various parts of pineapple possesses an antibacterial, antifungal, antiparasitic, antiviral, and anti-inflammatory effect. Based on that, pineapple waste could be used as a natural antioxidant. Active components contained in pineapple peel can be used as material for a functional

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beverage. Pineapple peel contains flavonoids, alkaloids, tannins and steroids that act as antioxidants (Kalaiselvi *et al.*, 2012). Previous studies have demonstrated that drying, an essential thermal process in producing herbal teas, may cause a significant effect on bioactive ingredients and the quality of the products (Taufik *et al.*, 2016; Nguyen and Chuyen, 2020).

Pineapple waste, especially the peel, can be processed to be a more valuable and health-promoting consumed product by drying process, such as herbal tea. Heat processing is a popular method to produce herbal tea (Nguyen and Chuyen, 2020). According to Li *et al.* (2014), pineapple peel contains polyphenolic compounds such as catechin, epicatechin, gallic acid and ferulic acid. Drying pineapple peel may be an essential step in processing these materials. The chemical and biochemical changes that may occur during this process should be studied. The objectives of this study were to find the most suitable drying temperature for the total biochemical content, antioxidant activity and antimicrobial activity of pineapple peel herbal tea. The results help to suggest a convenient procedure of drying to produce high-quality pineapple peel herbal tea.

2. Materials and methods

2.1 Materials

The *Ananas comosus L.* Merr fruit was collected from Pontianak, West Kalimantan, Indonesia, with a variety of Queen and fruit maturity levels of 20-40%, which is confirmed by the yellowish-green skin of the fruit. Pineapple peel was separated from the fruit using a knife and cut into small pieces (100 mm^3). The peels were then washed and heated using a cabinet dryer over 8 hours at 50, 60 and 70^oC and stored in a plastic jar until further analysis

2.2 Processing of pineapple peel herbal tea

The peel was separated from the fruit, washed with running water, and then drained. Next, the peel was sliced into 10 mm × 10 mm × 1 mm (Satriadi *et al*., 2015), and dried at 60, 80, 100, and 120^oC using a cabinet drying oven for 12 hrs. The dried pineapple peel samples obtained from different drying temperatures were then analyzed for phytochemical screening, total alkaloids, total flavonoids, and total phenols

2.3 Extraction

About 5 g of dried pineapple peel tea was macerated by 100 mL of solvent (methanol:aquadest ratio of 70:30) and stirred for 24 hrs at room temperature. The crude extract was filtered through Whatman No.1 paper. The residue was macerated again up to 2 times by the same procedure and collected. The extract was then concentrated using a rotary evaporator at 40^oC until viscous. The viscous extract was stored in the freezer for further analysis

2.4 Bioactive compound analysis

2.4.1 Phytochemical screening

The pineapple peel herbal tea extract was tested for the presence of alkaloids, flavonoids, phenolics, tannins and terpenoids based on the method described by (Prabhavathi *et al*., 2016). The qualitative result was expressed as + for the presence and - for the absence of phytochemicals

2.4.2 Total phenolic content

The total phenolic content evaluation was done using the Folin-Ciolcalteu method (Farhan *et al.*, 2012) with a minor modification. In brief, the sample (200 µL) was mixed with Folin-Ciocalteu reagent (1 mL) (1:10 v/v) followed by adding sodium carbonate (3 mL) (2% w/v). The solution was homogenized and incubated at room temperature for 30 mins. The absorbance was measured at a wavelength of 765 nm. Gallic acid standard solution (20-140 µg/mL) was prepared with the same procedure to obtain a calibration curve. Total phenolic content expressed as a percentage of total gallic acid equivalent per 1g extract (mg GAE /g).

