Journal of Functional Food and Nutraceutical

GARLIC PEEL EXTRACT PHYTOCHEMICAL EVALUATION AND EXTRACTION OPTIMIZATION

Della Rahmawati[1],
Dylan Andika[1],
Stacia Andani Fortunata[1]

[1] Department of Food Technology, Faculty of Life Sciences and Technology, Swiss German University, The Prominence Tower, Alam Sutera, Jalan Jalur Sutera Barat No 15, Tangerang 15143, Indonesia

ABSTRACT

Garlic plant has been known to have various beneficial properties beside as a condiment. However, the garlic peel is still considered as waste and the research of its functional properties are still very limited. The purpose of this study was to explore the potential phytochemical activities of garlic peel extract which might be utilized as natural food additive or even functional ingredient in the future. The experiment was divided to two phases. The first phase identifies and compares the phytochemical content and activities between aqueous and ethanolic extract. The presence of saponins was detected along with the absence of alkaloids on both aqueous and ethanolic extract. The aqueous extract possessed lower flavonoid content (7.593±0.299 mg/l quercetin equivalent), but higher phenolic content (64.688±1.865 mg/l GAE) and stronger antioxidant activity (17.042±0.380%) compared to the ethanolic peel extract. Aqueous extract showed antimicrobial activity against *S. cerevisiae*, while the ethanolic extract did not. Moreover, both types of extract also did not show any α-glucosidase inhibition activity. In the second phase, optimization attempts for extraction method were done and it was found that the highest amount of antioxidant activity along with flavonoid and phenolic content could be obtained in treatment of the raw material-solvent ratio to 20 gr/1000ml.

Keywords: *garlic peel extract, phytochemical activities, antioxidant, antimicrobial, extraction method.*

ABSTRAK

Bawang putih merupakan bumbu yang telah dikenal dengan berbagai keunggulannya. Meskipun demikian, kulit bawang putih sering dianggap limbah dan riset mengenai sifat fungsionalnya sangat terbatas. Pada penelitian ini potensi dari kandungan dan aktivitas fitokimia ekstrak kulit bawang putih akan dieksplorasi untuk penggunaannya di masa depan sebagai bahan tambahan pangan alami atau bahan baku pangan fungsional. Eksperimen yang dilakukan dibagi menjadi dua tahap. Pada tahap pertama dilakukan perbandingan antara kandungan dan aktivitas fitokimia dari eksrak air dan ekstrak etanol. Hasil penelitian menunjukkan keberadaan saponin pada kedua ekstrak, sedangkan keberadaan alkaloid tidak terdeteksi pada keduanya. Ekstrak air memiliki kandungan flavonoid yang lebih rendah (7.593±0.299 mg/l quercetin equivalent), namun memiliki kandungan fenolik yang lebih tinggi (64.688±1.865 mg/l GAE) dan kandungan antioksidan yang lebih kuat (17.042±0.380%) dibandingkan ekstrak etanol. Ekstrak air menunjukkan aktivitas antimikroba terhadap *S. cerevisiae*, yang tidak dimilik ekstrak air. Kedua ekstrak tidak menunjukkan aktivitas inhEXibisi terhadap enzim α-glukosidase. Pada tahap kedua, ekstraksi optimisasi dilakukan dan ditemukan bahwa aktivitas antioxidant terkuat yang didukung dengan kandungan flavonoid dan fenolik tertinggi diperoleh pada perlakuan rasio ekstraksi bahan baku terhadap solvent 20g/1000ml.

Kata kunci: *kulit bawang putih, aktivitas fitokimia, antioksidan, antimikroba, ekstraksi.*

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 1 Issue 1

Manuscript ID
v1n11928-2

Received Date
28 August 2019

Accepted Date
30 August 2019

Published Date
30 August 2019

DOI:
10.33555/jffn.v1i1.20

Corresponding author:
Stacia Andani Fortunata
Tangerang, Indonesia, 15143
Email:
staciafortunata@gmail.com

Citation:
Rahmawati, D., Andika, D., Fortunata, S.A. 2019. Evaluation of Phytochemical Activities of Aquaeous and Ethanolic Garlic Peel Extract. J. Functional Food & Nutraceutical, 1(1), pp.41-46.

