Verdant Legacy

Plant Protection and Agritech 1(1): 2026

ISSN ONLINE:
PLANT PROTECTION AND AGRITECH
A Research Journal of Plant Science
CHIEF EDITOR: Dr. Arusa Aftab

Research Article

In-silico Analysis of Influence of Foliar application of Silver Nanoparticles on Chlorophyll Proteins of Cabbage

Kinza Ramzan[1], Summera Jahan[1*], Lubaba Komal[1], Atif Kamran[1]

[1] Institute of Botany, University of the Punjab, Lahore, Pakistan

Corresponding author: summera.botany@pu.edu.pk

Abstract

This study was designed to explore how the interaction between green silver nanoparticles and chlorophyll proteins is still poorly understood. This study investigated the molecular binding of neem-derived saponin-coated AgNPs with the water-soluble chlorophyll protein of *Brassica oleracea*. The objective was to better understand the mechanism that may contribute to improved photosynthetic performance following nanoparticle application. We proposed that saponin-coated AgNPs could form stable interactions with WSCP and potentially influence its structural integrity, particularly under stress conditions. WSCP was chosen because of its well-established role in chlorophyll stabilization and photoprotective functions within plant cells. Molecular docking revealed strong binding between saponin from neem-derived silver nanoparticles and the water-soluble chlorophyll protein of cabbage, supported by multiple non-covalent interactions and a favorable binding affinity (−7.8). This interaction provides a molecular explanation for enhanced photosynthetic performance following AgNP application and underscores the potential of neem-based green nanotechnology in sustainable agriculture.

Key Words: AgNPs **,** Hydrogen bonding, metal ion ,Ligand ,Saponin

Received: 05 January 2026 **Accepted**: 25 January 2026 date: **Published**: 31 March 2026

Citation: Ramzan, K., S. Jahan, L. Komal and A. Kamran (2026). In-silico Analysis of Influence of Foliar application of Silver Nanoparticles on Chlorophyll Proteins of Cabbage. Plant Protection and Agritech. 1(1): 1-8

Introduction

Nanotechnology has introduced new opportunities in agriculture through the development of nanoparticles that can enhance plant growth, protect crops from pathogens, and improve stress tolerance (Zhao *et al*., 2020). Silver nanoparticles (AgNPs) have gained considerable attention due to their broad biological activity, high surface reactivity, and highly use in agriculture, medicine, and environmental applications (Bamal *et al*., 2021). In recent years, green synthesis of AgNPs using plant extracts has emerged as an eco-friendly alternative to chemical methods (Bhardwaj *et al*., 2020).

Neem (*Azadirachta indica*) leaves are widely used for the green synthesis of silver nanoparticles because they are contain bioactive compounds such as Saponin ,flavonoids, terpenoids, phenolics, and proteins (Ahmed *et al*., 2023). These phytochemicals act simultaneously as reducing, stabilizing, and capping agents, resulting in biologically active and stable AgNPs (Ahmed *et al*., 2016). Neem-mediated AgNPs have attracted considerable interest due to their enhanced biocompatibility and potential agricultural applications (Ezeh *et al*., 2024).

When applied to crop plants such as cabbage (*Brassica oleracea*), silver nanoparticles can enter plant tissues through roots or leaf surfaces and interact with cellular components (Siddiqi & Husen *et al*., 2022). Several studies have reported that AgNPs exposure may influence plant growth, photosynthesis, nutrient uptake, and oxidative balance, depending on nanoparticle concentration and size (Yan & Chen, 2019). In cabbage and other leafy vegetables, these effects are particularly important because of direct nanoparticle contact with edible tissues. We chose cabbage (*Brassica oleracea var. capitata*) because it is an important and widely cultivated vegetable, making it relevant for studying AgNP effects. The protein selected is chlorophyll-associated, critical for photosynthesis, allowing meaningful evaluation of AgNP impact on plant physiology.