2.4.3 Total flavonoid content

Total flavonoid was performed using a colourimetric method (Dewanto *et al.*, 2002; Sultana *et al.*, 2009). Briefly, 1 mL sample pineapple peel herbal tea extracts of plant material were mixed with 4 mL water in the test tube, followed by a 0.3 mL 5% $NaNO_2$ solution. After 5 mins, 0.3 mL of 10% $AlCl_3$ was added and let stand for 6 mins before adding 2 mL of 1 M NaOH. The solution was then diluted with 2.4 mL water and mixed well. The absorbance was measured at 510 nm using a spectrophotometer. Next, the quercetin standard solution (20 – 140 µg/mL) was prepared with the same procedure to obtain the calibration curve. Finally, the blank solution was distilled as a sample. Total flavonoid content was presented as percentage of total quercetin equivalent per 1 g extract (mg QE/g).

2.4.4 Total alkaloid content

The total alkaloid content evaluation was performed based on Li *et al.* (2015) with some modifications. Pineapple peel herbal tea extracts was dissolved in a 3 mL phosphate buffer solution of 4.5 pH, transferred into a separatory funnel, and mixed with 3 mL of bromocresol green solution 0.03 %. After 30 mins, 1, 2, 3 and 4 mL of chloroform was added to it and shaken for

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2 mins. The lower layer was separated after 10 mins, collected in a 10 mL volumetric flask, and diluted to the mark with chloroform. The extracts were measured for absorbance by using UV-Vis spectrophotometer at 415 nm. Berberine solution (20 - 140 µg/mL) was prepared with the same procedure to obtain a calibration curve. Total alkaloid content expressed as a percentage of total berberine equivalent per 1 g extract (mg BE/g).

2.5 Determination of antioxidant activity

Antioxidant activity was measured by DPPH radical scavenging method described by Dewi et al. (2022) with slight modification. Pineapple peel herbal tea extracts (4 mL) was added with 2 mL of 0.2 mM DPPH methanolic solution and incubated for 30 mins at room temperature in the dark. The mixture absorbance was measured at 517 nm wavelength (Dewi *et al.*, 2020). The percentage inhibition of the DPPH activity is calculated as:

$$\text{DPPH inhibition (\%)} = [(A_b - A_e)/ A_b] \times 100$$

Where A_b= absorbance of the control (blank) and A_e = absorbance of extract. DPPH radical scavenging activity's antioxidant activity was represented as the 50% inhibition (IC_{50}) value.

2.6 Determination of antimicrobial activity

The analysis of antimicrobial activity was carried out based on Brooks *et al.* (2005) by preparing pure culture of microbes, such as *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans* from the Institut Pertanian Bogor Culture Collection (IPBCC). Antimicrobial activity test by well diffusion methods was carried out based on (Garriga *et al.*, 1993). The microbial culture was used on colonies density 10^7 - 10^8 CFU/mL. Furthermore, inoculation of 0.2% starter culture into 20 mL Mueller Hinton Agar (*E. coli* and *B. subtilis*) and Sabouraud Dextrose Yeast Agar + Chloramphenicol (*C. albicans*) media and the number of colonies on each plate was 10^5 - 10^6 CFU/mL. Then, pour the medium to sterilized Petri dishes to solidify and make a well (5 wells/Petri dishes) by using a sterile Cock Borer with a diameter of 6 mm and a drop of 60 µl pineapple peel herbal tea extract (0 ppm, 500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm). Another two wells were used as positive controls for *E. coli* and *B. subtilis* (streptomycin sulphate 0.02% w/v), *C. albicans* (Ketoconazole 0.02% w/v), and negative controls (sterile Aquades). After that Petri dish was incubated at room temperature for 48 hours, and the inhibition zone was measured based on the diameter of the clear zone with the good diameter (mm)

2.7 Data analysis

The chemical characteristic assays were carried out in triplicate. The results were expressed as mean values and the standard deviation (SD). Determination of the best treatment was done by comparing the value of each treatment through the effectiveness index test following De Garmo *et al*. (1984).

