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The Potential of Herbal Plant Extracts Native to South Minahasa as Traditional Coconut Oil Anti rancid Agents

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Abstract
Cooking oil is often quickly becoming rancid, changing its taste and smell. Rancidity in cooking oil occurs because of the oxidation process, fat oxidation in the diet can lead to negative impacts on human health. The aim of this research were to get the plant extracts, to identify its secondary metabolites composition and to examine the potential antirancid on coconut oil made traditionally by the Minahasan, a tribe in North Sulawesi, Indonesia. The research was carried out in three stages namely extraction of plants, analysis of secondary metabolite content, application extracts on coconut oil and coconut oil quality analysis. Parameters analysis of coconut oil quality were the number of the peroxide content, free fatty acids and antioxidant activity. The result showed that all plant extracts were composed by phytochemicals namely alkaloids, flavonoids, saponins, tannins, streroid and triterpenoid. The composition of steroid and triterpenoid was highly found in the seeds of Pangi (local name). Highly intensity alkaloids was found in Serei (local name) leaf extracts as well as in Pangi seeds while the the highest content of flavonoid was found in Pandan leaf extract. On the third day after the treatment, the average content of a lowest peroxide number on coconut oil provided by Pangi seed extract was 5 g in 40 ml of oil (b/v). While the highest number of peroxide content shown on the coconut oil given by Serei extract was 5 g in 40 ml of oil (b/v). Pangi seed extract possesed free fatty acids content lower than in extracts of Pandan leaf and Serei leaf by comparison. Pangi seed extract also showed a better antioxidant activity than Serei and Pandan leaf extracts.
Keywords: serei leaf, pangi seed, pandan leaf, antioxidant, antirancid

1. INTRODUCTION

Naturally, all food contains antioxidant compounds from plants. All foodstuffs of animal or plant can be degraded both physically and chemically so that its function is reduced, for it needs to be added the antioxidants from the outside to protect the food from oxidation reactions (Milner, 2004; Mosquera et. al. 2007;). Antioxidants are necessary to preserve foods containing oil or fat with the nutritional value of the food is not reduced (Mc Cord, 2000; Mosquera et. al. 2007; Mokosuli 2008). Smell and taste of rancid are damage to food containing high lipids among other cooking oil. The process of Rancidity (rancidity) occurs due to the hydrolysis of triacylglycerols that enrich the content of fat-free asthma. At room temperature, free fatty acids will be oxidized to hydrocarbons, aldehydes or ketones, as well as epoxy and alcohol. Rancid smell is caused by a combination of oxidation products. Other than at room temperature, this process can occur during processing using high temperatures. The results of the oxidation of oils or fats in food do not only result in taste and smell bad, but it can also lose nutritional value because of defective vitamin a (carotenoids and tocopherols) and essential fatty acids in fats (Ketaren, 1986;)

Oxidation occurs in bonding is not saturated in the fatty acids. At room temperature up to a temperature of 100oC, each bond is not saturated can absorb two oxygen atoms, so the peroxide compounds formed are unstable. This peroxide radical outlines can not saturate intact so that formed two molecules of fertilization of the oxide. The process of the formation of peroxides is accelerated by the presence of light, atmosphere, humidity and an acid catalyst. Some types of metal salts or salts contained in the oil is a catalyst in the oxidation process, such as copper metal, iron, cobalt, vanadium, manganese, nickel, chromium, whereas aluminum small its influence on oxidation process (Ketaren, 1986).

