Extraction of sambung nyawa leaves (Gynura Procumbens [Lour. Merr] using methanol as solvent

Mersi Suriani Sinaga*, Putri Defriska Siagian and Shintia Meidinah

Department of Chemical Engineering, Faculty of Engineering, Universitas Sumatera Utara, Medan, Indonesia, 20155

*E-mail: mersisinaga68@yahoo.com

Abstract. Sambung nyawa leaves (Gynura Procumbens [Lour. Merr]) is used as traditional medicine. Sambung nyawa leaves contain flavonoid compound which can be used as natural antioxidant. This research was done to analyze the effect of the addition of Sambung Nyawa leaves extract as antioxidant and its ability to keep the quality of coconut oil. The variables used in this research were the ratio of raw material to solvent (m/v) and extraction temperature. First, extraction was done to extract flavonoid compound from Sambung Nyawa leaves. The extract of Sambung Nyawa leaves with the highest flavonoid compound was mixed with coconut oil for 3 days, 6 days, 9 days, 12 days and 15 days. Total flavonoid level from Sambung Nyawa leaves was analyzed by UV-Vis Spectrophotometry. Acid number, iodine number and peroxide number of the coconut oil were also analyzed in this research. The highest total flavonoid level obtained was 1.32% at 55°C by using 1:10 (m/v) of raw material to solvent ratio. The lowest acid number, the highest iodine number and the lowest peroxide number in coconut oil with Sambung Nyawa leaves extract after it was kept for 3 days were 0.45%, 7.90g I₂/100g and 4.40 mg O₂/100g respectively.

1. Introduction
The biodiversity in Indonesia causes it is potential to found new compound as antioxidant. Antioxidant is a compound or chemical component which inhibit damage caused by oxidation process in a certain amount [1]. Antioxidant is divided into 2 types, they are natural antioxidant and synthetic antioxidant. Example of the synthetic antioxidant is butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). They are very effective in inhibiting oxidation in oil and fat. But, the usage of BHA and BHT causes a lot of worries about the side effect [2]. The worries of the side effect of the usage of synthetic antioxidant encourage the chemists to find safer natural antioxidant. Existing antioxidant is usually extracted from fruits, spices and vegetables. One of them is Sambung Nyawa. Sambung Nyawa is shrub of Asteraceae which contains triterpenoid, polyphenols, saponin, steroids, chlorogenate acid, cafeat acid, vanillic acid, fumaric acid, para hydroxy benzoate, flavonoid and essential oil [3]. As herbal plant, Sambung Nyawa cannot stand high temperature so one of the ways to extract flavonoid from it is by using reflux extraction method. The extraction is done by using solvent in its boiling temperature for a certain time with a certain amount of solvent and with the present of reflux cooler constantly [4]. The efficiency of reflux method is determined by some factors such as particle size, stirring speed, ratio of raw material to solvent (m/v), extraction time, extraction temperature and type of solvent [5]. The solvents used are methanol, ethanol, ethyl acetate, acetone and water [6].

By considering the theory above, this research was done by reflux method and using methanol as solvent with different ratio of raw material to solvent and different extraction temperature to obtain total flavonoid level. Then, the extract with highest total flavonoid level was used as antioxidant in coconut oil. The acid number, iodine number and peroxide number of the coconut oil after the extract was added...
were analyzed and compared to Indonesia National Standard (SNI) of coconut oil 01-2902-1992 as shown in table 1.

| Characteristic           | Standard quality SNI |
|--------------------------|-----------------------|
| Water content (%)        | Max. 0.5              |
| Impurities level (%)     | Max. 0.05             |
| Iodine number (g I₂/100g)| 8-10                  |
| Peroxide number (mg O₂/100 g) | Max. 5 |
| Acid number (as lauric acid) (%) | Max. 5 |
| Saponification number (mg KOH/g) | 255-265 |
| Colour and smell         | normal                |

2. Materials and methods

2.1. Raw materials and tools
The main raw materials in this research were Sambung Nyawa leaves powder and methanol. The experimental equipments used in this research were hot plate, 40 mesh sieve, funnel, reflux condenser, three neck rounded flask, blender, electric scales, thermometer, filter paper, rotary evaporator, water bath, UV-Vis spectrophotometry and glassware.

