Antioxidant Activity and Total Phenolic Compound of Aged Garlic Extract

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ABSTRACT

Garlic (*Allium sativum*) is a well known and widely used medicinal herb in Asia. It contains several bioactive constituents and possesses health promoting properties. Extracts of fresh garlic are fermented for 13 months to produce aged garlic extract (AGE) that contain antioxidant phytochemicals to prevent oxidative damage. This study examined the antioxidant activities of aged garlic extract by using, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing antioxidant power (FRAP) and total phenolic compound. Results of the present study showed that the total phenolics compound was 56.02±5.29 µg TAE/mg plant extract and FRAP value of aged garlic extract was 119.44±18.79 µmol/g crude extract. The 50% inhibition of DPPH activity of aged garlic extract was 3.23±0.57 µg/ml. This indicated that the aged garlic extract produced by our laboratory showed high antioxidant activities both by DPPH and FRAP assays.

บทคัดย่อ

กระเทียม *(Allium sativum)* เป็นพืชสมุนไพรที่รู้จักกันดีและถูกนำมาใช้เป็นยาสมุนไพรอย่างแพร่หลายในภูมิภาคเอเชีย องค์ประกอบหลายชนิดในกระเทียมมีฤทธิ์ทางชีวภาพที่น่าสนใจและมีคุณสมบัติในการส่งเสริมสุขภาพ กระเทียมสดที่ถูกหมักเป็นระยะเวลานาน (13 เดือน) ให้ผลผลิตเป็นสารสกัดกระเทียมหมัก (AGE) ที่มีส่วนประกอบของสารต้านออกซิเดชันใช้สำหรับป้องกันความเสียหายอันเกิดจากอนุมูลอิสระ สำหรับการศึกษาครั้งนี้ทำการทดสอบฤทธิ์ต้านออกซิเดชันของสารสกัดกระเทียมหมักโดยใช้การทดสอบด้วยวิธีการทำลายอนุมูลอิสระดีพีพีเอช (DPPH assay) การวิเคราะห์ความสามารถในการรีดิวซ์เฟอร์ริกของสารต้านอนุมูลอิสระ (FRAP assay) และทำการวัดปริมาณสารประกอบฟีนอลิกรวม ผลการศึกษานี้แสดงให้เห็นว่าสารสกัดกระเทียมหมักมีสารประกอบฟีนอลิกรวมเท่ากับ 56.02 ± 5.29 ไมโครกรัม/มิลลิกรัมน้ำหนักแห้ง ค่า FRAP เท่ากับ 119.44 ± 18.79 ไมโครโมลต่อกรัมน้ำหนักแห้ง และค่าการยับยั้งอนุมูล DPPH ที่ 50% ของสารสกัดกระเทียมหมักเท่ากับ 3.23±0.57 ไมโครกรัมต่อมิลลิลิตร ผลที่ได้จากการศึกษาแสดงให้เห็นว่าสารสกัดกระเทียมหมักที่ผลิตโดยห้องปฏิบัติการของเรามีประสิทธิภาพในการต้านออกซิเดชันสูงทั้งวิธี DPPH และ FRAP assay

Key Words: Aged Garlic Extract, antioxidant activities (DPPH, FRAP), total phenolic compound

คำสำคัญ: สารสกัดกระเทียมหมัก ฤทธิ์ต้านอนุมูลอิสระ (DPPH, FRAP) สารประกอบฟีนอลิกรวม

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Introduction

Oxidative stress is caused by an imbalance between the production of reactive oxygen species or free radical and a biological system's ability to readily detoxify the reactive intermediates (Wickens, 2001). In humans, oxidative stress is involved in many diseases, such as cancer, atherosclerosis, myocardial infarction, Alzheimer's disease and others chronic diseases (Brace, 2002; Corzo-Martinez et al., 2007; Osawa et al., 1995; Sener et al., 2005). Antioxidants protect the cells from oxidative damage by scavenging free radicals. They convert free radicals into harmless substances and repair the cell damage from free radical. Many plants and animals contain a wide variety of free radical scavenging antioxidants such as vitamin C, vitamin E and enzymes including catalase, superoxide dismutase and peroxidase enzymes (Shahidi & Naczk, 1995). Currently, garlic or *Allium sativum* is used as a traditional medicine in several countries. However, many people avoid fresh garlic because of its lingering pungent smell. One of the better known garlic preparations is aged garlic extract (AGE), which is formed during garlic aging. During this time, unstable and highly odorous compounds in fresh garlic are converted into odourless aged garlic extract containing stable compounds and increased level of antioxidants (Borek, 2001). Because there are differences in antioxidant effects of aged garlic extract produced by many laboratories, therefore, the aim of this study was to investigate the *in vitro* antioxidant activities in our aged garlic extract by determination of total phenolic compounds, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP).