The plant contains many types of secondary metabolites that are capable of inhibiting the oxidation process. The secondary metabolite compounds known as antioxidants (Heldt and Heldt, 2005) Antioxidants are defined as compounds that can delay, slow down, and prevents lipid oxidation process. In a specific sense, antioxidants are substances that can delay or prevent the onset of the reaction of antioxidase free radicals in the oxidation of lipids (Kochhar and Rossell, 1990; Mokosuli, 2008). Antioxidant compounds are generally more reactive with free radicals or oxidants that can impede the process of rancidity. Active molecules of antioxidant compounds to work frustrate the formation of peroxide with the binding of oxygen. In addition to the compound or secondary metabolites of plants as a source of antioxidants. Synthetic antioxidants are also widely used, but the side effects from the use of synthetic antioxidants are still being explored (Mokosuli, 2008, Peng and Kuo,

2003). Antioxidants in classifying into two types, namely natural and synthetic antioxidants, the use of synthetic antioxidants as BHA (Butyl Hydroxy Anisol) and BHT (Butyl Hydroxy Toluene) is very effective for inhibiting oil or grease to prevent oxidation occurs. But the use of BHA and BHT many raises fears of side effects. The results of a test that has been carried out taking action against the use of BHT can cause that brings about the swelling organ the heart and affects the activity of enzymes in the liver. It also causes fatal bleeding cavity paternal peritoneal and pancreas (Arlee et. Al. 2013). Fears of side effects of synthetic antioxidants encourage experts to seek safer natural antioxidants. Antioxidants which are currently widely used are taken from the Spice ingredients because herbs are very odorless and tasteless so affect the aroma and flavor so need to look for antioxidants are safe but not much effect on aroma and flavor of food (Peng and Kuo, 2003).

As a tropical country, Indonesia has many areas that produce coconut crops. North Sulawesi in Minahasa Kabupetan in particular, production and utilization of coconuts by the rural community for the creation of coconut oil. On the creation of coconut oil is traditionally done by taking the coconut milk, soaked for a whole night and then fried at high temperatures. This process causes hydrolysis triacylglycerols acid-free fatty acids and other derivative compounds. Fatty acids and their derivatives caused smell rancid in storage because it is so easily oxidized. The compound, among others, free fatty acids, monoglyceride, diglyceride, phosphatide, substances, carbohydrate gum and other shit.

To improve the quality of coconut oil additives, it is necessary. One of them, namely antioxidants to prevent rancidity of coconut oil and fat. Prevention of rancidity is usually done by adding material anti-rancidity (antioxidants). There are antigenic materials are natural and some are synthetic. The raw materials and the process of making natural antioxidants are quite expensive. Yield or production results are also not too high when compared to the raw materials used. As a result, the price of its products much more expensive compared to synthetic antioxidant. It also became another disadvantage of the use of such materials. In an effort to get the natural antioxidants in order to repair the quality of cooking oil coconut then extract used in this study some selected plant species originating from the South Minahasa. This research was carried out with the aim of conducting bioprospection of plants potentially antioxidants because it contains a natural antioxidant compound as a material substitution in the manufacture of traditional coconut oil in order good-quality coconut oil is formed (not quickly undergo Rancidity). South Minahasa is a Regency in North Sulawesi that has inhabited a vast coconut cultivated therefore becomes one of the main agricultural commodities. Rising oil prices led community strives to make fried cooking oil from raw materials abundant coconut but the problem faced is the process of oxidation of coconut oil is faster compared to Palm oil. It is hoped the research will be found through the formulation of plant extracts that are potentially anti-rancidity

2. MATERIALS AND METHODS

2.1. Tools and Materials

There were several tools used in this research: Eppendorf centrifuge glass, tools, UV-Vis spectrophotometer Parkin Elmer, rotary evaporator heidolp, micropipette, homogenizer, oven mammert and tools glasses. The materials used include coconut oil is made in the traditional way are obtained from the population in South Minahasa, serei leaf, pandanus leaf, seed pangi, glacial acetic acid, sodium thiosulfate cloroform-0.01 N sodium hydroxide, Potassium Iodide, amylum indicator 1%, phenols, alcohol phtalin and aquadest.

2.2. Research Methods

2.2.1.The extraction of Herbs, pandan leaf, serei and pangi seed. The extraction was done by maceration method where plants simplisia mashed/grinded into powder form was then soaked with the solvent for 2 x 24 hours at room temperature on a shaker with a speed of 50 rpm. After 2 x 24 hours, filtered then the solvent was evaporated with the rotary evaporator and the residue is thrown.

