Extraction of tannin from garlic skins by using microwave with ethanol as solvent

C. Pardede¹, Iriany¹,², R Tambun¹*, M D Fitri¹ and R Husna¹
¹Department of Chemical Engineering, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia
²Center of Excellence for Natural Resources-Based Technology, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia

*Email: rondang@usu.ac.id

Abstract. Tannins are phenolic compounds which have benefits as an anti-oxidant, anti-diarrhea, anti-bacterial and astringent. This study aims to determine the tannin content and antioxidant activity of garlic skins by microwave assisted extraction method. In this research, ethanol is used as solvent and the various experimental consist of 1:6 g/ml, 1:8 g/ml and 1:10 g/ml of garlic skin (solid) to ethanol (liquid) ratio; 30 s, 60 s, 120 s, 180 s and 240 s of extraction time; and 100 W and 180 W of microwave power. The tannin content of garlic skins is determined with Folin-Ciocalteu method. The antioxidant activity of garlic skins is evaluated with DPPH method. The results showed that extraction conditions had significant effect on the extraction content of tannin compounds, and the optimum condition is found at solid to liquid ratio of 1:8; extraction time of 180 s and microwave power of 100 W. Tannin content under this condition is about 1.77% and the antioxidant activity index of garlic skin extract is 1.994. The results showed that microwave-assisted extraction method is suitable and effective to extract tannin from garlic skin.

1. Introduction
Garlic (Allium sativum) is usually used as a flavour of food and traditional medicine that has been known for a long time to overcome various diseases. The components contain in garlic could be used as anti-microbial, anti-cancer, anti-oxidant, immune boosting activity, antiaging, and antiplatelet and its potential role in preventing cardiovascular disease [1]. The use of garlic produces garlic skin as a waste.
Garlic skin could be used to remove Cu²⁺ from water [2], a coating on fruits and vegetables [3], strong antioxidant activity [4], new cellulose source [5], and anode material for Li–ion [6]. Garlic skin also could be used as a new inhibitor of tyrosinase (S)-N-trans-feruloyloctopamine [7] and ethanolic extract of garlic peel demonstrated both antioxidant and antibacterial activities in cooked beef [8].
Tannins are defined as water-soluble phenolic compounds that have molecular weights between 500 and 3000. In addition to providing the usual phenolic reactions, tannins have special properties such as the ability to precipitate alkaloids, gelatine and other proteins [9]. Tannin has benefits as an anti-oxidant, anti-diarrhea, anti-bacterial and astringent.
Microwave has high frequency electromagnetic waves with frequency ranging from 0.3 to 300 GHz. Microwaves are electromagnetic waves consisting of two oscillating perpendicular fields, namely electric field and magnetic field. The principle of heating using microwave is based on the interaction of the electrical field with compounds of a material which is governed by two phenomenon’s, they are ionic conduction and dipole rotation [10-11]. Microwave assisted extraction (MAE) method is recently developed technique which has been widely applied to extract the organic compound of plants.
Ethanol used in this research because ethanol can absorb and transfer more microwave energy to the plant matrix leads to cell disruption to remove bioactive components as tannin and this solvent has high electric constant about 24.3 [10]. The ability of a solvent to absorb microwave energy depend on the dissipation factor (tan δ) for which tan δ = ε''/ε' where ε'' is the dielectric loss and ε' is dielectric constant. This research aims to determine the highest tannin and antioxidant activity with the variable such as solid (garlic skin) to liquid (ethanol) ratio, extraction time, and microwave power. Determination of tannin content is analyzed using UV-Vis Spectrophotometric and antioxidant activity is evaluated with DPPH method.

2. Methods
Materials used in this study are garlic skin (from the market of Medan), ethanol 70%, FeCl₃ 5%, gelatine, folin-ciocalteu, gallic acid, DPPH, vitamin C. The equipment used in this research consist of microwave, blender, 50 mesh sieve of tray, Whatman no. 41 filter paper, analytical balance, beaker glass, rotary evaporator, the Fourier Transform Infrared (FTIR), and UV-Vis Spectrophotometric.