Methodology

Plant material and chemicals

Aged garlic extract (AGE) were developed by the Center for Research and Development of Herbal Health Products (CRD-HHP), Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. It is prepared by soaking fresh native garlic (*Allium satifum*) in 30% ethanol for 13 months at room temperature. The extract is then filtered and evaporated by the alcohol out, dried as a powder and stored at low temperature before use. All chemicals in this study were analytical grade.

2,2-Diphenyl-1-Picrylhydrazyl radical (DPPH) scavenging capacity assay

2,2-DPPH assay was used to demonstrate the scavenging activity (Katsube et al., 2004). Each aged garlic extract was mixed with methanol and DPPH methanol solution, respectively. The mixture was kept at room temperature for 15 minutes. The decrease in absorbance of DPPH solution was measured at 517 nm. using a UV-visible spectrophotometer. The capability to scavenge the DPPH radical was calculated using the following equation :

$$\text{Inhibition } \% = [(A_0 - A)/ A_0] \times 100$$

A_0 was the absorbance of DPPH without sample and A was the absorbance of sample with DPPH.

IC_{50} values calculated denote the concentration of plant extract required to decrease the absorbance at 517 nm by 50%. Vitamin C and vitamin E were used as the reference antioxidants.

Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay was used to determine the antioxidant capacity of AGE (Benzie & Strain, 1996) based on the reduction of Fe^{3+}TPTZ complex to blue

colored Fe^{2+}TPTZ solution by electron donating substance under acidic condition. Plant extract was allowed to react with the FRAP solution under dark condition for 30 minutes. The absorbance of assay solution was measured at 593 nm. for 10 minutes at 37^{O}C. The final absorbance was calculated with the standard curve. The data were expressed as µmolFe^{2+}/g crude extracts. A high FRAP value indicated higher antioxidant activity of the sample.

Determination of total phenolic compounds (TPC)

Total phenolic compounds were determined with Folin-Ciocalteu's reagent (Singleton & Rossi, 1965). Tannic acid was used as the standard in this study. Each aged garlic extract was mixed with distilled water, Folin–Ciocalteu reagent and Na_2CO_3, respectively. Mixture was kept at room temperature for 40 minutes. The absorbance was then measured at 725 nm. Total phenolic compounds were expressed as µg of equivalent tannic acid (TAE) per mg crude extract.

and 7.63 ± 0.26 µg/ml, respectively) (Table 1). By FRAP assay, the result was shown as Fe^{2+}equivalent (µmol/g crude extract) using the equation based on the calibration curve (y = 0.2307x + 0.0872, R^2 = 0.9995). The reducing ability of the aged garlic extract was in the ranges of 119.44 ± 18.79 µmol Fe^{2+}/g crude extract (Table 1).

Total phenolic compounds

The result of total phenolic compounds is shown in table 1, using the equation based on the calibration curve (y = 35.17x, R^2 = 0.9908). The aged garlic extract contain considerable amount of phenolic compounds (56.02 ± 5.29 µg TAE/mg crude extracts) (Table 1)

Discussion and Conclusions

DPPH assay is a technique to measure the radical scavenging activity of any antioxidant. It is a quick, reliable and reproducible method to determime the *in vitro* antioxidant activity of pure compounds as well as plant extracts (Mosquera et al., 2007). The ability of aged garlic extract to act as donors of electrons in transformation of DPPH radical into its reduced form DPPH-H was investigated. The aged garlic extract was able to reduce the stable, purple coloured radical, $DPPH^{\bullet}$, into the yellow-coloured diphenyl picrylhydrazine, DPPH-H, reaching 50% of

Table 1 Antioxidant activities and total amount of plant phenolics

	Total phenolics, µg TAE/mg plant extract[a]	FRAP µmol/g extract (in Fe^{2+})[a]	DPPH IC_{50} (µg /ml)[a]
Vit. C	-	-	3.11 ± 0.02
Vit. E	-	-	7.63 ± 0.26
Aged Garlic Extract	56.02 ± 5.29	119.44 ± 18.79	3.23±0.57

