



Preparation Of Garlic Peel Extract and Its Free Radical Scavenging Activity

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ABSTRACT

Garlic (*Allium sativum*) has played an important dietary and medicinal role for centuries. Even today the medicinal use of garlic peel is widespread and growing. Studies have conducted the consumption of garlic could treat various ailments such as heart disease, arthritis and pulmonary complaints. Garlic peel extract has been found to possess significant free radical scavenging activity due to the presence of various bioactive compounds such as phenolics, flavonoids, and sulphur-containing compounds like alliin and allicin. Studies have shown that garlic peel extract exhibits potent antioxidant activity, which helps in preventing oxidative damage to cells and tissues. Free radicals are highly reactive molecules that can damage cells and tissues in the body, leading to various diseases such as cancer, cardiovascular disease, and ageing. Garlic peel extract has also been found to have antimicrobial and anti-inflammatory properties, which makes it useful in treating various diseases and infections. Overall, the free radical scavenging activity of garlic peel extract is attributed to its rich content of bioactive compounds, and it may have potential therapeutic applications in the prevention and treatment of various diseases. The extract from the garlic peel has been reported to show strong antioxidant activity and could be used as an herbal medicine. Garlic peel extract is prepared using the powder, distilled water was added, left for for 24 hours and then added ethanol to it. The extract is boiled for 40 minutes. Then keep it in the shaker. The colour change was observed. This resulted in an almost equal rate of percentage inhibition when compared to the standard. Thus garlic peel extract showed excellent antioxidant activity due to the presence of compounds like phenols and flavonoids in large amounts. The aim of this study was to assess the free radical scavenging activity of the garlic peel extract.

Keywords: *Garlic, antioxidant potential, phenols, flavonoids*

INTRODUCTION

Garlic (*Allium sativum*) Is considered to be one of the best disease preventive foods, based on its potential and varied effects (1). Data that characterise the antioxidant properties related to phenolic and flavonoid compounds of garlic were recorded. Reports showed that garlic and garlic peel extracts possess antioxidant activity, and they supply protection against free radical damage in the body. Since ancient times, garlic has been used extensively in food and medicine. Garlic is still used for therapeutic purposes widely and increasingly. Garlic use has been shown to be effective in treating a number of conditions including cancer risk reduction, heart disease, arthritis, respiratory complaints, stomach (uterine) growths, diarrhoea and worm infestation . Throughout human history, garlic has attracted a lot of interest in Korea as an essential component of diet (2)

Phenolics, flavonoids, Allin, Allicin, organosulfur volatiles, S allyl - L - Cysteine steroid saponins and saponinins were the main bio active components in garlic. One of the most physiologically active components of garlic is allicin, Which is a substantial component of newly cut garlic. Allicin is created when the enzyme allinase reacts with allin. Allicin then undergoes rapid metabolism to produce ajoene, S- allylmercaptocysteine and S - allyl cysteine (3).

Allicin is believed to reduce systolic blood pressure , serum cholesterol, triglycerides and exhibit antibacterial, antifungal, anti parasitic and antiviral activities (2,3). Oxidative stress is thought to be a factor in various diseases, including age-related neurological diseases, cardiovascular disease and cancer. Oxidative stress is caused by an excess of oxidative radicals, such as reactive oxygen species (ROS) or reactive nitrogen species (RNS). Fruits and vegetables include a variety of bio active substances (Antioxidant phytochemicals) for health promotion and illness prevention in addition to the necessary nutrients needed for everyday life .