2.2.2. Analysis of secondary metabolite content of plant extracts

Analysis of the content of secondary metabolites of plants is done by using the method of Harborne 1996.

2.2.3. The application extracts on coconut oil

The use of selected plant extracts as an antioxidant to prevent the onset of Rancidity, by adding extracts on coconut oil. Comparison between extract and coconut oil is 1:8 (b/v) or 5 g extract added to 40 ml of coconut oil. This mixture is then in the oven on a humid circumstances at 110oC, it is intended to speed up the process of rancidity of oils. The last stage is a storage mix coconut oil with plant extracts for 3 x 24 hours (3 days).

2.2.4. Coconut oil quality analysis

Analysis of quality of oil includes the following: a. an analysis of the content of free fatty acids (FFA) with AOAC method, b. analysis of peroxide number, c. analysis of physical parameters (color, odor, and density) and d. analysis of antioxidant activity

3. RESULTS AND DISCUSSION

3.1. The content of secondary metabolites.

Simplisia plants used in the dry state and then made into a flour form. Ethanol extracts of leaf extracts of greatest weight show serei leaf i.e. 17.32 g whereas most extracts a small weight is the weight of the ethanol extract of pandan leaf i.e. 12.25 g (table 1). Moisture content that still present on simplisia extracted extraction results either by solvent ethanol is polar or n-heksan which is nonpolar.

Table 1. The results of the extraction to extract some spice plants

No	Herbs	The type of extract	The type of simplicia	Initial weight (g)	The weight of the extract (g)	Colours of extract
1	Pandan leaf	Ethanol (Repeat 1)	Dry	300	13,5	Brownies green
		Ethanol (Repeat 2)	Dry	300	12,25	Brownies green
2	Serei Leaf	Hexane (Repeat 1)	Dry	300	15,23	Blackish brown
		Ethanoll (Rrepeat 1)	Dry	300	17,32	Blackish brown
3	Pangi seed	Hexane (Repeat1)	Dry	300	15,25	Blackish brown
		Ethanol (Rrepeat 1)	Dry	300	16,35	Blackish brown

Note: the comparison of solvent: simplicia (solute) = 1:5

Extract the rough acquired further used for the analysis of the content of the phytochemicals. All types of extract show the content of the spread of the phytochemicals namely alkaloids, flavonoids, saponins, tannins, steroid and triterpenoid. The content of secondary metabolites has a steroid and triterpenoid namely lipids with the high intensity found in the seeds of the pangi. The content of alkaloids by high intensity was found in extracts of serei leaf and pangi seeds while the flavonoid content of high intensity found in the extract of pandan leaf (table 2).

Table 2.The result of Secondary Metabolites Analysis

Phytochemical compounds	The Intensity Of The Content Of The Compound						
	Polar Pandan leaf extract	Non PolarPandan leaf extract	Polar Serei Leaf extract	Non Polar Serei Leaf extract	Polar extract of Pangi seeds	Non Polar extract of Pangi seeds	Green tea (comparison)
Alkaloid	+	+	++	+	++	++	++
Flavonoid	+++	++	++	++	++	+	+++
Saponin	++	+	++	+	+	+	++
Steroid	+	++	+	+	++	++	+
Triterpenoid	+	++	+	+	+	++	+/-

Description: the number of the +, sign indicates the relative color intensity that describes the intensity of the content in the samples in analysis

Analysis of UV-Vis spectrophotometer pandan leaf extract showed a peak absorption at a wavelength of 287.42 nm; 384.58 nm and 401.4 nm whereas serei leaf extract showed a peak absorption at 220.72 nm; 327.23 nm and 384, 54 nm (Figure 4)