3. Results and discussion

3.1 Bioactive compounds

The effect of thermal exposure to bioactive compounds has been investigated in several plant species. Most of these have indicated the degradation of alkaloids on exposure to heat and an enhancement of phenols (Nantongo *et al*., 2018). Enhancement of phenolic compounds has been associated with the thermal destruction of cell walls and sub-cellular compartments, resulting in increased levels of free phenolic compounds, the formation of novel compounds, or the inactivation of deteriorative enzymes polyphenol oxidases (Yi and Wetzstein, 2011). Alkaloids, on the other hand, are not very stable. Therefore, they undergo degradation or decomposition on exposure to air, light, moisture and heat or chemicals (Hossain *et al*., 2016).

3.1.1 Phytochemical screening

The information regarding phytochemical compounds from plants is generally obtained by phytochemical screening. Qualitative tests by phytochemical screening on plant extracts showed significant indication of the presence of specific metabolites (Soni *et al*., 2018) and our results showed in Table 1. The pineapple peel extract showed the presence of all tested phytochemical compounds, except for terpenoids. However, different results showed by a previous study by (Sharma *et al*., 2017) showed that pineapple dry at room temperature and then ground to form powder has reported the presence of terpenoid beside alkaloid, flavonoid, phenolic and tannin. According to (Husain *et al.,* 1987), the phytochemical compound is useful for maintaining human health and possessing antioxidant activity.

Table 1. Phytochemical screening of pineapple peel herbal tea extract.

Parameters	Drying Temperature		
	50°C	60°C	70°C
Alkaloids	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+
Tannins	+	+	+
Terpenoids	-	-	-

3.1.2 Total phenolic content

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According to Marinova *et al*. (2005), polyphenols are a large group of phytochemicals in edible fruits. Additionally, Khadem and Marles (2010) reported that polyphenols molecules might have one aromatic ring, thus one phenol, or more leading to polyphenols. The changes observed from pineapple peel to hot controlled drying temperature effect on the pineapple peel herbal tea total polyphenol content are delivered in Table 2.

Table 2 shows an increase in the drying temperature of pineapple peel to accelerate of total phenolic content. For example, the total phenolic content of methanolic extract of pineapple peel herbal tea obtained by temperature drying of 50, 60, and 70°C was 43.33±8.87 mgGAE/g extract, 54.58±7.94 mgGAE/g extract, and 59.17±0.72 mgGAE/g extract, respectively.

Table 2. Total phenols, flavonoids, and alkaloids of pineapple peel extract.

Parameters	Drying Temperature		
	50°C	60°C	70°C
Total phenols (mg GAE/g)	43.33±8.87	54.58±7.94	59.17±0.72
Total flavonoid (mg QE/g)	10.88±3.35	11.79±1.14	13.91±2.08
Total alkaloid (mg BE/g)	1.57±0.13	2.09±0.11	1.65±0.08

Elevating the drying temperature of pineapple peel from 50 to 70°C is thought to cause more considerable destruction of the cell tissue matrix. As a result, the phenol components are more easily released when extracted and produce a higher total phenol content. Que *et al.* (2008) indicated that the formation of phenolic substances occurred during drying at 70°C and mentioned that the formation of phenolic compounds might be due to the availability of precursors of phenolic molecules by non-enzymatic interconversion between phenolic molecules. The elevation of total phenolic content may be due to the sensitivity of bioactive compounds to heat. Phytochemicals extracted from the plant were affected by solvent polarity, the ratio of solvent and plant materials, material particle size, extraction method, temperature and sample preparation (Azwanida, 2015; Kannamba *et al.*, 2017).

3.1.3 Total flavonoid content

The total flavonoid content of methanolic extract of pineapple peel herbal tea at varied temperature drying (50, 60, and 70°C) was about 10.88±3.35 mgQE/g, 11.79±1.14 mg QE/g, and 13.91±2.08 mgQE/g, respectively (Table 2). Increasing the heating temperature used to dry the peels, the more the cell-matrix will be damaged. As a result, many flavonoid group compounds with low molecular weight will be extracted during the extraction with methanol and cause the flavonoid content to increase. Aside from solvent and extraction methods, the maturity of the plant material also affects the phytochemical extraction. Several studies showed that phytochemical content quantity increased as plant growth, particularly fruit at the ripening stage, possesses total phenolic and flavonoid content (Mahmood *et al.*, 2012). The present study showed that the higher the drying temperature on pineapple peel, the higher the flavonoid content. According to (Rababah *et al.*, 2015), the increase in pre-heating temperature decreases flavonoids' enzyme activity to degrading enzymes such as polyphenol oxidase, which caused the increase of flavonoids.