Molecular docking is a powerful *in silico* technique used to predict interactions between nanoparticles (or released Ag^+ ions) and biological macromolecules such as proteins and enzymes (Abdelsattar *et al*., 2021). Although initially designed for small organic ligands, molecular docking is now increasingly applied to nanoparticle research by modeling silver atoms or small silver clusters as ligands interacting with target proteins (Kar *et al*., 2024). This approach helps identify potential binding sites, interaction energies, and key amino acid residues involved in Ag–protein binding. In plants, AgNPs are known to interfere with antioxidant defense mechanisms by interacting with enzymes such as superoxide dismutase, catalase, and peroxidases, leading to oxidative stress and metabolic disturbances (Liu *et al*., 2020). Molecular docking reveals how neem-derived silver nanoparticles interact with cabbage proteins at the molecular level, explaining their physiological effects. Thus, docking-based analysis serves as an important link between nanoparticle exposure and plant biochemical behavior. Molecular docking of neem-derived silver nanoparticles with cabbage proteins helps to predict nanoparticle-induced effects and supports their safe use in sustainable agriculture. (Yan & Chen, 2019; Kar *et al*., 2024). we focused only on AgNP interactions with the protein, and Ag^+ interactions were not analyzed, but we will consider exploring this distinction in future work. In the current study, our work is limited to *in silico* docking predictions, and no experimental validation has been performed. We recognize the importance of linking computational results with laboratory experiments and will consider such validation in future studies to strengthen the biological relevance and applicability of our findings.

Material Method

Fresh leaves of neem (*Azadirachta indica*) 25g were collected from university of the Punjab, Lahore Pakistan (geographical coordinates 31.5°N, 74.3°E).Then the prepared neem leaf extract was mixed with 0.01 M $AgNO_3$ solution in a 1:4 volume ratio (20 ml neem extract to 80 ml $AgNO_3$ solution). The pH of the reaction mixture was adjusted to 8 by dilute NH_4OH. The flask was then placed on a rotary shaker at 80 rpm for 4 hoursand boiled in deionized water to prepare an aqueous extract.The reaction mixture was gently shaken until a visible color change confirmed nanoparticle formation, after which the particles were centrifuged, purified, and oven-dried (Namratha & Monica, 2013).

The experimental design was randomized complete block design with nine treatment combinations and three replicate plots per treatment. Each individual sub-plot measured to be 42 m^2 and consisted of four rows; within each rowcabbage (*Brassica oleracea* var. capitata Hybrid F1) 11seeds were sown. Seed sowing was done at a depth of 2 cm in the soil.

Molecular docking

Saponin was observed as a major compound in green synthesized silver nanoparticles. This compound was further used as ligand for molecular docking to study its role in improving physiological or morphological attributes of cabbage. Its molecular structure was retrieved from Pubchem (https://pubchem.ncbi.nlm.nih.gov) with Compound ID:198016, molecular formula C58H94O27 and molecular weight 1223.3 g/mol. Receptor protein selected for docking was based on photosynthetic responses observed in the study. Water-soluble Chlorophyll Protein (WSCP) was reported in literature (Agostini *et al*., 2019). Its PDB formate was retrieved from protein data bank with Protein ID 6S2Z (pdb_00006s2z) (https://www.rcsb.org/structure/6S2Z). Both the molecules were cleaned in Pymol software and docking was performed through AutoDock Vina in AMDock software. Biovia Discovery Studio was used to visualize the compounds and to generate 2D structure of docked molecules. Docking interactions and bonds were analyzed through PLIP web (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index).

Results

Fig 1 presents the pictorial frames of compounds used in molecular docking sowing raw protein (Fig a), ligand (Fig b), cleaned protein (Fig c), docking interface between ligand and protein (Fig d and 1e), and 2-dimensional (2D) presentation of docking results (Fig f). The 2D diagram showed that there are alkyl, van der waal interactions, conventional hydrogen and carbon-hydrogen bonds formed when saponin (ligand) interacts with the water-soluble chlorophyll protein (WSCP). The affinity (-7.8) at three binding sites depicted strong interactive capacity between receptor protein and ligand at lowest activation energy (Fig 2a). The interactions observed from PLIP website (Fig 2b) presented four strong hydrogen bonds (Fig 2c). The hydrogen bonds were formed between amino acids threonine, leucine, and serine with ligand residues 48A, 49A, 84A respectively. The docking results were presented using AutoDock software, supported by visualization and interaction analysis to ensure clarity and transparency. In the revised manuscript, we have further strengthened the interpretation of these findings by linking specific interactions and binding affinity values to their biological relevance.