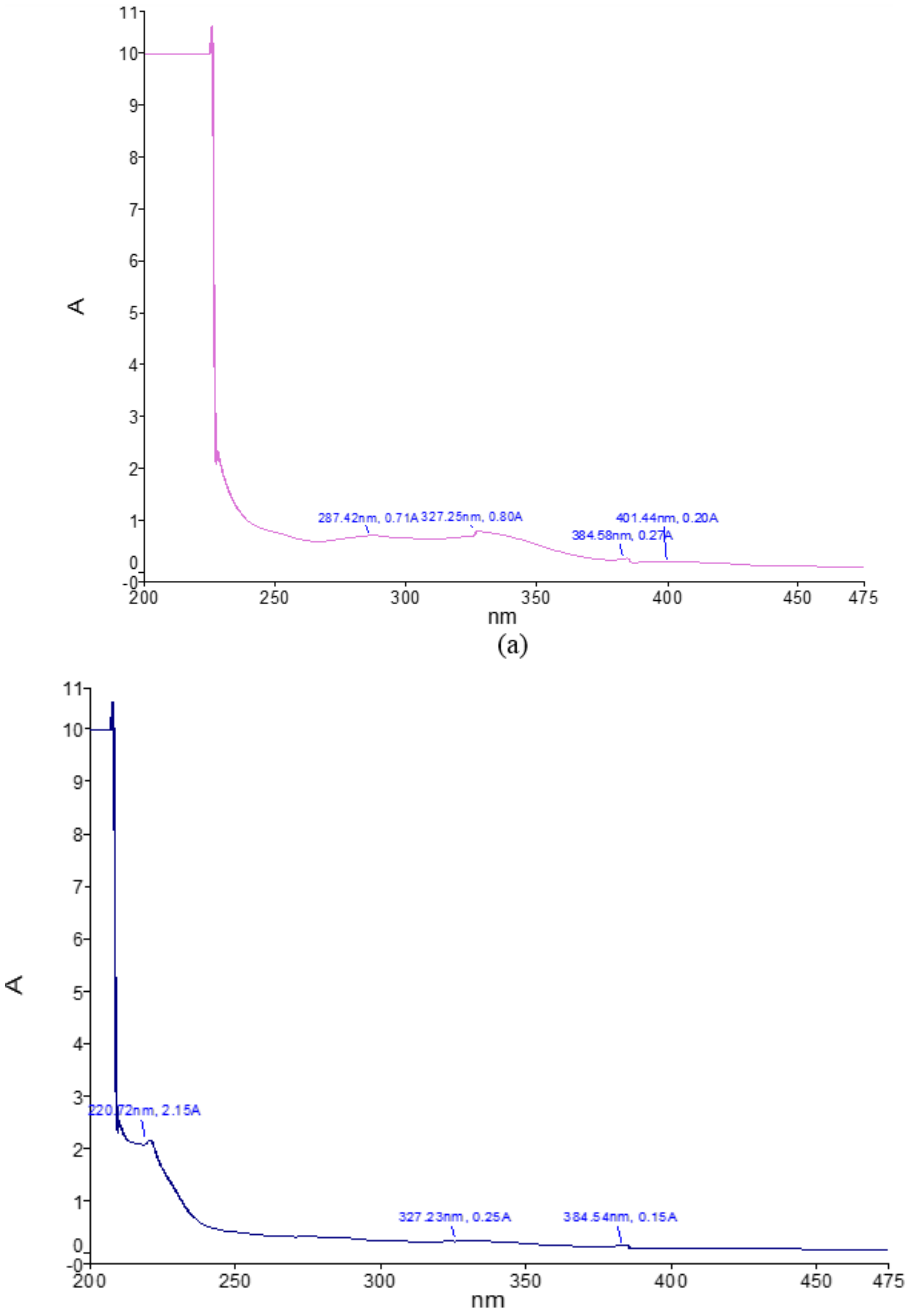
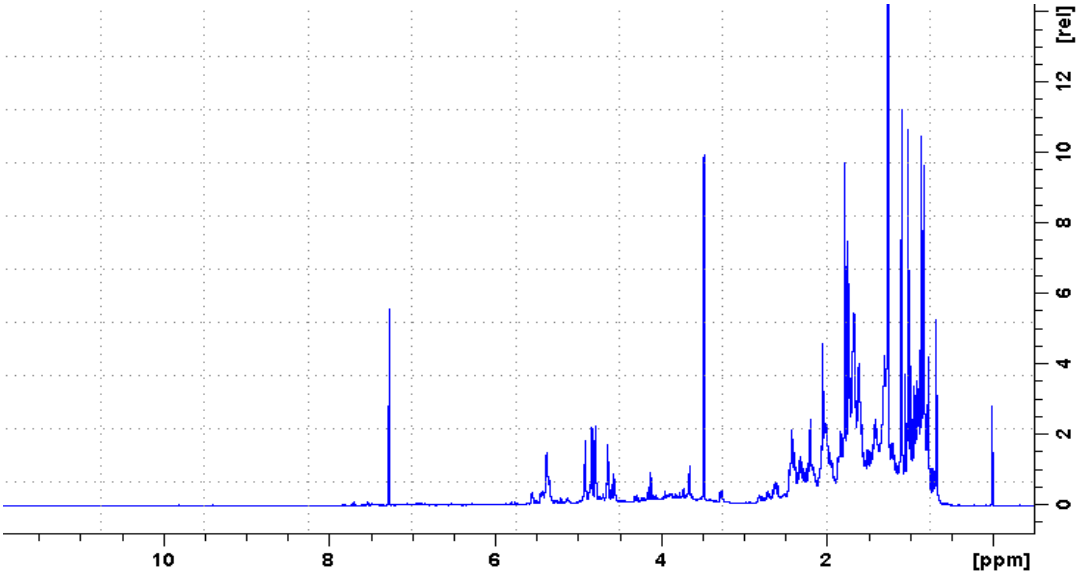