INTRODUCTION

Garlic plant has been known since the ancient times to have many benefits. Through various studies, different parts of the plants have been shown to possess many properties which are valuable for functional ingredients for food and promoting health-beneficial effects (Durairaj, S., Srinivasan, S., & Lakshmanaperumalsamy, P. 2009; Amagase, H. et al. 2001). However, behind the well-known usefulness of garlic plant, there is one part of it which is still widely considered as waste. The garlic peel/skin is usually discarded directly without any further use. Moreover, in 2015 Indonesia had been reported to produce at least 23,991 tons of garlic (BPS. 2015). Therefore, the amount of garlic peel waste produced was also very significant.

There have been several efforts to study the potential of garlic peel even though they are still very limited. Garlic peel has been proven to contain six phenyl propanoids which act as strong antioxidants (Ichikawa M, et al. 2003). Another research has also reported the antioxidant and antimicrobial activities of garlic peel extract (Ifesan et al. 2014). Moreover, it is also able to reduce blood sugar level of laboratory mice which have been induced by alloxan (Wijayanti, R. et al. 2015).

Based on the evidence provided by the studies above, there might be a possibility to utilize garlic peel extract as a natural food additive or even as a functional food in the future. However, natural extracts can only give their maximum benefits if they are obtained by proper extraction methods. Since the research on the potential of garlic peel is still very limited, the optimum extraction method for garlic peel is yet unclear. Therefore, in the hope that it may provide basic knowledge for the future applications of garlic peel extract, this study was conducted to evaluate the phytochemical activities of garlic peel extracts obtained by using various extraction methods (aqueous and ethanolic extraction by maceration) and try to improve the quality of the extract by optimizing the extraction method (re-maceration and ratio change). The extraction method of garlic peel can be optimized once the phytochemical activities have been observed, and the optimization technique can be guided to produce better extract. The optimization was aimed to improve its phytochemical activities and yield.

MATERIALS AND METHOD

The study was divided into two phases. The first phase was intended to identify the phytochemical properties of garlic peel extract obtained by using water and ethanol 96% as extraction solvents using maceration process. The extraction was done according to modified method by Ifesan et al. 2014.

Garlic peel extraction

Twenty gram of ground garlic peel was macerated using 500 ml of solvent for 24 hours. The extracts obtained using both solvents were then filtered and concentrated to an equivalent concentration of 200mg/ml and analyzed for phytochemical activities.

Qualitative analysis

The analyses consisted of qualitative alkaloid content (Wagner's test), qualitative saponin content (foam test), total phenolic content (Folin-Ciocalteau assay), total flavonoid content (aluminium chloride assay), antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Aspergillus niger* (well diffusion method), antioxidant activity (DPPH scavenging assay) and alpha-glucosidase inhibition activity. Based on the result obtained from the first phase, the extraction method which produces extract with better activities was chosen for optimization attempts in the second phase.

Optimization of extraction process

In the second phase, the optimization for extraction method was done through two different approaches. Extraction method with better activities in the first phase was modified using re-maceration and raw material-solvent ratio change. Re-maceration technique was divided into two variations. The solvent was changed and collected every 8 hours and every 12 hours during 24 hours extraction using the same ratio of 20 gram/500ml. The raw material-solvent ratio change was divided into 3 different concentrations (20gram/1000ml, 20 gram/1500ml, 20 gram/2000ml). The extracts obtained from each treatment were then concentrated and the phytochemical activities of the extracts were analyzed and compared to the result obtained in the first phase. Only activities regarded significant or potential in the first phase will be analyzed in phase two.

RESULTS AND DISCUSSION

The phytochemical activity analyses conducted in first phase indicated that both type of extracts were distinctive from each other. This phytochemical analysis will check whether the garlic peel has same bioactive compound with garlic bulb or not. A health property of garlic depends upon the bioactive compounds such as organo-sulfur and non-sulfur compounds (Kim et al., 1997; Rahman, 2007; Sendl et al., 1992; Tepe et al., 2005). Those compounds are vitamins, fatty acids, glycolipids, phospholipids, essential amino acids, phenolics, and flavonoids (Fenwick and Hanley, 1985; Tsiaganis et al., 2006).