Discussion

In this study, the protein was prepared by cleaning to remove unnecessary ligands after downloading from the Protein Data Bank, and no further structural modifications were performed. The term "activation energy" was reported based on observations from molecular docking using AutoDock software. Among bio-organic compounds in AgNPs, saponin is reported as a major derivative of neem extract (Dash *et al*., 2017). The saponins have a compact structure composed of four hydrocarbon rings to which sugar is bound in groups of one or two not more than ten units. Saponins comprise a large class of plant-based natural products, including triterpene (C30), steroids (C27), and glycosides, which are further divided into 11 main carbon-skeleton-based subgroups (Bailly and Vergoten, 2020). A significant number of plant families synthesize saponin (Hussain *et al*., 2019). Various plant sections have explicit saponin concentrations and types (Zhou *et al*., 2018). The age and mechanisms of plants determined the kind of saponin molecules, leaf produces more types and amounts of saponins than shoots or flowers (Wei *et al*., 2018). Plants possess water-soluble chlorophyll-binding proteins (WSCPs) that are not directly involved in light harvesting or electron transport during photosynthesis. Instead, these proteins are thought to play a protective and regulatory role, especially when plants are exposed to stress conditions (Theiss *et al*., 2007). Water-soluble chlorophyll-binding proteins (WSCPs) are grouped into two main classes based on their photo conversion ability. Class I proteins can alter the absorption properties of bound chlorophyll when exposed to light, whereas Class II proteins lack this capability. Class II WSCPs are further divided into two subclasses. Subclass IIA predominantly binds chlorophyll a, while subclass IIB can bind both chlorophyll a and b with comparable efficiency (Maleeva *et al*., 2019).

For observing its role as a compound in AgNPs, molecular docking reported that it has strong affinity with proteins playing role in photosynthesis. Water-soluble Chlorophyll protein (WSCP) was reported in *Brassica oleracea,* that has a critical role in activity of Chlorophyll b pigments (Agostini *et al*., 2019) Hence, results observed for improved photosynthetic rate with AgNPs were attributed to saponins affinity with WSCPs in cabbage. On the other hand, the study reports computational interactions between ligand and protein. These responses may vary in-vivo cell cultures and needs more accurate monitoring in future studies.

Conclusion

Molecular docking showed that saponin from neem-derived silver nanoparticles binds strongly with the water-soluble chlorophyll protein of cabbage, forming several hydrogen bonds and exhibiting a favorable binding affinity (−7.8). This interaction helps explain the improved photosynthetic performance observed after AgNP application and demonstrates the usefulness of docking studies for understanding nanoparticle effects in sustainable agriculture.

Author Contributions:

Conceptualization, KR.; methodology, SJ.; software, KR.; validation, SJ. formal analysis, LK.; investigation, SJ; data curation, LK and AK.; writing—original draft preparation

Funding: The author states that no external funding was received for this research.

Conflicts of Interest: The authors declare no conflicts of interest

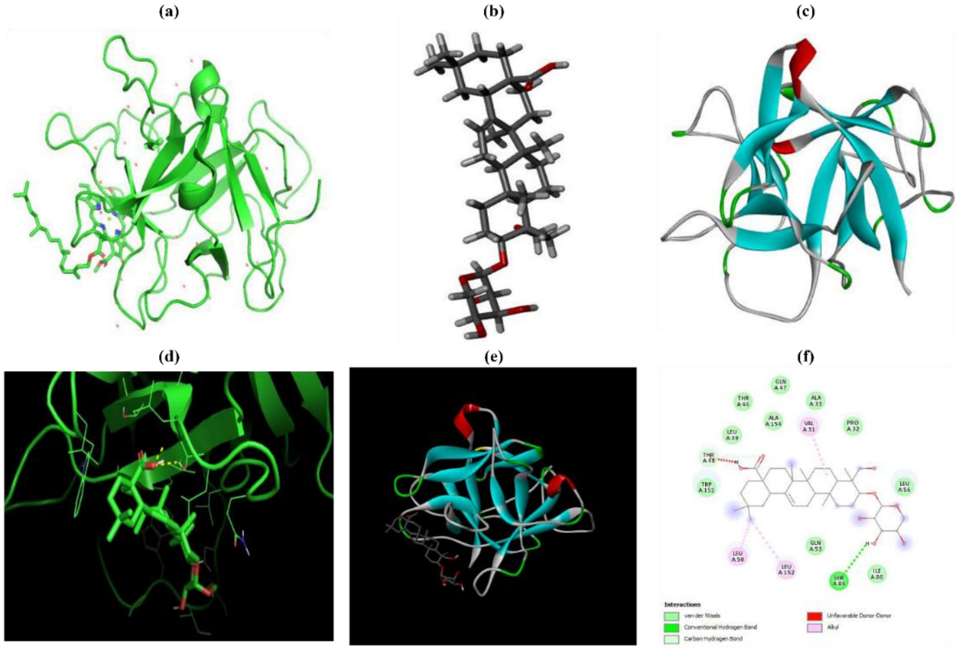
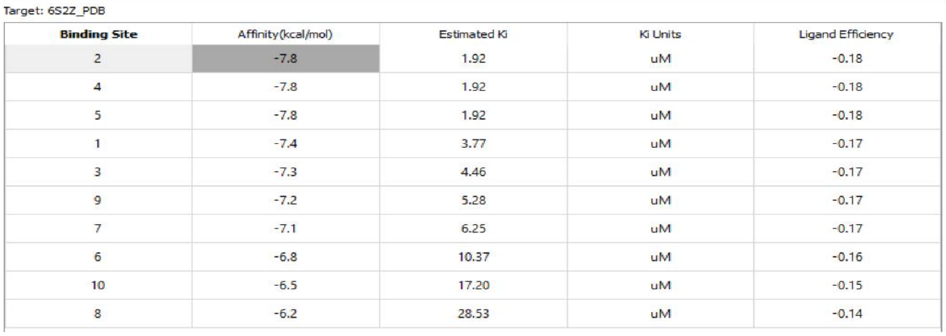