According to reports garlic peel extracts exhibit significant antioxidant activity and may be used as a herbal remedy (4). In simple words, antioxidant activity can be defined as a limitation or innovation of nutrient oxidation (especially lipids and proteins) by restraining oxidative chain reactions. They prevent or delay some type of cell

damage. They are also found in fruits and vegetables. They are also available as dietary supplements. Example: beta-carotene. Our team has extensive knowledge and research experience that has translate into high quality publications(5–13)

MATERIALS AND METHODS

Preparation of extract

Garlic peels were collected and dried. It was ground into fine powder with a blender. Distilled water was added to it and kept aside and dark for 24 hours. Then, ethanol (70%)was added and kept aside for the next 24 hours. The next day it was heated for 40 minutes until the extract boiled well, showing colour change. Then, it was covered with a foil and put in the shaker for 24 hours. Next day after all the particles were separated, the extract was transferred into a centrifugal tube and kept for centrifugation. The final extract was evaporated until the solvents were completely removed. The extract was collected and stored for further use.(14)

Antioxidant activity

DPPH method

DPPH Assay was used to test the antioxidant activity of biogenic synthesis zinc oxide nanoparticles. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of *Allium sativum* extract was mixed with 1 ml of 0.1mM DPPH in methanol and 450 µL of 50 mM tris HCL buffer (PH 7.4) and incubated for 30 minutes. Later the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm(15,16).Ascorbic acid was used as a standard. The percentage inhibition was determined by the formula:

$$\text{Percentage inhibition} = \left(\frac{\text{Absorbance control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \right)$$

Hydroxyl radical scavenging assay

All solutions were prepared freshly. 1 ml of The reaction mixture contained 100 µL of 28 mM of 2 deoxy 2 ribose (dissolved phosphate buffer, PH 7.4), 500µL Solution of various concentrations of the garlic peel extract(10µL,20µL,30µL,40µL,50µL) 200µL of 200 µM FeCl₃ and 1.04 mM EDTA(1:1 V/V),100 µL H₂O₂(1.0 mM) and 100 µL

Ascorbic acid (1.0 mM). After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532 nm against the blank solution. Vitamin E was used as a positive control.

FRAP assay

Reagents for FRAP assay

- a) Acetate buffer 300 mM pH 3:3 : weigh 3.1 g sodium acetate trihydrate and at 16 mL of glacial acetic acid and make the volume to 1 L with distilled water.
- b) TPTZ (2,4, 6-tripyridyl-s-triazine) (M.W 312.34), 10mM in 40 mM Hcl (M.W36.46)
- c) FeCl₃.H₂O : (M.W270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before taking the test. Standard was FeSO₄.7H₂O: 0.1-1.5 mM in methanol. All the reagents were prepared from MERCK (Germany company)(17)

Procedure

FRAP solution (3.6 mL) is added to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution mixed with a certain concentration of the garlic peel extract(10µL,20µL,30µL,40µL,50µL)and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO₄, 7H₂O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