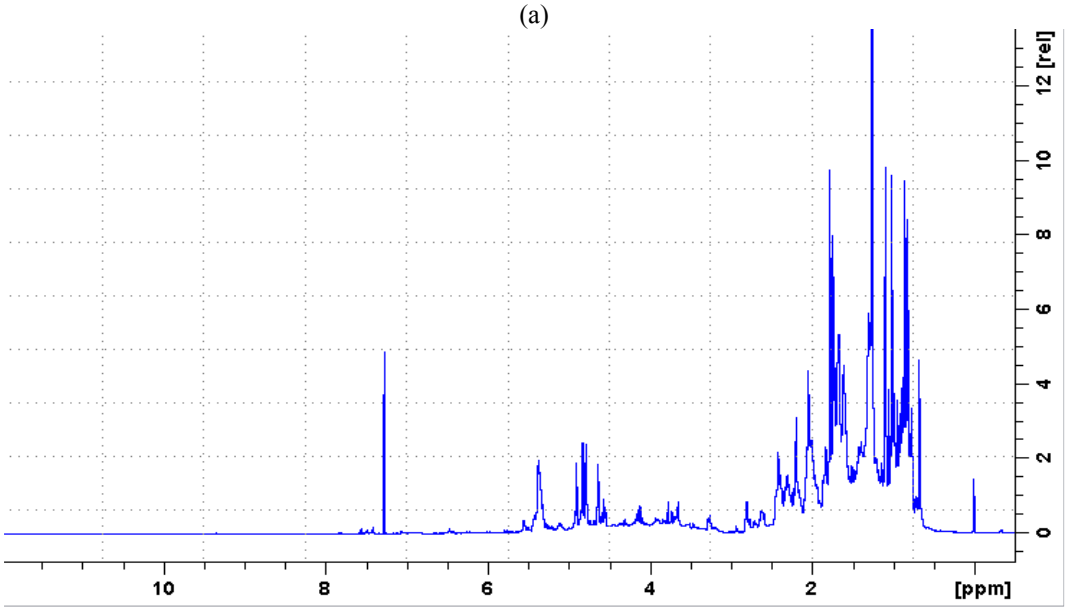