2.2. Preparation of Sambung Nyawa leaves powder
The Sambung Nyawa Leaves were washed to remove the contaminant. Then, the leaves were aerated to dry. After they were dry, the leaves were ground into powder by using blender and then the powder was sifted by using 40 mesh sieve.

2.3. Extraction of Sambung Nyawa leaves powder
Sambung Nyawa leaves powder was weighed for 25 g and was put into three neck rounded flask. Then, methanol solvent was poured into the flask at the raw material to solvent ratio of 1:5 (m/v). Sambung Nyawa leaves powder was extracted by reflux for 3 hours by using 300 rpm stirring speed at 35°C. After the extraction was done, the extract was separated from its solvent by using rotary evaporator. Then, the extract was concentrated by water bath at 50°C until viscous extract was obtained. This procedure was repeated for 1:10 and 1:15 raw material to solvent ratio and 45°C, 55°C and 65°C extraction temperature.

2.4. Procedure of making coconut oil
Thick coconut milk from old coconut was prepared as much as 4 L. The coconut milk was cooked for 5 hours. Then, the coconut oil was separated from the impurities.

2.5. Procedure of utilization of Sambung Nyawa leaves extract as antioxidant in Coconut oil
The coconut oil was weighed as much as 15 g. The extract of Sambung Nyawa leaves was added to the coconut oil at the amount of 5% of coconut oil. The mixture was kept according to the keeping time variable (3 days, 6 days, 9 days, 12 days and 15 days). The acid number, peroxide number and iodine number of the mixture was analyzed.
3. Result and discussion

3.1. Effect of raw material to solvent ratio and extraction temperature to total flavonoid level

The relation of raw material to solvent ratio and extraction temperature to total flavonoid level can be seen in figure 1.

Figure 1 shows the effect of raw material to solvent ratio of 1:5, 1:10 and 1:15 (m/v) and extraction temperature to total flavonoid level. It shows that the total flavonoid level increased at 35-55°C but decreased at 55-65°C. Generally, the higher the solid-liquid extraction temperature and the higher raw material to solvent ratio will resulted higher total flavonoid level [8]. The increasing of temperature causes flavonoid dissolved easier by solvent and carried it out from the Sambung Nyawa leaves so that it increased the diffusion of solvent and increased the total flavonoid level. Meanwhile, if solvent to total flavonoid level ratio is the bigger than raw material to solvent ratio, the higher the flavonoid yield will be obtained [9].

According to Tan and Ho (2013), extraction temperature affects the flavonoid yield of henna plant. At the extraction temperature of 25-55°C with methanol as solvent, the flavonoid yield increased. The result of the research stated that the bigger the extraction temperature, solvent concentration and extraction time, the more flavonoid produced [10]. Meanwhile, according to Weirong (2010), extraction with raw material to solvent ratio of 1:5, 1:10, 1:15, 1:20 and 1:25 (m/v) and different extraction temperature of 60°C, 70°C, 80°C and 90°C with ethanol solvent showed the higher the extraction temperature, solvent concentration, extraction time and raw material to solvent ratio, the bigger the flavonoid yield of Opuntia milpa alta plant [8].

The increasing of extraction temperature until 55°C resulted the increasing of total flavonoid level. However, the increasing of extraction temperature to 65°C did not give significant enhancement but significantly decrease. This can be caused by the damage of the raw material because of too high temperature while processing it. So the best temperature in this research is 55°C.

The increasing of raw material to solvent ratio until 1:10 enhance the total flavonoid level to the highest. This can be caused by the ratio of the raw material to the solvent was enough, so that the solvent can penetrate well to the material and flavonoid can be dissolved by the solvent.

This research shows at 55°C and raw material to solvent ratio of 1:10 was the best condition to obtain the highest total flavonoid level. The result of the total flavonoid level from Sambung Nyawa leaves extract in this research was 1.32%.