2.1. Preparation of garlic skins powder and crude extract
Garlic skin is pulverised to powder. The garlic skin powder is sieved by using sieve tray of 50 mesh. The powder is taken as much as 10 g then mixed with 70% ethanol solvent with variation of solid to liquid ratio of 1: 6 g/ml, 1: 8 g/ml and 1:10 g/ml; extraction time of 30 s, 60 s, 120 s, 180 s and 240 s; microwave power of 100 W and 180 W. After extracted, the extract is filtered with Whatman no. 41 filter paper. Temperature and pH are measured after extraction process. The extract is thickened by using rotary evaporator at the temperature of 40 °C – 60 °C until become a paste form. The concentrated extract is analyzed qualitatively and quantitatively.

3. Results and discussion

3.1. Qualitative and quantitative analysis
The analysis of tannin in garlic skin is done by qualitative test using FeCl₃ 5%, HCL, gelatine and NaCl which is summarized in Table 1. The presence of tannins is characterized by changes in colour due to the addition of chemicals to the sample.

| Chemical Test | Sample Colour Before Chemical Test | Sample Colour After Chemical Test | Result |
|---------------|-----------------------------------|----------------------------------|--------|
| FeCl₃ 5% [12-15] | ![Image](example1.png) | ![Image](example2.png) | Blackish-green colour indicates the presence of tannins |
| Gelatin 1% + NaCl [13] | ![Image](example3.png) | ![Image](example4.png) | Sediment indicates the presence of tannins |
HCl [15]

Red colour indicates the presence of condensed tannins

The chemical characteristics of garlic skin are described at Figure 1, and FTIR is used to analyze chemical compounds of garlic skin. Each functional group in a material will absorb a different spectrum. Figure 1 shows that the spectrum of tannin where it can find a strong absorption around 3700 cm\(^{-1}\) and 3000 cm\(^{-1}\) with a wide and strong band centered at 3383.1 cm\(^{-1}\) and 3421.72 cm\(^{-1}\) respectively. The bands are assigned to the hydroxyl groups (OH) stretching vibrations and due to the wide variety of hydrogen bonding between OH. In this spectrum it can notice a sharp peak at 2920.23 cm\(^{-1}\) and 2939.52 cm\(^{-1}\), a small shoulder at 2858.51 cm\(^{-1}\) associated with the symmetric and antisymmetric \(-C-H-\) stretching vibrations of CH\(_2\) and CH\(_3\) groups respectively. The signal characteristics bands of carbonyl groups: \(C=O\) stretching vibration at 1735.93 cm\(^{-1}\) and \(C-O\) at 1327.03 cm\(^{-1}\) - 1033.85 cm\(^{-1}\). At peak 1454.33 cm\(^{-1}\) – 1419.61 cm\(^{-1}\) stretching vibrations of \(-C-\) Caromatic groups appear in both spectrums respectively, the alkaline group (C≡C) at the peak of 817.82 cm\(^{-1}\).

![FTIR analysis of garlic skin.](image)

3.2. Determination of total tannin content

Determination of tannin content is done by using UV-Vis Spectrophotometric. The tannin content is graphically calculated by plotting the extract concentration vs. the absorbance, and the folin-ciocalteu as reagent and gallic acid as standard solution. The wavelength obtained of tannin is 765 nm.

3.3. Effect of solid to liquid ratio

Figure 2 shows that tannin content of garlic skin is affected by solid to liquid ratio. The amount of solvent has to be considered in an extraction process because it should be sufficient to immerse all the solid. Generally, in a conventional extraction method the amount of high solvent can increase the
extract, but excessive solvent used in microwave actually can decrease the amount of extract. The highest of tannin content in garlic skin of 1.77% is obtained when the solid to liquid ratio is 1:8.

3.4. Effect of extraction time
Extraction time in MAE is very short compared to the conventional extraction. Optimizing time is another important criterion because excessive exposure to microwave radiation will reduce extraction results. Generally, by increasing the extraction time, the extraction yield will increase, although there is a risk that degradation may occur [17]. Figure 2 shows the effect of extraction time on percentage extraction of MAE. The results indicate that the tannin content increase with the increasing of extraction time for all solid to liquid ratio until 180 s, but decrease after 240 s.