3.1.4 Total alkaloid content

The total alkaloid content of pineapple peel with different drying temperature treatment extracts was shown in Table 2. The extract's total alkaloid content varied from 1.57±0.13 mg BE/g, 2.09±0.11 mg BE/g, and 1.66±0.08 mg BE/g, respectively, with the highest result obtained by drying temperature at 60°C. In most studies, it was recommended that the drying temperature be not higher than 60°C (Sukrasno, 2014). This result is the opposite of other research that the absence of alkaloid content in pineapple peel when it has dried at 40°C (Fitriyanti *et al.*, 2019).

3.2 Antioxidant activity

The antioxidant activity of pineapple peel herbal tea extract dried at a temperature of 50, 60 and 70°C was evaluated (Table 3). The correlation of concentration of pineapple peel herbal tea methanol extract was shown in Figure 1. The IC_{50} was interpreted as the required antioxidant concentration from the sample to scavenge 50% of the radicals of DPPH. Thus, the smaller the IC_{50} value indicated, the more substantial the antioxidant power of the test subject.

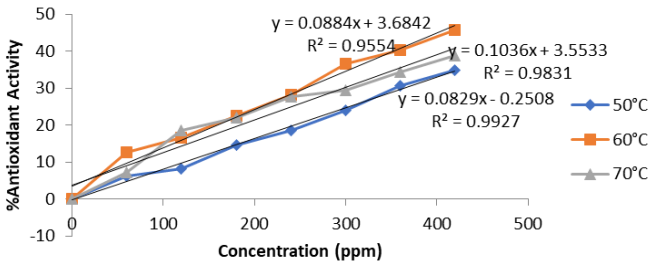

Figure 1. Correlation of concentration of pineapple peel herbal tea ethanolic extract to the oxidation inhibition.

The present study in Table 3 showed that The IC_{50} value of pineapple peel herbal tea methanol extract varied from 606.14 ppm, 448.31 ppm, and 523.95 ppm. The lowest IC_{50} value of antioxidant activity was 60°C

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then followed by 70 and 50°C. The highest antioxidant activity was that pineapple peel herbal tea dried at 60°C.

Table 3. Antioxidant Activity in IC_{50} of pineapple peel herbal tea extract at various drying temperature.

Drying temperature (°C)	IC_{50} (ppm)
50	606.14
60	448.31
70	523.95

Figure 1 shows the linear regression equation for pineapple peel herbal tea dried temperature at 50, 60 and 70°C. Interestingly, in Figure 1, the graph showed a high correlation (upper than 0.9) between the concentration of methanol extract and antioxidant activity at all dried temperature degrees. These results indicated that methanol extract contains polar compounds as phenolic compounds and serves as an antioxidant. According to (Li *et al.*, 2014), four polyphenolic compounds exhibited their antioxidant capacities: catechin, epicatechin, gallic acid, and ferulic acid in methanolic extract in a ventilated oven at 60°C for 48 hrs dried Bali pineapple peel which is grown in China. The ability of pineapple peel extract to scavenge DPPH radicals is believed due to the presence of phenolic compounds. Phenolic compounds have hydroxyl groups, and ideal structure chemistry for free radical scavenging activity by donating hydrogen atoms or electrons to a free radical and extended conjugated aromatic system to delocalize an unpaired electron (Stankovic *et al.*, 2012). Thus, herbal teas with a brewing process practically easy to dissolve antioxidant compounds and are considered beneficial as a health drink