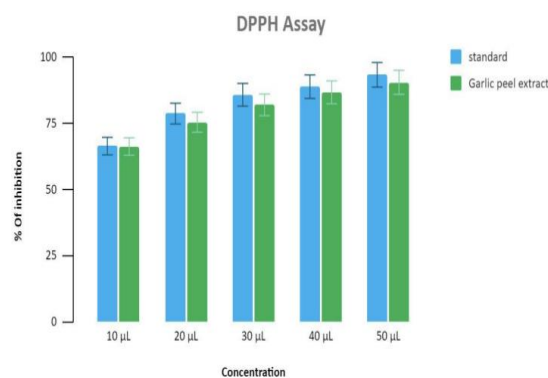


FIGURE 2: Comparison of DPPH assay of garlic peel extract with standard



FIGURE 3: Garlic peel powder

RESULTS & DISCUSSION

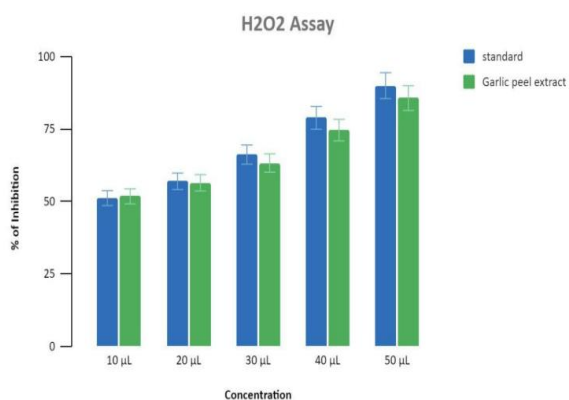


FIGURE 1: Comparison of H₂O₂ assay of garlic peel extract with standard

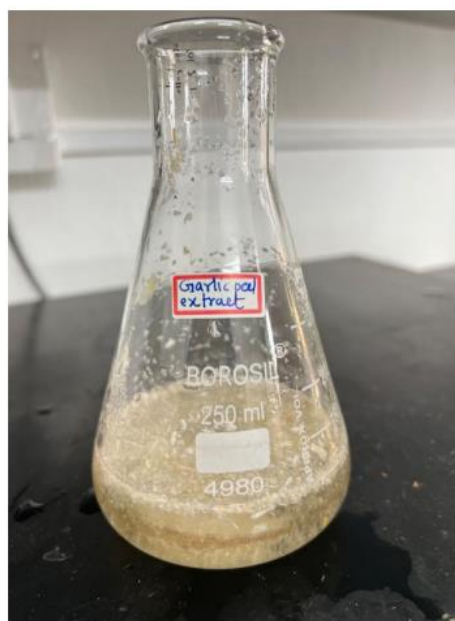


FIGURE 4: Extract preparation using ethanol



FIGURE 5: Prepared Garlic Peel extract

The garlic peel extract showed excellent antioxidant activity when compared to the standard due to the presence of compounds like phenols and flavonoids in large amounts. The above results clearly state that the antioxidant potential of the garlic peel extract can be used to cure various heart diseases and cancer.

The extraction of garlic peeled by methanol (70%) yielded maximum in the studies conducted by (4). Our study also showed maximum yield methanolic garlic peeled extract. Due to the hydroxyl substituent on the aromatic ring in phenolic compounds, these compounds can operate as antioxidants to scavenge oxygen radicals and the other highly reactive oxygen species. Additionally, I have already found a positive and very significant association between total phenolics and antioxidant activity (18).

According to the report of (19) The total final content of methanolic extract was found to be 55.48 ± 0.40 . Although reports on the bioactive compounds and antioxidant capacity of garlic peels are scarce, some studies have indicated that total flavonoids were higher than the values reported for garlic bulbs (0.6–75 mg quercetin/g) obtained from different regions of Morocco (20). Though garlic peel is considered a waste, it can be a good source of bioactive compounds such as caffeic, *p*-coumaric, ferulic, and di-ferulic acids (21).

The values of total phenolic compounds are very similar to the data obtained for husks (28.35 ± 0.07 mg GAE/g dm) and different varieties of garlic bulbs (17.16–42.53 mg GAE/g) (22). Three flavonoids namely myricetin,

isoquercitrin, and isorhamnetin were isolated and identified from Laba garlic. The isolated compounds were investigated on the protective effects against H₂O₂-induced oxidative damages in hepatic L02 cells and apoptosis inducing mechanism in hepatic cancer cells HepG2 by using MTT assay, flow cytometry and western blotting analysis. Results suggested that the flavonoids from laba garlic could be a promising agent towards the development of functional foods (23).

The antioxidant activity (AA) of garlic is caused by the presence of polyphenol and sulphur components. The purpose of this study was to assess the AA of raw garlic, as well as its commercialised products and shelf life. Three different techniques were used to assess the antioxidant activity of fresh garlic (FG) and its products, including chopped with salt (CGS), chopped without salt (CG), fried (FRG), and mixed garlic (FG with dehydrated garlic; MG). These techniques were the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, -carotene/linoleic acid assay, and Rancimat® method. Fried garlic has the highest antioxidant activity of all the products evaluated. During the shelf life of all examined products, the free radical-scavenging activity decreased, which was correlated with the decline in the overall polyphenol content. Our results imply that substances other than phenol may have contributed to this result.(24)

Results of DPPH radical scavenging activity conducted by Hyun Joo Jung, showed a decreasing tendency according to the extraction solvent (DW > ethanol > chloroform) (25). Results show that the antioxidant activity of extracts was IC₅₀ = 0.240.00 mg/mL. At a concentration of 0.1%, the garlic peel extract demonstrated strong efficacy, removing almost 90% of all the DPPH radicals. Similar results of FRAP of fractions were generally observed in the studies conducted by(14), Compared with those of DPPH radical scavenging activity. FRAP values of extract were observed to be correlated with their total phenol contents and total flavonoid contents. The FRAP value for methanolic garlic peeled extract was found to be (0.82 ± 0.01), lower than that of ascorbic acid (26).