3.5. Effect of microwave power

Microwave power and irradiation time are two things that influence each other. In general, low or medium microwave power with a long extraction time may increase extracts, or conversely extraction efficiency can be increased by increasing microwave power but with a shorter extraction time [10].
Figure 3 shows the effect of microwave power and extraction time on increasing temperature. The temperature is an important factor contributing to increase recoveries, not only for MAE but for all extraction techniques [18]. Figure 4 shows the effect of time and microwave power when solid to liquid ratio of 1:8, and the highest of tannin content is obtained when the microwave power of 100 W and the extraction time of 180 s.

![Figure 4. The effect of microwave power when solid to liquid ratio of 1:8.](image)

Figure 4 shows that the tannin content increases considerably when the microwave power of 180 W in 120 s and decrease drastically at 180 s. This is because high power with longer extraction time causes the temperature rise so that the extract is degraded. While when the power of 100 W is used, the tannin content begins to decline at 240 s.

3.6. Antioxidant activity
Antioxidant activity is done quantitatively by using DPPH method (2,2-diphenyl-1-picrylhydrazil). The maximum DPPH wavelength used is 300 nm because it provides the maximum absorption of the test solution. Antioxidant activity on DPPH method is expressed in IC$_{50}$. The IC$_{50}$ is calculated graphically by plotting the extract concentration vs. the corresponding scavenging effect. The antioxidant activity is expressed as the antioxidant activity index (AAI), and calculated by using equation (1) [19].

$$\text{AAI} = \frac{\text{Final DPPH concentration (\mu g mL}^{-1})}{\text{IC}_{50} (\mu g mL}^{-1})$$

(1)

The values of IC$_{50}$ are 19.76 and the AAI of 1.994 signified strong antioxidant activity. Previous research on garlic skin informed that the extract of garlic skins showed strong antioxidant activity [4]. Vitamin C has strong antioxidant activity and as a comparator, it has IC$_{50}$ of 10.94 with AAI of 3.601. It can be concluded that the antioxidant activity of garlic skin extract is weaker than vitamin C.

3.7. Mass transfer coefficient
Mass transfer occurs because of concentration difference when the component in the mixture moves in the same phase or from one phase to another, and determination of mass transfer coefficient aims to
study the rate of diffusion. The mass transfer coefficient can be calculated by the relationship between concentration and extraction time by the equation (2) [20].

\[
\frac{N_A}{V_0} = K (C_{AS} - C_A) \\
\frac{V d(c_A)}{dt} = N_A = AK_C (C_{AS} - C_A) \tag{2}
\]

Equation (3) is integrated with the boundary of \( t = 0 \) and \( C_A = 0 \) until \( C_A = C_A \) so that equation (4) is obtained.

\[
\int_{C_A_0}^{C_A} \frac{d(c_A)}{dt} = \frac{AK_C}{V} \int_0^t dt \\
\ln \frac{C_{AS} - C_A}{C_A - C_{A_0}} = - \left( \frac{K_c A}{V} \right) \times t \tag{4}
\]

Figure 5. Relationship \( \ln \frac{C_{AS} - C_A}{C_A - C_{A_0}} \) and time when solid to liquid ratio of 1:8 is used.

By plotting \( t \) as abscissa and \( \ln \frac{C_{AS} - C_A}{C_A - C_{A_0}} \) as ordinate at Figure 5, the \( K_c A \) can be obtained from slope of \( \frac{K_c A}{V} \), where \( C_{AS} \) is saturation concentration and \( C_A \) are concentrations at a given time, \( K_c \) is mass transfer coefficient, \( V \) is volume, and \( t \) is extraction time. From slope \( \frac{K_c A}{V} \) at Figure 5, the 0.86 cm.s\(^{-1}\) of \( K_c A \) is obtained.

4. Conclusion

The microwave-assisted extraction method is suitable and effective to extract tannin from garlic skin. The highest of tannin content in garlic skin of 1.77% is obtained when extraction time of 180 s, the garlic skin (solid) to ethanol (liquid) ratio of 1:8, and microwave power of 100 W. IC\(_{50}\) as antioxidant activity in garlic skin extract is 19.76 and AAI of 1.994 signified strong antioxidant activity. The mass transfer coefficient \( (K_c A) \) is 0.86 cm.s\(^{-1}\).

References

[1] Santhosha SG, Jamuna P, Prabhavathi M 2013 Bioactive components of garlic and their physiological role in health maintenance: A review Food Bioscience 3 pp 59-74.