Fig 1. Pictorial presentation of molecules use in molecular docking showing **A)** raw protein **B)** ligand **C)** cleaned protein **D-E)** docking interface **F)** 2D structure of docked molecule

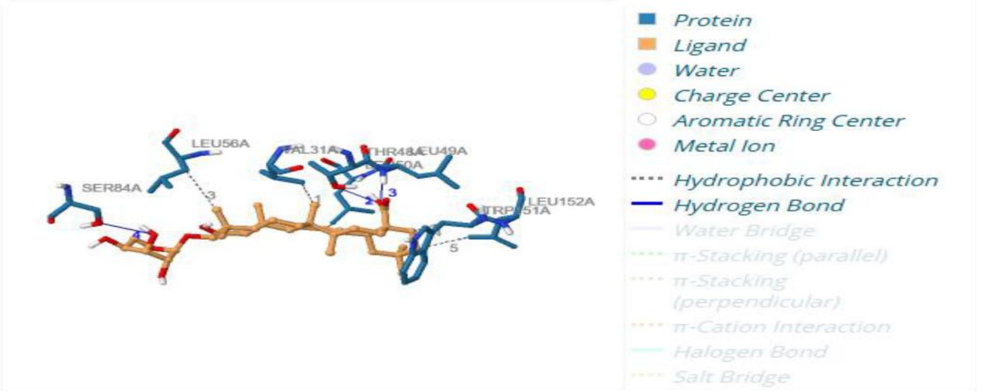
(a)