3.2. Preservation of coconut oil
This research was done to analyze the effect of the addition of Sambung Nyawa leaves extract as antioxidant and keeping time to acid number, iodine number and peroxide number. These three parameters are the most important analysis to know the quality of the coconut oil. The characteristic of coconut oil used in this research is shown in table 2 below.

| Analysis                        | Result of the research | Quality standard (SNI 01-2902-1992) [8] |
|---------------------------------|------------------------|------------------------------------------|
| Acid number                     | 0.20                   | Max. 0.5                                 |
| Iodine number (g I₂/100 g)      | 8.38                   | 8-10                                     |
| Peroxide number (mg O₂/100 g)   | 4.00                   | Max. 5                                   |

3.3. Coconut oil acid number analysis

Effect of addition of Sambung Nyawa leaves extract and keeping time to acid number can be seen in figure 2 below.

![Figure 2. Acid number analysis result of coconut oil.](image)

Figure 2 shows the effect of addition of Sambung Nyawa leaves extract to acid number of coconut oil obtained. The chart shows that acid number increased as the keeping time increased. The acid number for 3 days, 6 days, 9 days, 12 days and 15 days of keeping time is 0.50%, 0.71%, 1.45%, 1.50% and 1.70% respectively. Meanwhile, with the addition of Sambung Nyawa leaves extract resulted 0.45%, 0.65%, 1.20%, 1.35% and 1.55%.

The research about utilization of katuk leaves flavonoid extract as antioxidant in coconut oil which was done by Cikita (2015) showed that the addition of katuk leaves extract caused the acid number increased as the keeping time increased and the bigger the total flavonoid level, the smaller the acid number obtained [11].

This research result has met the theory where the addition of Sambung Nyawa leaves extract caused the acid number lower than the acid number of coconut oil without the addition of Sambung Nyawa leaves extract. This shows that Sambung Nyawa leaves extract is effective to be used as natural antioxidant.

3.4. Coconut oil iodine number analysis
Effect of the addition of Sambung Nyawa leaves extract and keeping time to the iodine number of coconut oil can be seen in figure 3 below.

![Iodine Number Analysis of Coconut Oil](image.png)

**Figure 3.** Result of iodine number analysis of coconut oil.

Figure 3 shows the effect of the addition of Sambung Nyawa leaves extract to the iodine number obtained. The chart shows that iodine number decreased as the longer keeping time used. The iodine number of coconut oil which was kept for 3 days, 6 days, 9 days, 12 days and 15 days were 7.61 g I₂/100 g, 7.30 g I₂/100 g, 6.73 g I₂/100 g, 6.25 g I₂/100 g, 5.96 g I₂/100 g respectively. However, by the addition of Sambung Nyawa leaves extract, the iodine number become 7.90 g I₂/100 g, 7.68 g I₂/100 g, 7.17 g I₂/100 g, 6.73 g I₂/100 g, and 6.54 g I₂/100 g for each keeping time respectively.

The decrease of the iodine number is related to the peroxide compound formed as a result of oxidation and polymerization oxidation happened. This is happened because of the double bond of unsaturated fatty acid decreased because it was added by oxygen atom to form peroxide. So, the higher the peroxide number of oil will cause the iodine number decrease. The decrease of the iodine number is also caused by polymerization of free fatty acid and fatty acid which was bond in triglyceride where the double bond was detached to merge with another unsaturated fatty acid component [11]. The higher the iodine number indicates the better quality of oil [12].

Cikita (2015) has done a research about the utilization of katuk leaves flavonoid extract as antioxidant in coconut oil, the addition of the extract will cause the iodine number decrease as the keeping time gets longer and the bigger the total flavonoid level used caused the bigger the iodine number of coconut oil obtained [11].

This research’s result has met the theory where the addition of Sambung Nyawa leaves extract caused the decrease of iodine number in coconut oil. This indicates that Sambung Nyawa leaves extract is effective to be used as natural antioxidant.