[2] Chowdhury A, Bhowal A, Datta S 2012 Equilibrium, thermodynamic and kinetic studies for removal of copper (II) from aqueous solution by onion and garlic skin Water Journal 4 pp 37-51.
[3] Hershko V, Weisman D, Nussinovitch A 2008 Method for Studying Surface Topography and Roughness of Onion and Garlic Skins for Coating Purposes Journal of Food Science. 63(2) pp 317-321.

[4] Ichikawa M, Ryu K, Yoshida J, Ide N, Kodera Y, Sasaoka T, Rosen R T 2003 Identification of Six Phenylpropanoids from Garlic Skin as Major Antioxidants Journal of Agricultural and Food Chemistry 51(25) pp 7313–7317.

[5] Reddy JP, Rhim JW 2014 Isolation and Characterization of Cellulosa Nanocrystals from Garlic Skin Materials Letters 129 pp 20-23.

[6] Mao R, Guo H, Tian D, Zhao D, Yang X, Wang S, Chen J 2013 2D SnO$_2$ Nanorod Networks Templated by Garlic Skins for Lithium Ion Batteries Material Research Bulletin 48(4) pp 1518-1522.

[7] Wu Y, Wu ZR, Chen P, Yang-Li, Deng WR, Wang YQ, Li HY 2015 Effect of the tyrosinase inhibitor (S)-N-trans-feruloyloctopamine from garlic skin on tyrosinase gene expression and melanine accumulation in melanoma cells Bioorganic & Medicinal Chemistry Letter 25(7) pp 1476-1478.

[8] Ifesan BOT, Fadipe EA, Ifesan BT 2014 Investigation of Antioxidant and Antimicrobial Properties of Garlic Peel Extract (Allium sativum) and Its Use as Natural Food Additive in Cooked Beef Journal of Scientific Research & Reports 3(5) pp 711-721.

[9] Vermerris W and Nicholson R 2009 Phenolic Compound Biochemistry, Springer Science+Business Media B.V.

[10] Mandal V, Mohan Y, Hemalatha S 2007 Microwave Assisted Extraction-An Innovative and Promising Extraction Tool for Medicinal Plant Research Pharmacognosy Reviews 1(1) pp 7-18.

[11] Letellier M and Budzinski H 1999 Microwave assisted extraction of organic compounds. Analysis 27(3) pp 259-270.

[12] Jaradat N, Hussen F, Al Ali A 2015 Preliminary Phytochemical Screening, Quantitative Estimation of Total Flavonoids, Total Phenols and Antioxidant Activity of Ephedra alata Decne. Journal of Materials and Environmental Science 6(6) pp 1771-1778.

[13] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H 2011 Phytochemical screening and Extraction: A Review Internationale Pharmaceutica Sciencia 9(1) pp 98-106.

[14] Bargah KR 2015 Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn Journal of Pharmacognosy and Phytochemistry 4(1) pp 7-9

[15] Tambun R, Christamore E, Pakpahan YF, Haryanto B 2018 Banana peel utilization as the green corrosion inhibitor of Iron in NaCl medium IOP Conference Series: Materials Science and Engineering 420 012059

[16] Harborne JB 1998 Phytochemical Methods: A guide to modern techniques of plant analysis, Chapman and Hall, London.

[17] Mandal V, Dewanjee S, Mandal SC 2009 Microwave Assisted Extraction of Total Bioactive Saponin Fraction from Gymnema sylvestre with Reference to Gymnemagenin Phytochemical Analysis 20(6) pp 491-497.

[18] Eskilsson CS and Bjorklund E 2000 Analytical-Scale Microwave-Assisted Extraction Journal of Chromatography A 902(1) pp 227–250.

[19] Mijanur R, Shahdat H, Asiqur R, Nusrat F, Taslima N, Borhan U, Mafroz AB 2013 Antioxidant Activity of Centella asiatica (Linn.) Urban: Impact of Extraction Solvent Polarity Journal of Pharmacognosy and Phytochemistry 1(6) pp 27-31

[20] Richard GG 2002 Transport Phenomena and Unit Operations: A Combined Approach, John Wiley and Sons, Inc, New York.