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Research Article

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Phytochemical screening and antioxidant activities of 31 fruit peel extract from Sumatera, Indonesia

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ABSTRACT

Antioxidants are compounds that inhibit the oxidation processes and protect cells from the harmful effects of free radicals. Phytochemical and antioxidant activity screening has been done to 31 types of fruits peel extract. Phytochemical screening performed to determine the pharmacological active ingredient such as: alkaloids, steroids and triterpenoid, flavonoids, saponins and phenolic especially those with antioxidant activity. Phytochemical screening performed by standard methods while antioxidant activity using 1-1-diphenyl-2-picrihydrazil (DPPH). Result showed that the chemical compounds of avocado fruit peel extract and mangosteen positive for alkaloids, steroid-triterpenoids, flavonoids, saponins and phenolic. Alkaloid compounds are also found in fruit peel extract of Cytrus hystrix, Carica papaya, Ipomoea batatas L, Annona muricata L, Manihot utilissima, Mangifera odorata and Manilkara zapota. Steroid-triterpenoid compounds also presented in the bark Salacca sumatrana, Mangifera odorata, Alium cepa L and Citrus sinensis L. Flavonoids are also found in Hylocereus polyrhizus , Cytrus hystrix, Ipomoea batatas L, and Citrus sinensis L. Saponins are also found in Carica papaya, Ananas comocus, Ipomoea batatas L , Annona muricata L, Dimocarpus longan, Musa paradisiacal, Manihot utilissima , Musa paradisiacal , Mangifera odorata and Alium cepa L. Phenolics were found in Cytrus hystrix, Annona muricata L, Dimocarpus longan, Solanum betaceum, Mangifera odorata, Citrus aurantifolia , Passiflora edulis, and Citrus sinensis L. From this research it is known that the peel of Persea Americana and Garcinia mangostana contained the highest antioxidant activity.

Keywords: Sumatra fruit peel extract, DPPH, Antioxidant, Phytochemical

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INTRODUCTION

Medicinal plants particularly conceive several different pharmacological active compounds that may act individually, additively or in synergy to improve health. Recently there has been an increase of interest in the potential of plants as the source of antioxidants in reducing free radical induced tissue damage [1] .Unstable reactive oxygen species (ROS) react rapidly and could destruct the biomolecules such as protein, lipid, DNA, RNA, lead to

lipid peroxidation and resulting cell structural damage, tissue injury or gene mutation [2]. Free radicals may be related to various illnesses such as arteriosclerosis, rheumatic arthritis, aging, circulatory disease, neurodegenerative diseases, alteration of DNA, could cause different type of cancer and hundreds of other pathologies [3].

The damage generally reduced by endogenous antioxidants, but additional protection is necessary, and nutritive elements from food are critical in disease prevention [4] . There are two types of antioxidants which are natural antioxidant and synthetic antioxidant . Synthetic antioxidant is manmade antioxidant through chemical process while natural antioxidant is produce by human body or plant and normally regarded as safe [5]. Fruit is a natural product and Indonesia's remarkable tropical biodiversity includes fruits that have provided significant contributions to national development programs such as those involving food, nutrition, drugs and pharmaceutical agents [6]. Fruit was known as a source of antioxidant that are beneficial for for health because it contains compounds or molecules that can scavenging and prevent free radicals and reactive oxygen species such as ascorbic acid, β-carotene, lycopene and others [7].

Sumatera has a variety of fruits that are consumed for food and health. Various fruits contain may chemical compounds that plays an important role in supporting the usefulness of the fruit crops, especially as a medicinal traditional plant. The chemical compounds which are flavonoids and phenolic compounds that have a various of benefits as an antioxidant, hypo-allergenic, antidiabetic and anti inflammatory [8]. The chemical compounds that act as antioxidant are phenolic compounds and phenolic. That group of compounds are widely abundant in nature, especially in fruits and herbs that have ability to scavenging free radicals. DPPH (1,1- diphenyl 2-picrylhydrazyl) method is a conventional method in testing the antioxidant. This method is a conventional method and has been used for determination of the activity of antioxidant compounds.