Figure 1. UV-Vis spectrograph, extract of Pandan leaf (a) and Serei leaf extract (b)

H-NMR results showed a high content of hydroxy and methoxy group cluster on both types of extracts. The main cluster is a cluster of hydroxy on flavonoids (Figure 2). This cluster can act as electron donors in the reaction of oxidation so that they can act as antioxidants (Mokosuli, 2008).



(a)



(b)

Figure 2. H-NMR results Spectrograph Pandan leaf (a) and Serei leaf extract (b)

3.2. Coconut Oil Quality Analysis

3.2.1. The Number of Peroxides

Coconut oil storage after the treatment is done at a temperature of 1100C to speed up the process of Rancidity. The addition of extract affects levels of a number of peroxide coconut oil. On the third day after the treatment, the average content of a lowest peroxide number on coconut oil provided pangi seed extract 5 g in 40 ml of oil. While the highest number of peroxide content shown on the coconut oil given extract serei 5 g in 40 ml of oil. Compared with the control treatment without extracts or indeed a third type of plant extracts with comparison 1:8 (b/v) between extract and coconut oil still produces levels of peroxide better numbers (table 3).

Table 3. Number of Peroxide on day 3 of coconut oil After being given the treatment extract

Extract	Repetition	The weight of coconut oil (ml)	The weight of extract (g)	The number of peroxide on day3
Pangi Seed	1	40	5	12,532
	2	40	5	12,532
Serei Leaf	1	40	5	14,623
	2	40	5	14,726
Pandan Leaf	1	40	5	13,625
	2	40	5	13,432
Control	1	40	5	18,634
	2	40	5	20,635

The lower number of peroxides obtained means antioxidants derived from extracts of the dried powder betel leaf it high. Flavonoids and phenolic groups have strong antioxidant activity. On the third type of extracts the retrieved content of flavonoids and phenolic compounds.

3.3.2. Free fatty acids

The content of free fatty acid (FFA) on the third type of extract is added to the coconut oil extract shows the capabilities in absorbing the oxidants which cause Rancidity in coconut oil. Refer to the average content of free fatty acid of coconut oil that is named extract treatment, the addition of seed extract pangi still shows the content of free fatty acids which lower than the addition of serei leaf extract and extract the pandan leaf (table 2). This indicates that the secondary metabolite contained in the seeds of the inhibitory activity of hydrolysis gives the pangi seed triacylglycerols into fatty acids.

Table 4. Content Of Fatty Acids (%) Coconut oil After being given the treatment extracts

Extract	Repetition	The weight of coconut oil (ml)	The weight of extract (g)	FFA (0 hari)	FFA (on day 3)	The difference of content of FFA
Pangi Seed	1	40	5	0,294	0,5423	0,248
	2	40	5	0,187	0,342	0,155
Serei Leaf	1	40	5	0,284	0,6532	0,370
	2	40	5	0,263	0,5632	0,300
Pandan Leaf	1	40	5	0,243	0,6432	0,400
	2	40	5	0,183	0,7642	0,581
Control	1	40	5	0,199	1,273	1,0743
	2	40	5	0,215	1,362	1,14758

3.3.3. Antioxidant activity

Potential antioxidant herbs extracts analyzed by getting the concentration power of drag free radicals extracts by 50% against free radicals from DPPH (IC50). As a comparison or positive used BHT, control used as synthetic antioxidants in foodstuffs especially cooking oil. Just extract the ethanol that is used to test the antioxidant activity in coconut oil. Extract of n-heksan is not recommended as a solvent plant materials that are used for food. Ethanol extract seeds pangi shows the activity of free radical attenuation is best with IC50 values 90.25 µ g/l, the ethanol extract of Serei leaf with IC50: 102.03 µg/l and ethanol extract of pandan leaf 126.7 µg/l (Figure 6). Compared to the BHT as a comparison, still showed the activity of the free radical DPPH curbs better (IC50 35.6 µ g/l).