CONCLUSION

As a conclusion, 70% methanolic garlic extract showed excellent antioxidant activity. Total

phenol, flavonoid contains were also assessed, by DPPH scavenging activities and IC 50. It was found that methanolic extract had the maximum phenolic content. They inhibit thermal deterioration of oil by improving its hydrolytic stability and inhibiting double bond conjugation. Therefore for garlic peel extracts can be considered as natural antioxidants. Extracts with high flavonoid and total phenol concentration demonstrated potent antioxidant action. The presence of well-known antioxidant components including phenols and flavonoids in significant amounts is primarily responsible for the remarkable antioxidant activity of the 70% ethanol extract. We believed that further research should be done on these peel fractions to determine whether they might offer cancer and cardiovascular disease protection.

REFERENCE

1. Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *J Am Coll Nutr.* 2000 Jun;19(3 Suppl):312S – 319S.
2. Amagase H. Clarifying the real bioactive constituents of garlic. *J Nutr.* 2006 Mar;136(3 Suppl):716S – 725S.
3. Chung LY. The Antioxidant Properties of Garlic Compounds: Allyl Cysteine, Alliin, Allicin, and Allyl Disulfide [Internet]. Vol. 9, *Journal of Medicinal Food.* 2006. p. 205–13. Available from: <http://dx.doi.org/10.1089/jmf.2006.9.205>
4. Kim GH, Department of Biological Science and Technology, Yonsei University, Seoul, Korea, Duan Y, et al. Assessment of Antioxidant Activity of Garlic (*Allium sativum* L.) Peels by Various Extraction Solvents [Internet]. Vol. 33, *Journal of the Korean Oil Chemists' Society.* 2016. p. 204–12. Available from: <http://dx.doi.org/10.12925/jkocs.2016.33.1.204>
5. Samuel SR, Kuduruthullah S, Khair AMB, Al Shayeb M, Elkaseh A, Varma SR, et al. Impact of pain, psychological-distress, SARS-CoV2 fear on adults' OHRQOL during COVID-19 pandemic. *Saudi J Biol Sci.* 2021 Jan;28(1):492–4.
6. Gowhari Shabgah A, Amir A, Gardanova ZR, Olegovna Zekiy A, Thangavelu L, Ebrahimi Nik M, et al. Interleukin-25: New perspective and state-of-the-art in cancer prognosis and treatment approaches. *Cancer Med.* 2021 Aug;10(15):5191–202.
7. Muthukrishnan L. Multidrug resistant tuberculosis - Diagnostic challenges and its conquering by nanotechnology approach - An overview. *Chem Biol Interact.* 2021 Mar 1;337:109397.
8. Kanniah P, Radhamani J, Chelliah P, Muthusamy N, Joshua Jebasingh Sathiyar Balasingh E, Reeta Thangapandi J, et al. Green synthesis of multifaceted silver nanoparticles using the flower extract of *Aerva lanata* and evaluation of its biological and environmental applications. *ChemistrySelect.* 2020 Feb 21;5(7):2322–31.
9. Ramesh Kumar KR, Shanta Sundari KK, Venkatesan A, Chandrasekar S. Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study. *Am J Orthod Dentofacial Orthop.* 2011 Oct;140(4):479–85.
10. Ganapathy D, Ramadoss R, Yuwanati M, Karthikeyan M. Rarity of mucormycosis in oral squamous cell carcinoma: A clinical paradox? *Oral Oncol.* 2022 Feb;125:105725.
11. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. *Am J Cancer Res.* 2020 Aug 1;10(8):2546–54.
12. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. *J Endod.* 2021 Aug;47(8):1198–214.
13. Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O, et al. Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles [Internet]. Vol. 129, *Inorganic Chemistry Communications.* 2021. p. 108647. Available from: <http://dx.doi.org/10.1016/j.inoche.2021.108647>
14. Chaudhary BU, Lingayat S, Banerjee AN, Kale RD. Development of multifunctional food packaging films based on waste Garlic peel extract and Chitosan. *Int J Biol Macromol.* 2021 Dec 1;192:479–90.
15. Ifesan B. Investigation of Antioxidant and Antimicrobial Properties of Garlic Peel Extract (*Allium sativum*) and Its Use as Natural Food Additive in Cooked Beef [Internet]. Vol. 3, *Journal of Scientific Research and Reports.* 2014. p. 711–21. Available from: <http://dx.doi.org/10.9734/jsrr/2014/5726>
16. Ogunjobi AA, Fagade OE, David OO. Antimutagenic and potential anticarcinogenic activities of aloe-vera gel and aqueous garlic extract in the bacterial reverse mutation test