EXPERIMENTAL SECTION

The Peel Samples

The peel samples that used in this study were *Hylocereus polyrhizus*, *Cytrus hystrix, Daucus carota* L, *Carica papaya, Ananas comocus, Ipomoea batatas* L, *Annona muricata* L, *Dimocarpus longan, Solanum betaceum, Manihot utilissima, Musa paradisiacal, Persea Americana, Salacca sumatrana, Garcinia mangostana, Mangifera odorata, Citrus aurantifolia, Manilkara zapota, Passiflora edulis, Alium cepa* L, *Citrus sinensis* L, *Archidendron pauciflorum, Spondias dulcis, Annona squamosa* L, *Luffa acutangula, Phaseolus vulgaris, Sechium edule, Theobroma cacao* L, *Psidium guajava, Parkia speciosa, Morinda citrifolia* L.

Samples preparation

The dry peel samples were mashed using food blender, macerated with methanol (1 :10) for 24 hours, then filtered using filter paper. The filtrate then macerated with the same solvent to achieve methanol extract of peel samples. The methanol extract then concentrated using rotary evaporator at 55^{o}C to obtain crude extract.

Phytochemical screening

The phytochemical screening which conducted in this study include alkaloid test, steroid / triterpenoid test, saponins test, phenolic test, and flavonoid test.

Test for alkaloids

Two milliliters of ammonium chloroform and 30 g of crude extract were mixed in the test tube, stirred and filtered into capped test tube. Ten drops of concentrated sulphuric acid was added and shake well. The acidic phase at the top and organic phase at bottom layer were formed. Mayer's reagent was used to test the titrated acidic phase and a white precipitate indicates the presence of alkaloids [9].

Test for steroid and triterpenoid

Two milliliters of sulphuric acid was added to crude chloroform extract. Then ten drops of acetic anhydride was added to the mixture and the changes were observed. The colour changed from violet to blue or green indicated the presence of steroids, the colour changed from violet to red indicated the presence of triterpenoids [9].

Test for saponins
Approximately 5 mL of distilled water was added into 0,5 g crude extract, then filtered into test tube. The filtrated was shake vigorously. The stable persistent froth for 30 minutes was indicated the positive for the presence of saponins [9].

Test for flavonoid
The aqueous extract of the samples was pipette into test tube. 0,1 g Mg and a few drops of acid chloride was added. The presence of red or orange colour indicated the flavonoid

Test for phenolic
The aqueous extract of the samples was pipette into test tube. A few drops of ferric chloride was added. The presence of blue or violet colour indicated the phenolic.

Antioxidant activity of peel extract using DPPH method
Preparation of solution of peel extract and DPPH
The solution of peel extract (1000µg/mL) obtained by dissolve 10 mg crude extract into 10mL methanol. DPPH solution 0,1mM obtained by dissolving 3,95 mg of DPPH powder into 100mL methanol and keep in dark and closed container.

Antioxidant activity
The serial dilutions of peel samples (1000 µg/mL) was performed with various concentration ranging from 62,5; 125; 250 and 500 µg/mL. 100 µL of 0,1mM DPPH was added using micropipette. 100 µL methanol mix with 100 µL DPPH 0,1mM as a blank. The mixture was shaken vigorously using vortex and left to stand for 30 minutes at room temperature in a dark room. Absorbance was read using spectrophotometer at 517 nm. The scavenging effects on the DPPH radical were calculated using the following equation (1) :

$$\textbf{DPPH free radical scavenging (\%)} = \frac{Ao-As}{Ao}\ x\ 100\%$$

A_0 = Blank absorbance at 517 nm (Methanol)
As = Samples absorbance at 517 nm
The antioxidant activity experiment was conducted in triplicate.

RESULTS AND DISCUSSION

Phytochemical analyses
The result for the determination of alkaloids, steroid/triterpenoid, flavonoids, saponin, and phenolics are in **Table.1**. from all samples, 12 samples (38,7%) contains alkaloids, 10 samples (32,2%) contains steroid, 11 samples (35,4%) contains triterpenoids, 19 samples (61,2%) contains saponin, 21 samples (67,7%) contains phenolic.