Qualitative analyses showed in Table 1. showed that alkaloid was not present in both ethanolic and aqueous garlic peel extract. This may indicates that both types of extract possess a very low quantity of alkaloids that it could not be detected visually, or they do not contain alkaloids at all. While saponin were detected in both of them. Saponins from garlic have been found to have pharmacologic activities such as antifungal, antibacterial, anti-inflammatory and hypocholesteremic influences (Lacaille-Dubois and Wagner 1996). However, antimicrobial activity was only existed in aqueous extract against *S. cerevisiae*.

Tabel 1. Comparative Qualitative analysis data of the phytochemicals of the garlic

Secondary metabolites	Ethanol Extract Garlic Peel	Water Extract Garlic Peel	Methanol Extract Garlic Bulb*
Alkaloids	-	-	+
Saponins	+	+	+
AGI	-	-	NA
Antimicrobial activity (*S. cerevisiae*)	-	+	NA
Flavonoid	+	+	+
Antioxidant activity	+	+	NA
Phenolics	+	+	+

+ indicates presence, - indicates absences

*Divya BJ, Suman B, Venkataswamy M, Thyagaraju K. (2017)

Antimicrobial activity of aqueous garlic extract against *Saccharomyces cerevisiae* has already been previously studied (Durairaj et al., 2009) and according to the research by Kivanc & Kunduhoglu (1997), *Saccharomyces cerevisiae* exhibit the highest sensitivity against various plant juices including the garlic juice, compared to other type of yeasts and bacteria. This may possibly have a connection to the antimicrobial activity of aqueous garlic peel extract against *Saccharomyces cerevisiae.*

There were also no alpha-glucosidase activity inhibitions detected in both extract (Table 1). Negative results were obtained possibly due to the absence of inhibitor and the presence of sugar in the extract, therefore the absorbance of sample solution will be considerably high. Since sugar is far more soluble in water than in ethanol, the negative percentage in the aqueous extract will be greater as well, because more of the sugar will be broken down by the enzyme.

The flavonoid in both extracts were detected. The ethanolic extract of garlic has higher flavonoid content than aqueous extract, based on quantitative analysis of flavonoid content. The flavonoid content (dilution factor/DF=1) of aqueous and ethanolic extract were respectively 7.593±0.299 mg/l quercetin equivalent and 14.019±0.539 mg/l quercetin equivalent. These results show that flavonoids in garlic peel are tend to be more soluble in less polar solvent such as ethanol. This might happen since various kinds of phenolic compounds, including flavonoids have different solubility in various solvents (Ganora, 2009).

The total phenolic content of aqueous extract higher than ethanolic extract. The total phenolic content (DF=5) of aqueous and ethanolic extract were respectively 64.688±1.865 mg/l gallic acid

equivalent (GAE) and 47.043±0.750 mg/l GAE. Based on the result, it can be concluded that the phenolic compounds contained in garlic peel can be more optimally extracted using water as solvent.

The antioxidant activity (DF=5) of aqueous and ethanolic extract were respectively 17.042±0.380% and 13.706±0.668%. This antioxidant activity may be heavily related to the phenolic compounds of garlic peel, especially phenyl propanoids which have been already identified and studied previously by Ichikawa et al (2003). Based on the experimental results, the antioxidant activity seems to be directly proportional to the total phenolic compound. As the total phenolic compound of aqueous extract was higher, the antioxidant activity was also higher as expected. Aqueous extraction was chosen for the second phase. Since the antioxidant properties of aqueous extract was regarded significant, only antioxidant activity, total flavonoid content and total phenolic content were analyzed and compared in second phase. Moreover, extraction method using water is cheaper, more flexible for processing and it will not have any problem with halal certification if it is applied for food or pharmaceutical industry in the future.

In the second phase optimization was performed. The optimization process of extraction procedure is important due to changes in conditions such as extraction technique, ratio of solvent and sample determine the extraction yield of individual chemical constituents extractable (Hinneburg and Neubert, 2005). In other hand, different temperature, extraction time were also affected the yields.