- (ames assay) [Internet]. Vol. 10, African Journal of Biomedical Research. 2010. Available from: <http://dx.doi.org/10.4314/ajbr.v10i3.50654>
17. Elsom GK. An antibacterial assay of aqueous extract of garlic against anaerobic/microaerophilic and aerobic bacteria [Internet]. Vol. 12, Microbial Ecology in Health and Disease. 2000. p. 81–4. Available from: <http://dx.doi.org/10.1080/089106000435464>
 18. Demircan B, Velioglu YS. Optimization of Drying Parameters to Maximize Antioxidant Activity, Total Phenolic and Anthocyanin Content of Purple Carrot Puree Powder [Internet]. Available from: <http://dx.doi.org/10.21203/rs.3.rs-1580258/v1>
 19. Kaur S. Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants [Internet]. Vol. 1, Journal of Microbiology & Experimentation. 2014. Available from: <http://dx.doi.org/10.15406/jmen.2014.01.00005>
 20. Carreón-Delgado DF, Hernández-Montesinos IY, Rivera-Hernández KN, Del Sugeyrol Villar-Ramírez M, Ochoa-Velasco CE, Ramírez-López C. Evaluation of Pretreatments and Extraction Conditions on the Antifungal and Antioxidant Effects of Garlic () Peel Extracts. Plants [Internet]. 2023 Jan 3;12(1). Available from: <http://dx.doi.org/10.3390/plants12010217>
 21. Soto-Hernández M, Tenango MP, García-Mateos R. Phenolic Compounds: Natural Sources, Importance and Applications. BoD – Books on Demand; 2017. 458 p.
 22. Charles DJ. Antioxidant Properties of Spices, Herbs and Other Sources. Springer Science & Business Media; 2012. 612 p.
 23. Gao X, Yanan J, Santhanam RK, Wang Y, Lu Y, Zhang M, et al. Garlic flavonoids alleviate H₂O₂ induced oxidative damage in L02 cells and induced apoptosis in HepG2 cells by Bcl-2/Caspase pathway. J Food Sci. 2021 Feb;86(2):366–75.
 24. Koch HP, Lawson LD. Garlic: The Science and Therapeutic Application of Allium Sativum L. and Related Species. Lippincott Williams & Wilkins; 1996. 360 p.
 25. Jang HJ, Lee HJ, Yoon DK, Ji DS, Kim JH, Lee CH. Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. Food Sci Biotechnol. 2018 Feb;27(1):219–25.
 26. Aydın S, Kahyaoğlu DT. Antioxidant Effect Potential of Garlic In Vitro and Real Food System: Effects of Garlic Supplementation on Oxidation Stability and Sensory Properties of Butter [Internet]. Vol. 122, European Journal of Lipid Science and Technology. 2020. p. 1900261. Available from: <http://dx.doi.org/10.1002/ejlt.201900261>