The **Tables 1** shown the phytochemical screening of the samples. The presence of these secondary metabolites classes is known to have therapeutic activity against several diseases and therefore could suggest its traditional use for the treatment of various illnesses [10]. Boakye *et al* [11] has been investigated the total phenols and phytochemical constituent of four tropical fruits ; *Irvingia gabonensis, Artocarpus altilis, Annona muricata* and *Annona squamosa*. Tannins, triterpenoids, saponins, sterols, cardiac glycoside and flavonoid and coumarins were detected in the most of the fruits extracts. It revealed that these tropical fruits have considerable free radical scavenging (antioxidant) activity and an array of phytochemicals necessary to significantly health of consumers.
Saponins were detected in 61,3% of samples. Saponins are common in most plants and have been revealed that shave a wide range of biological activity such as antioxidant, anticarcinogenic and immunostimulant. Triterpenoids was detected in 32,23% of total samples. Varadharajan *et al* [12] reported that wound healing properties responsible for contraction of wound and epithelialisation by triterpenoids. Flavonoids were detected 29,03 % of total samples. Flavonoids are recognized for their antioxidant and antimicrobial activity [13]. Phenolic were found in 67,74% of total samples. Phenolic are acclaimed for their high free radical scavenging ability, antimicrobial, antiviral and anti inflammatory activity [14].

Table.1 Phytochemical screening of 31 peel samples

No	*Peel extracts*	alkaloids	Steroid / triterpenoids		flavonoids	saponin	phenolics
1	*Hylocereus polyrhizus*						
2	*Cytrus hystrix*	✓		✓	✓		✓
3	*Daucus carota L*						
4	*Carica papaya*	✓				✓	
5	*Ananas comocus*					✓	
6	*Ipomoea batatas L*	✓		✓	✓	✓	
7	*Annona muricata L*	✓				✓	✓
8	*Dimocarpus longan*					✓	✓
9	*Solanum betaceum*						✓
10	*Manihot utilissima (inner peel)*	✓					
11	*Manihot utilissima outer peel)*					✓	
12	*Musa paradisiacal*			✓	✓	✓	
13	*Persea Americana*	✓		✓		✓	✓
14	*Salacca sumatrana*		✓				
15	*Garcia mangostana*	✓	✓		✓	✓	✓
16	*Mangifera odorata*	✓	✓			✓	✓
17	*Citrus aurantifolia*						✓
18	*Manilkara zapota*	✓					
19	*Passiflora edulis*			✓		✓	✓
20	*Alium cepa L*		✓			✓	✓
21	*Citrus sinensis L*		✓		✓		✓
22	*Archidendron pauciflorum*	✓			✓	✓	✓
23	*Spondias dulcis*		✓				✓
24	*Annona squamosa L*			✓		✓	✓
25	*Luffa acutangula*					✓	✓
26	*Phaseolus vulgaris*					✓	✓
27	*Sechium edule*		✓	✓		✓	✓
28	*Theobroma cacao L*		✓	✓			✓
29	*Psidium guajava*		✓	✓	✓		✓
30	*Parkia speciosa*	✓	✓		✓	✓	✓
31	*Morinda citrifolia L*	✓		✓	✓	✓	✓

Table.2 Percentage of DPPH free radical scavenging of peel samples

No	Peel samples	[samples]				
		1000 µg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62,5 µg/mL
1	*Garcia mangostana*	79,369	79,028	79,284	81,245	82,95
2	*Persea Americana*	73,402	78,858	78,858	81,756	81,33
3	*Ipomoea batatas* L	81,074	79,795	68,968	38.789	6,479
4	*Cytrus hystrix*	83,205	61,381	39,045	26,257	9,548
5	*Mangifera odorata*	78,261	79,113	77,749	79,113	78,66
6	*Annona muricata* L	77,287	77,054	79,457	57,442	36,36
7	*Citrus sinensis* L	77,752	73,256	52,791	32,093	17,75
8	*Dimocarpus longan*	82,326	84,961	85,581	85,504	84,5
9	*Solanum betaceum*	85,891	84,109	84,109	75,349	76,74
10	*Citrus aurantifolia*	61,318	42,248	25,349	14,031	8,682
11	*Theobroma cacao* L	71,786	38,567	20,705	6,94	0,91
12	*Sechium edule*	80,887	59,841	39,932	18,66	7,053
13	*Passiflora edulis*	81,473	80,543	67,752	41,938	26,67
14	*Luffa acutangula*	37,315	20,592	12,173	12,17	5,119
15	*Annona squamosa* L	80,091	79,977	79,522	74,856	73,49
16	*Archidendron pauciflorum*	81,115	81,911	81,911	79,75	73,95
17	*Parkia speciosa*	81,115	77,133	79,75	77,82	71,33
18	*Alium cepa* L	54,894	23,83	23,546	22,411	16,45
19	*Spondias dulcis*	69,787	24,255	19,858	15,177	11,49
20	*Psidium guajava*	68,085	24,823	23,404	16,17	6,667
21	*Phaseolus vulgaris*	54,468	26,525	17,021	13,901	8,794
22	*Morinda citrifolia* L	73,759	68,085	48,085	34,894	14,47
23	*Positive control (Ascorbic acid)*	80,851	81,56	81,844	76,17	76,74