[a]Data represent mean ± standard deviation of three separate measurements

Results

Antioxidative activities

Antioxidative activities of aged garlic extract measured by DPPH assay demonstrated that the extracts exhibit low activity in comparison with the IC_{50} of standards vitamin C and vitamin E (3.11 ± 0.02

reduction (Bozina et al., 2008). Our results indicated that the aged garlic extract showed high antioxidative activity by DPPH assay in comparison to the standard vitamin C and vitamin E. Furthermore, by FRAP assay the extract considered to have antioxidative activity under the classification of Wong et al. (2006). They classified the medicinal plants into four categories on the basis of their antioxidant activities: extremely high (>500 μmol Fe^{2+}/g), high (100–500 μmol Fe^{2+}/g), medium (10–100 μmol Fe^{2+}/g), and low (<10 μmol Fe^{2+}/g). Therefore, our result revealed the high antioxidative activity of the extract by FRAP assay. Antioxidant potential of aged garlic extract is also determined by their content of phenolic compounds (Park et al., 2009). Although our aged garlic extract produced slightly low phenolic content, their antioxidative effects were high. Various studies showed the different amount of phenolic compound in aged garlic extract (Park et al., 2009; Nencini et al., 2011). This may suggest that the phenolics were largely affected by process and time of fermentation. Moreover, other constituents of aged garlic extract are likely to contribute to their radical-trapping and antioxidant properties. These are mainly sulfur compounds such as S-allyl cysteine, S-allyl mercaptocysteine, and allicin (Benzanger-Beauquesne & Delelis, 1967), which are claimed to constitute the bioactive components of commercial preparations of garlic extract (Lawson et al., 1991). The high reducing power of aged garlic extract by FRAP suggests that the total phenolic content measured by the Folin-Ciocalteu procedure does not give a full picture of their antioxidant capacity. They may be some interferences arising from other chemical components presented in the extract. Aged garlic extract is made by aging for prolonged period at high temperature thought to cause the major phenolic loss that typically occurs during aging process. Previous studies showed that fruits and vegetables tend to lose certain aspects of their bioactivities when stored at high temperatures or cooked (Pedraza-Chaverri et al., 2006). In addition, the reduction of phenolic content could be due to oxidative enzymes such as polyphenoloxidases and peroxidases contained in different amounts in the assayed species (Nencini et al., 2011; Shahidi & Naczk, 1995).

In summary, Aged garlic extract showed antioxidant capacity *in vitro*; However, chemical compound, mechanism of action, and toxicity for new development should be further evaluated.

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References

Benzanger-Beauquesne L, Delelis A. 1967. Flavonoids from Allium ascal- onicum bulbs. Compt. Rend. Ser. D. Sci. Nat. 265: 2118-2119.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. (239): 70-76.

Borek C. 2001. Antioxidant health effects of aged garlic extract. J. Nutr. (131): 1010S-1015S.

Bozina B, Mimica-Dukic N, Samojlik I, Gorand A, Igic R. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chem. (111): 925-929.

Brace LD. 2002. Cardiovascular benefits of garlic (*Allium sativum* L). J. Cardiovas. Nurs. (16): 33-49.

Corzo-Martinez M, Corzo N, Villamiel M. 2007. Biological properties of onions and garlic. Trends Food Sci. Tech. (18): 609-625.

Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, et al. 2004. Screening for antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. J. Agric. Food Chem. (52): 2391-2396.

Lawson LD, Wood SG, Hughes B. 1991. HPLC of Allicin and other thiosulinates in garlic clove homogenates. Planta. Med. (57): 263-270.

Mosquera OM, Correa YM, Buitrago DC, Nino J. 2007. Antioxidant activity of twenty five plants from Colombian biodiversity. Mem. Inst. Oswaldo. Cruz. (102): 631-634.

Nencini C, Menchiari A, Franchi GG, Micheli L. 2011. *In vitro* Antioxidant Activity of Aged Extracts of some Italian Allium Species. Plant Foods Hum. Nutr. (66): 11-16.

Osawa T, Yoshida A, Kawakishi S, Yamashita K, Ochi H. 1995. Protective role of dietary antioxidants in oxidative stress. In: Cutler RG, Packer L, Bertman J, Mori A (eds) Oxidative Stress and Againg Birkhauser Verlag, Basel, Switzerland. 367-377.

Park JH, Park YK, Park E. 2009. Antioxidative and Antigenotoxic Effects of Garlic (*Allium sativum* L.) Prepared by Different Processing Methods. Plant Foods Hum. Nutr. (64): 244-249.

Pedraza-Chaverri J, Medina-Campos ON, Avila-Lombardo R, Berenice Zuniga-Bustos A, Orozco-Ibarra M. 2006. Reactive oxygen species scavenging capacity of different cooked garlic preparations. Life Sci. M. (78): 761-770.

Sener G, Sehirli AO, Ipci Y, Cetinel S, Cikler E, Gedik N. 2005. Chronic nicotine toxicity is prevented by aqueous garlic extract. Plant Foods Hum. Nutr. (60): 77-86.

Shahidi F, Naczk M. 1995. Food phenolics, sources, chemistry, effects, application. Technomic Publishing Co. Inc., Lancaster. 75-107.

Singleton VL, Rossi JA Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Viticult. (16): 144-158.

Wickens AP. 2001. Ageing and the free radical theory. Res. Physiol. (128): 379-391.

Wong CC, Li HB, Cheng KW, Chen F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. (97): 705-711.