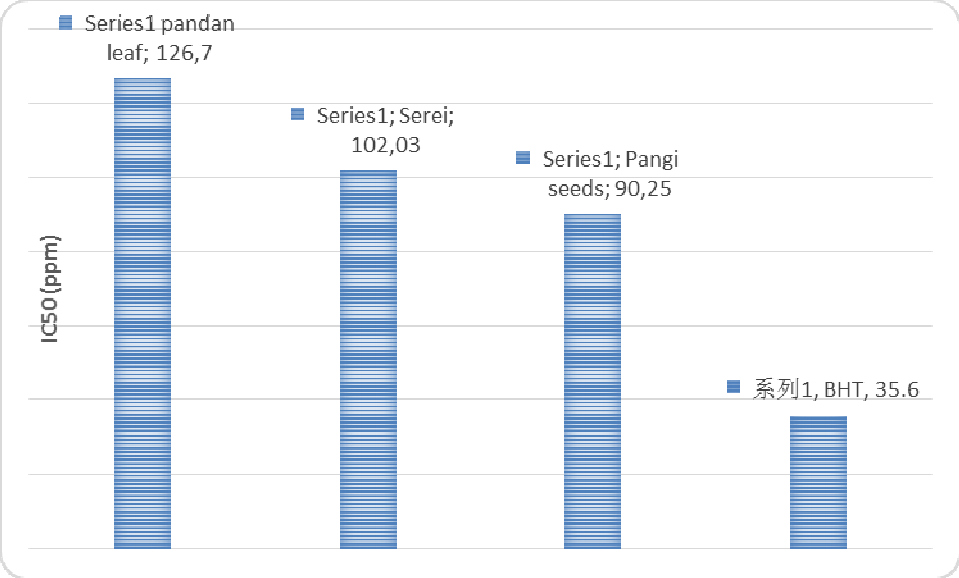


Figure 3. The antioxidant activity

Discussion

Although these three types of extracts are added in comparison with coconut oil 1:8 (b/v) indicates a good influence on the process of anti rancidity compared with controls, but those results are still under the Indonesian National Standard (SNI) (Wijana, 2010). According to SNI numeral peroxide content of coconut oil is 2 meg/kg and free fatty acid 0.3% Max. Peroxide can accelerate the process of emergence of the rancid smell and flavor that is undesirable in foodstuffs. If the number of peroxides more than 100 peroksid meq/kg of oil will be very toxic and has a bad odor. The increase in the number of peroxides is an indicator that the oil will smell rancid (Ketaren 1986).

This research has not been made on the treatment of a wide range of concentrations of the extracts so that research can be done in the future treatment of the distribution of the concentration of the extract to get the content of free fatty acid and peroxide number levels that meet standards for coconut oil. However in this research it is known that these three types of extracts were developed as a potential source of antitengik on coconut oil (figure 4 and figure 5). Research in previous years it has been known on the toxicity of extract based on toxicity test on Larval Shrimp categorized is not toxic or LC50 value of these three types of extract < 1000 ppm (Repi and Mokosuli, 2001).