### 3.5. Coconut oil peroxide number analysis

The effect of addition of total flavonoid extract from Sambung Nyawa leaves and keeping time to the peroxide number of coconut oil can be seen in figure 4.
Figure 4 shows the effect of the addition on Sambung Nyawa leaves extract to peroxide number obtained. The chart shows that the peroxide number increased as the keeping time gets longer. The peroxide number of coconut oil for 3 days, 6 days, 9 days, 12 days and 15 days of keeping time were 5.6 g O₂/100 g, 7.2 g O₂/100 g, 8.0 g O₂/100 g, 8.4 g O₂/100 g, and 10 g O₂/100 g respectively. Meanwhile, when the extract of Sambung Nyawa leaves was added, the peroxide number become 4.4 g O₂/100 g, 6.4 g O₂/100 g, 7.2 g O₂/100 g, 7.6 g O₂/100 g and 8.8 g O₂/100 g respectively for each keeping time. Peroxide number shows the rancidity of oil as a result of oxidation and hydrolysis. Peroxide number is used to measure the peroxide and hydro peroxide formed at the early stage of oil or fat oxidation. High peroxide number indicates fat or oil has been oxidized [13]. Peroxide number increases as the keeping time of coconut oil gets longer [14].

Cikita (2015) has done a research of utilization of katuk leaves flavonoid as antioxidant in coconut oil [11]. It resulted that the addition of the extract caused the peroxide number increased as the keeping time was longer and the higher the total flavonoid level used caused the peroxide number decreased.

The result of this research has met the theory where the higher the total flavonoid level used caused peroxide number decreased. The addition of Sambung Nyawa leaves extract caused the peroxide number of coconut oil increased as the keeping time was longer. This indicates that Sambung Nyawa leaves extract is effective to be used as natural antioxidant.

4. Conclusion
By this research, it can be concluded that Sambung Nyawa leaves extract is effective to be used as antioxidant in coconut oil because of the flavonoid content in Sambung Nyawa leaves. The highest flavonoid level obtained in this level was 1.32 %, extracted at 55°C by using 1:10 raw material to solvent ratio and 2 hours of extraction time. The lowest acid number, the highest iodine number and the lowest peroxide number obtained when the coconut oil was added by antioxidant and kept for 3 days was 0.45%, 7.90 g I₂/100 g and 4.40 g O₂/100 g respectively. It also resulted that the longer the keeping time of the coconut oil, the acid number and the peroxide number increased but the iodine number decreased.

Acknowledgement
Many thanks to my students for helping me in realizing this research.
References

[1] Sayuti K and Yenrima R 2015 Andalas University Press 7
[2] Hermiati R, Manalu N, Sinaga M 2012 Jurnal Teknik Kimia USU 2 37-43
[3] Priamsari M R, Susanti M M, Farmasi A, Semarang T, Atmaja A H 2016 Journal of Pharmacy 5 29-33
[4] Setiaji G 2014 Karakterisasi dan Uji Aktivasi Antioksidan Minyak Hasil Ekstraksi Biji Honje, Univeristas Islam Negeri Syarif Hidayatullah
[5] Miryanti, Arry Y I P, Sapei L, Budiono K, Indra S 2011 Ekstraksi Antioksidan dari Kulit Buah Manggis (Garcinia Mangostana L) Universitas Katolik Parahyangan
[6] Damanik D D P, Surbakti N, Hasibuan R 2014 Jurnal Teknik Kimia USU 3 10-14
[7] Standar Nasional Indonesia (SNI) Minyak Kelapa Virgin 1992 Badan Standarisasi Nasional
[8] Cai W, Gu X, Tang J 2010 Czech J. Food Sci. 28 108-116
[9] Prasetyo, Susiana, Henny, Sunjaya, Yanuar Y 2012 Lembaga Penelitian dan Pengabdian Kepada Masyarakat Perjanjian No: III/LPPM/2012.02/09-P.
[10] Tan M C, Tan C P, Ho C W 2013 Int. Food Research 20 3117-3123
[11] Cikita I 2015 Pemanfaatan Flavonoid Total dan Waktu Kontak Flavonoid Ekstrak Daun Katuk sebagai Antioksidan pada Miinyak Kelapa, Universitas Sumatera Utara
[12] Triyanto A 2013 Peningkatan Kualitas Minyak Goreng Bekas Menggunakan Arang Ampas Tebu Teraktivasi dan Penetralan dengan NaHSO₃, Universitas Negeri Semarang
[13] Laitupa F, Susane H 2010 Pemanfaatan Eugenol dari Minyak Cengkeh untuk Mengatasi Ranciditas pada Miinyak Kelapa, Universitas Diponegoro
[14] Madhavi N, Saroja T D 2014 Int. Journal of Pharma and Bio Sciences 5 437-440