Maceration was chosen as method of extraction. Maceration is one of suitable widely used technique for extractions of plant material. Therefore, in this study the optimization process was used re-maceration technique. Purpose of re-maceration technique is to maximizing yields of the compounds of interest, while minimizing the extraction of unwanted compounds with two time maceration (Sibul F.S, et al. 2016)

The result of total flavonoid content, total phenolic content and antioxidant activity of aqueous extract after optimization attempts are shown in Tables 2,3,4,5,6,7.

Table 2 Total flavonoid content after ratio change

Optimization Method	Total Flavonoid Content (mg/l quercetin equivalent), DF=1
Ratio 20 gr/1000ml	17.617±0.486
Ratio 20 gr/1500ml	8.528±0.256
Ratio 20 gr/2000ml	9.393±0.162

Table 3 Total flavonoid content after re-maceration

Optimization Method	Total Flavonoid Content (mg/l quercetin equivalent), DF=1
Re-maceration 8 hours	6.121±0.377
Re-maceration 12 hours	6.331±0.299

Table 4 Total phenolic content after ratio change

Optimization Method	Total Phenolic Content (mg/l GAE), DF=5
Ratio 20 gr/1000ml	80.817±1.609
Ratio 20 gr/1500ml	59.207±0.529
Ratio 20 gr/2000ml	56.923±0.999

Table 5 Total phenolic content after re-maceration

Optimization Method	Total Phenolic Content (mg/l GAE), DF=5
Re-maceration 8 hours	71.394±0.506
Re-maceration 12 hours	69.879±0.418

Table 6 Antioxidant activity after ratio change

Optimization Method	Antioxidant Activity (%), DF=5
Ratio 20 gr/1000ml	25.856±0.408
Ratio 20 gr/1500ml	21.881±0.305
Ratio 20 gr/2000ml	23.168±0.186

Table 7 Antioxidant activity after re-maceration

Optimization Method	Antioxidant Activity (%), DF=5
Re-maceration 8 hours	23.834±0.326
Re-maceration 12 hours	21.904±0.683

Based on the experimental results, optimization process showed significant difference in total flavonoid content compared to the control. Changing the ratio between raw material and solvent gave significant improvement towards flavonoid content, while doing re-maceration decreased the flavonoid extracted.

The re-maceration process should increase the yield of substances extracted theoretically. However, both extract with 8 hours and 12 hours of re-maceration similarly exhibit lower flavonoid content. This phenomenon may be explained by the fact that in phase one, the flavonoids in garlic peel tend to be less polar (higher flavonoid value with ethanolic extraction).

The flavonoids, as one of the phenolic compounds, have aglycones forms which are less polar than glicosidic flavonoids. These less polar aglycones flavonoids may bound with saponin which was also present in aqueous extract. Saponin is a natural surfactant which may help dissolving less polar substances into polar solvent. Since the basic principal of re-maceration is changing the solvent over time and the fact that saponin is hydrophilic, the saponin may have been exhaustedly extracted in earlier extraction solvent (Gonzalez-valdez et al.,2013).

As the result, in the later extraction solvent, flavonoids were not extracted optimally and the flavonoid content was lower in total. This did not happen to the extraction method which used the same solvent during 24 hours extraction (without re-maceration).

The optimization process of aqueous garlic peel extraction also showed significant difference in total phenolic content compared to the control. The re-maceration process significantly increased the amount phenolic content as expected. However, the change in raw material-solvent ratio indicated lower values.

The irregularly high phenolic content of 20 g/ 1000 ml extract may also be related to the mishandling issue. However, it is unclear why the 20 gr / 1500 ml extract and 20 gr/2000 ml extract exhibited lower values compared to the control extract. Moreover, the phenolic content also seemed to decrease as the amount of solvent gets higher. This may come from the evaporation process, which involves longer time to evaporate higher volume sample. The smaller ratio of raw-material-solvent requires higher volume to achieve the same concentration of 200 mg raw material/ ml solvent and consequently will lengthen the evaporation time. This longer time will expose more of the extract to the heat and degrade more of the phenolic compounds.

In general, all of the optimization process increased the antioxidant activity of aqueous garlic peel extract despite of several reduction in phenolic and flavonoid content mentioned in Table 6 and 7. This may indicate that other compounds which act as antioxidant were also extracted in higher amount besides flavonoid and phenolic compounds.