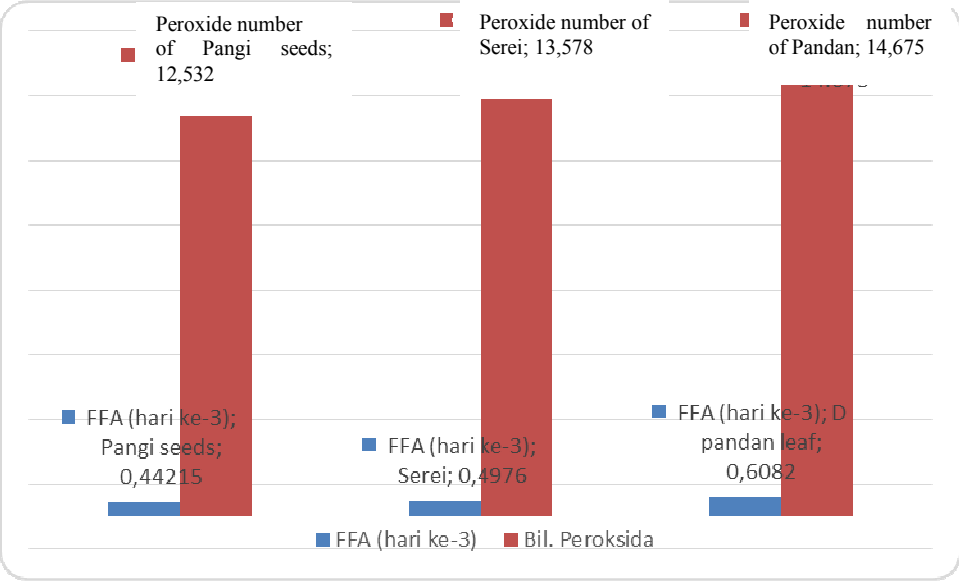
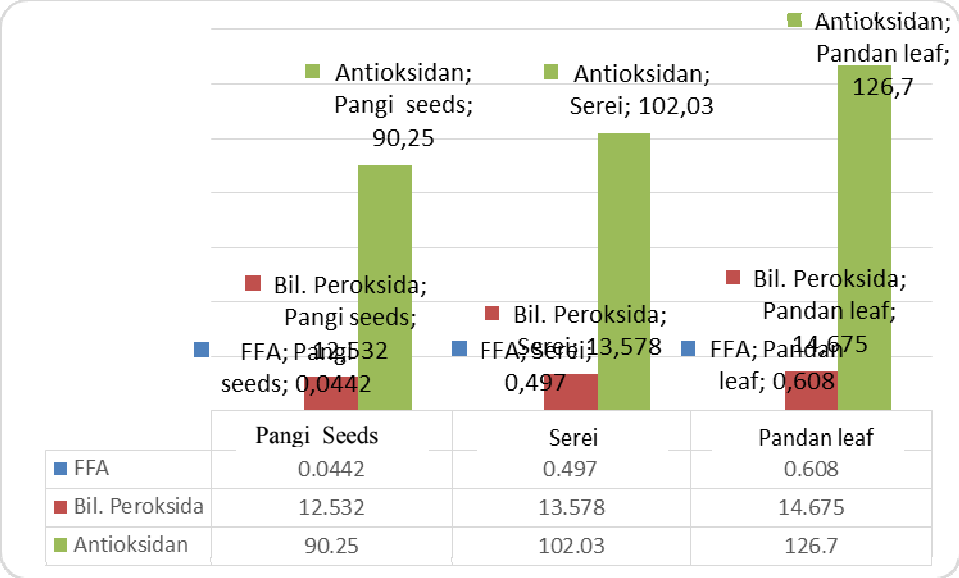


Figure 4. Fatty acid content (%) and number of peroxide coconut oil on the 3rd day of treatment



	Pangi Seeds	Serei	Pandan leaf
FFA	0.0442	0.497	0.608
Bil. Peroksida	12.532	13.578	14.675
Antioksidan	90.25	102.03	126.7

Figure 5. Comparison of free fatty acid content, the number of peroxide and antioxidant activity of coconut oil after 3 days of treatment to extract

Pangi seed extract also showed a better antioxidant activity than with serei leaf extract and extract of pandan leaf (images.). Although the content of flavonoids by high intensity found at pandan however activity of free radical curbs on coconut oil seed extract given pangi still better (IC50 = 90.25). Thus the content of secondary metabolites such as tannins, saponins, steroid and triterpenoid also influential in dampening oil oxidation by oxygen.

CONCLUSIONS

From the results of this research it can be concluded that:

1. All kinds of extracts showed the distribution of phytochemical contents of alkaloids, flavonoids, saponins, tannins, streroid and triterpenoids. High-intensity of lipid contents in the form of steroids and triterpenoids was found in pangi seeds. While for alkaloids was found in serei extract and pangi seed, and flavonoid was found in pandan leaf extract.
2. On the third day after treatment, the average content of a lowest peroxide number on coconut oil provided by pangi seed extract was 5 g in 40 ml of oil. While the highest number of peroxide content shown on serei was 5 g in 40 ml of oil.
3. Pangi seed extract possesed free fatty acids content lower than in extracts of Pandan leaf and Serei leaf by comparison..
4. Pangi seed extract also demonstrated better antioxidant activity than serei leaf extract and pandan leaf extract.

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