DPPH Free Radical Scavenging

From 31 peel samples, 22 showed a more extensive phytochemical content, so these samples were chosen for determination of percentage of DPPH free radical scavenging. The result was shown in **Table.2** and **Fig.1**

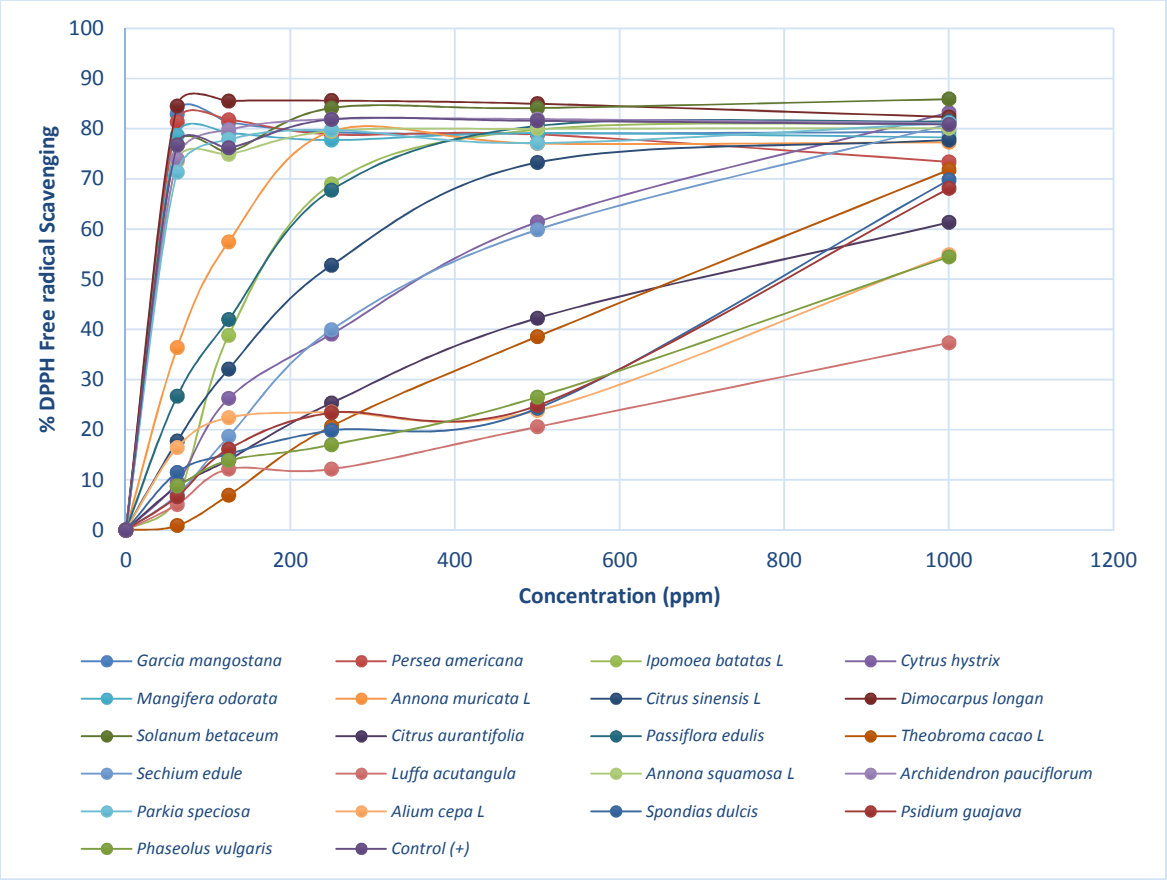

Fig.1. %DPPH free radical scavenging by 22 peel samples

Fig.1 shows that from 22 extract peel samples, 9 extract was showing the potentially highest antioxidant activity, which were *Cytrus hystrix*, *Ipomoea batatas* L, *Dimocarpus longan*, *Solanum betaceum*, *Passiflora edulis*, *Sechium edule*, *Annona squamosa* L, *Archidendron pauciflorum*, *Parkia speciosa*. This result have similar result that have been reported by several authors. Wungsintaweekul *et al* [15] reported that the methanol extracts of *C.hystrix* (leaf and peel) had potent antioxidant activity by the radical scavenging DPPH method with IC_{50} of 24,6 and 66,3 respectively. Ghasemzadeh *et al* [16] have been investigated the antioxidant activities of 6 varieties of *I.batatas* and the highest activities of antioxidant activity was shown by the leaf Vardaman varieties (IC_{50} = 450,46 µg/mL). The fruit of *Dimocarpus longan* was known to used as traditional medicine for different treatment such as promoting blood metabolism, soothing nerves and relieving insomnia. Longan pericarp tissues also contain high amounts of bioactive compounds, such as phenolic acid, flavonoid, polysaccharides, antibacterial, antiviral and antioxidant [17, 18]. The antioxidant activity of WLP (water extract longan pericarp) was determined by DPPH radical scavenging and the scavenging activity was ranging from 50-200µg/mL [17].

It was known that *Solanum betaceum* has a significant amount of phenolics, flavonoids and carotenoids which contribute to the antioxidant activity of the fruit. Nalakurumban *et al* [19] have determined the antioxidant activity by FRAP (Ferric Reducing Antioxidant Power) assay and showed that the antioxidant activity of *Solanum betaceum* was 208 AAeq/100g. The acceptable amount of phytochemicals in the fruits showed that *Solanum betaceum* is one of the richest sources of antioxidant properties that can enhance human health.