The considerably high antioxidant activity of 20 gr/ 1000 ml extract is believed to be related to mishandling issue mentioned before. The antioxidant activities of re-macerated extracts were significantly higher than control extract and directly proportional to the total phenolic compound mentioned in Table 7. The antioxidant activities of re-macerated extract were also generally higher than the extract with ratio-changing.

Based on the results above, optimization technique (changing ratio of solvent) in general were able to improve the phytochemical activities of aqueous garlic peel extract compared to the results previously obtained in the first phase with the highest total flavonoid content, total phenolic content and antioxidant activity can be achieved by using 1000 ml solvent for 20 mg sample. However, re-maceration technique was not able to improve activities.

CONCLUSION

Aqueous garlic peel extract had better phytochemical activity in terms of total phenolic content, antioxidant activity and antimicrobial activity compared to the ethanolic extract. Nevertheless, there were no alpha-glucosidase inhibition activity detected in both extract. Total flavonoid content, total phenolic content and antioxidant activity of garlic peel extract could be optimized by changing amount of solvent to 1000 ml for 20 mg sample. However, optimization by using re-maceration technique was not able to improve activities.

REFERENCES

Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., & Itakura, Y. 2001. Intake of Garlic and Its Bioactive Components. Journal of Nutrition, 131, 955–962.

BPS. 2015. Produksi Bawang Putih Menurut Provinsi, 2011-2015

Durairaj, S., Srinivasan, S., & Lakshmanaperumalsamy, P. 2009. In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. Electronic Journal of Biology, 5(1), 5–10.

Hinneburg, I. and Neubert R. H. 2005. Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (Fagopyrum esculentum) herb. J Agric Food Chem.12 53(1):3-7.

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), 7313–7317.

Ifesan, B. O. T., Fadipe, E. A., & Ifesan, B. T. 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), 711–721.

Ganora, L. 2009. Herbal Constituents: Foundations of Phytochemistry : a Holistic Approach for Students and Practitioners of Botanical Medicine. US: Herbalchem Press.

Gonzalez-valdez, L. S., Almaraz-abarca, N., Proal-nájera, J. B., Robles-martinez, F., Valencia-del-toro, G., & Quintos-escalante, M. 2013.Surfactant Properties of the Saponins of Agave durangensis, Application on Arsenic Removal.International Journal of Engineering and Appled Sciences, 4(2), 87–94.

Kim, M., Kim, Y. and Chung, S. 2005. Identification and in vitro biological activities of flavonols in garlic leaf and shoot : inhibition of soybean lipoxygenase and hyaluronidase activities and scavenging of free radicals. Journal of the Science of Food and Agriculture 85: 633.

Kivanc, M. and Kunduhoglu, B. 2016. Antimicrobial Activity of Fresh Plant Juice on the Growth of Bacteria and Yeasts. Journal of Qafqaz University: 26-53

Lacaille-Dubois, M.-A. and Wagner, H. 1996. A review of the biological and pharmacological activities of saponins. Phytomedicine 4: 363–386.

Rahman, K. 2007. Effects of garlic on platelet biochemistry and physiology. Molecular Nutrition & Food Research, 51, 1335–1344.

Sibul, F. S., Dejan, Z. O., Emilija, S., Neda, M. and Mimica, D. 2016. Optimization of extraction conditions for secondary biomolecules from various plant species. Hem. Ind. 70(4): 473–483.

Tepe, B., Daferera, D., Sokmen, A., Sokmen, M. and Polissiou, M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). Food Chem. 90: 333-340.

Tsiaganis, M.C., Laskari, K. and Melissari, E. 2006. Fatty acid composition of Allium species lipids. Journal of Food Composition and Analysis 19: 620-627.

Wijayanti, R., & Rosyid, A. 2015. Efek Ekstrak Kulit Umbi Bawang Putih (Allium sativum L.) terhadap Penurunan Kadar Glukosa Darah pada Tikus Putih Jantan Galur Wistar yang Diinduksi Aloksan. Jurnal Ilmu Farmasi & Farmasi Klinik, 12(1), 47–52.