3.3 Antimicrobial activities

In this study, the pineapple peel herbal tea extract showed varied antimicrobial activity at different drying levels (Table 4). The higher the drying temperature used affected the inhibitory activity of the microbes produced. However, the higher of oven drying temperature used, the higher the antimicrobial activity in inhibiting *B. subtilis.* Inversely, the antimicrobial activity in *C. albicans* substantially decreased with increasing temperature. In this study, the extract of pineapple peel herbal tea showed antimicrobial activity against the tested foodborne microorganisms. Phenolic extract of this fruit peel at different drying temperatures was the most effective in suppressing the growth of the pathogenic fungus *C. albicans*, as evidenced by the highest percentage of inhibition of 7.5±0.17 mm at a concentration of 2000 ppm extract, while for *B. subtillis* of 5.75±0.1 mm. However, the pineapple peel herbal tea can negatively inhibit the growth of Gram-negative bacteria of *E. coli.*

The test data raises the suspicion that the bioactive compounds contained in the pineapple peel extract are more suitable for suppressing the growth of Gram-positive pathogenic bacteria and pathogenic fungi. Results showed that was a significant difference in the inhibition zone of *B. subtilis* and *C. albicans*. The drying temperature significantly affected the inhibitory activity of microorganisms. However, there were differences in the effectiveness of extract in the two microorganisms. The increasing drying temperature had an impact on the inhibitory activity of *B. subtilis*. In contrast, *C. albicans* showed a decrease in line by increasing the drying temperature used in processing. This illustrated that temperature variation significantly affects the improvement of bioactive compounds extracted. It was previously reported that an increase in temperature up to 60°C might increase phenols, flavonoids, and tannins (Artati and Fadilah, 2007), but most likely not the 2 groups of compounds that act as anti-fungi.

The antimicrobial property of the pineapple peel extract is due to the presence of many active phytochemicals, including vitamins, terpenoids, carotenoids, coumarins, flavonoids, lignin, saponin and plant sterol (Li *et al.*, 2006). Phenolic compounds

Table 4. Determination of antimicrobial agents of pineapple peel herbal tea extract at various drying temperature.

Concentration (ppm)	Temperature Oven (°C)	Inhibition Zone (mm)		
		Bacillus subtilis	*Escherichia coli*	*Candida albicans*
500	50	0	0	0
	60	0	0	0
	70	0	0	0
1000	50	4.25±0.03	0	6.00±0.30
	60	4.50±0.09	0	5.80±0.20
	70	5.75±0.33	0	5.63±0.13
1500	50	4.25±0.03	0	7.75±0.06
	60	5.25±0.06	0	4.63±0.10
	70	5.38±0.04	0	5.00±0.04
2000	50	4.50±0.01	0	6.87±0.28
	60	5.38±0.04	0	5.00±0.10
	70	5.75±0.10	0	7.50±0.17

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Table 5. Effectiveness index of pineapple peel extract.

Variable	VQ	NQ	50°C		60°C		70°C	
			EV	TV	EV	TV	EV	TV
IC_{50}	1.00	0.21	0.00	0.00	1.00	0.21	0.52	0.11
Total phenolics	0.90	0.19	0.00	0.00	0.71	0.13	1.00	0.19
Total flavonoids	0.80	0.17	0.00	0.00	0.30	0.05	1.00	0.17
Total alkaloids	0.70	0.15	0.00	0.00	1.00	0.15	0.17	0.03
Antimicrobial	0.70	0.15	0.00	0.00	0.17	0.02	1.00	0.15
Total	4.10			0.00		0.56		0.63

VQ: Variable quality, NQ: Normal quality, EV: Effectivity value, TV: Treatment value

present in the sample have been reported as the main bioactive components for antimicrobial activity. Most plant phenolic compounds are not toxic for human consumption; therefore, they could prevent the growth of many foodborne and food spoilage microorganisms in foods.

3.4 Effectiveness index of pineapple peel extract

According to Table 5, pineapple peel drying temperature at 60°C is considered the most widely accepted value based on bioactive components, antioxidant activity, and antimicrobial activity, which promises utilization as a herbal tea product.

4. Conclusion

As a high amount of waste products of the pineapple processing, the peel can be produced to be a pineapple peel herbal tea by applying the drying temperature at 60°C.

Conflict of interest

The authors declare no conflict of interest.

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