Some species of the genus *Passiflora* L especially *P.edulis, P.incarnata* and *P.alata* recognized as human food and phytomedicine. Montero *et al* [20] reported the antioxidant capacity of some *Passiflora* species, ranging from 28% to 95% of DPPH scavenging activity. *P.laurifolia* and *P.coccinea* have greater free radical scavenging activity than commercially used species (*P.edulis, P.incarnata* and *P.alata*). It revealed that Passiflora should be considered as a good source of natural antioxidant.

Table.2 shown that *Sechium edule* was one of the samples which have high antioxidant capacity (80,887µg/mL). the previous study reported that the leaves extract of *Sechium edule* had the highest FRAP capacity with EC_{50} 759ppm and there were positively high correlation between total phenolic content in *Sechium edule* leaves extract with their antioxidant activity using FRAP and DPPH assays [21].

Annona squamosa (Annonacea) is large evergreen, straggling shrub or small tree, commonly occurring in India and Indonesia. In Indonesia it is known as srikaya include in this member is sirsak (*Annona muricata*), apel mete (*Annona glabra*) and buah nona (*Annona reticulata*) [22]. The previous study reported that ethanolic leaf extract of *Annona squamosa* recorded the DPPH radical scavenging activity was 77,14% [23]. The present study show that the peel extracts of *Annona squamosa* have higher antioxidant (80,091%). This result indicate that the peel extract of *Annona squamosa* may become an important source of compound with high potential to protect health.

Table.2 shows that the pods extract of *Perkia speciosa* has a high antioxidant activity (81,115%). The antioxidant activity of *P.speciosa* was also present in the seeds and leaves, but with lower activities when compared to the activity in the pod and seed mixtures. This suggest that the pods retain greater antioxidant content than the other parts of the plant [24].

CONCLUSION

The antioxidant analysis with DPPH assay of 22 of 22 fruit peel extracts, 9 extracts was showing the potentially highest antioxidant capacity, which were *Cytrus hystrix, Ipomoea batatas* L, *Dimocarpus longan, Solanum betaceum, Passiflora edulis, Sechium edule, Annona squamosa* L, *Archidendron pauciflorum, Parkia speciosa*. The result indicate that the peel extract of the fruits or plants are promising perspectives for the exploitation of the fruit species, studied that showed the antioxidant capacity. And also useful for nutritionist to estimating the daily intake of the antioxidant compounds that have positive impact on health.

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