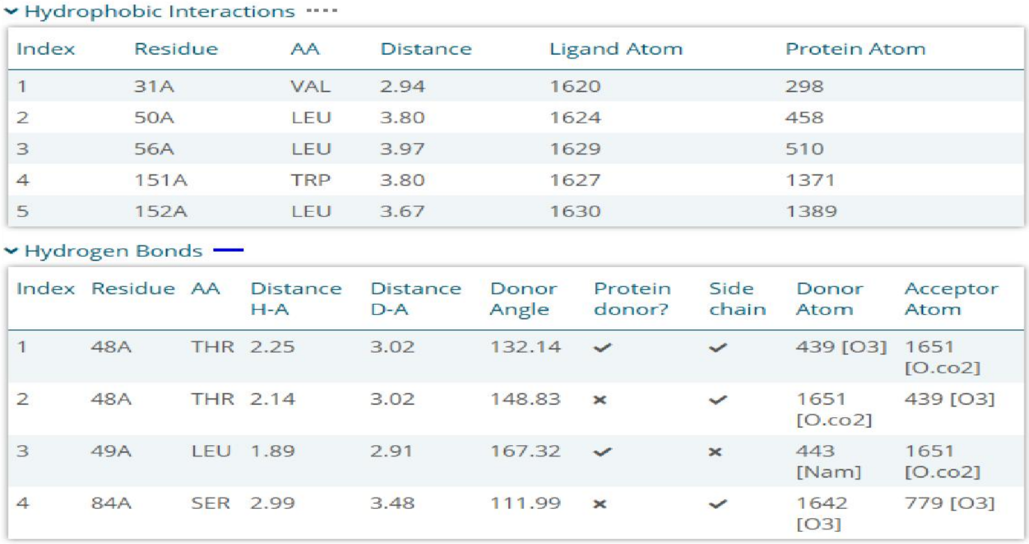
Target: 6S2Z_PDB

Binding Site	Affinity(kcal/mol)	Estimated Ki	Ki Units	Ligand Efficiency
2	-7.8	1.92	uM	-0.18
4	-7.8	1.92	uM	-0.18
5	-7.8	1.92	uM	-0.18
1	-7.4	3.77	uM	-0.17
3	-7.3	4.46	uM	-0.17
9	-7.2	5.28	uM	-0.17
7	-7.1	6.25	uM	-0.17
6	-6.8	10.37	uM	-0.16
10	-6.5	17.20	uM	-0.15
8	-6.2	28.53	uM	-0.14

(b)



(c)



Hydrophobic Interactions

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	31A	VAL	2.94	1620	298
2	50A	LEU	3.80	1624	458
3	56A	LEU	3.97	1629	510
4	151A	TRP	3.80	1627	1371
5	152A	LEU	3.67	1630	1389

Hydrogen Bonds

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	48A	THR	2.25	3.02	132.14	✓	✓	439 [O3]	1651 [O.co2]
2	48A	THR	2.14	3.02	148.83	✗	✓	1651 [O.co2]	439 [O3]
3	49A	LEU	1.89	2.91	167.32	✓	✗	443 [Nam]	1651 [O.co2]
4	84A	SER	2.99	3.48	111.99	✗	✓	1642 [O3]	779 [O3]

Fig 2. Docking results showing **A)** affinities **B)** interactions and **C)** bonds

References

Abdelsattar, A. S., Dawoud, A., & Helal, M. A. (2021). Interaction of nanoparticles with biological macromolecules: A review of molecular docking studies. *Nanotoxicology*, *15*(1), 66-95 doi.org/10.1080/17435390.2020.1842537

Agostini, A., Meneghin, E., Gewehr, L., Pedron, D., Palm, D. M., Carbonera, D., ... & Collini, E. (2019). How water-mediated hydrogen bonds affect chlorophyll a/b selectivity in Water-Soluble Chlorophyll Protein. Scientific reports, 9(1), 18255 doi.org/10.1038/s41598-019-54520-4.

Ahmed, M., Marrez, D. A., Mohamed Abdelmoeen, N., Abdelmoneem Mahmoud, E., Ali, M. A. S., Decsi, K., & Tóth, Z. (2023). Studying the antioxidant and the antimicrobial activities of leaf successive extracts compared to the green-chemically synthesized silver nanoparticles and the crude aqueous extract from Azadirachta indica. *Processes*, *11*(6), 1644 doi.org/10.3390/pr11061644

Ahmed, S., Ahmad, M., Swami, B. L., & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of advanced research*, *7*(1), 17-28 doi.org/10.1016/j.jare.2015.02.007

Bailly, C., & Vergoten, G. (2020). Proposed mechanisms for the extracellular release of PD-L1 by the anticancer saponin platycodin D. *International Immunopharmacology*, *85*, 106675 doi.org/10.1016/j.intimp.2020.106675

Bamal, D., Singh, A., Chaudhary, G., Kumar, M., Singh, M., Rani, N., ... & Sehrawat, A. R. (2021). Silver nanoparticles biosynthesis, characterization, antimicrobial activities, applications, cytotoxicity and safety issues: An updated review. *Nanomaterials*, *11*(8), 2086 doi.org/10.3390/nano11082086

Bhardwaj, B., Singh, P., Kumar, A., Kumar, S., & Budhwar, V. (2020). Eco-friendly greener synthesis of nanoparticles. *Advanced pharmaceutical bulletin*, *10*(4), 566 doi: 10.34172/apb.2020.067

Dash, S. P., Dixit, S., & Sahoo, S. (2017). Phytochemical and biochemical characterizations from leaf extracts from Azadirachta Indica: an important medicinal plant. Biochem Anal Biochem, 6(323), 2161-1009 DOI: 10.4172/2161-1009.1000323

Ezeh, C. K., Eze, C. N., Dibua, M. U. E., & Emencheta, S. C. (2024). A review on Azadirachta indica (neem) plant mediated biosynthesis, characterisation and antimicrobial activity of silver nanoparticles. *International Journal of Biomedical Nanoscience and Nanotechnology*, *5*(1), 15-36 doi.org/10.1504/IJBNN.2024.139351

Hussain, M., Debnath, B., Qasim, M., Bamisile, B. S., Islam, W., Hameed, M. S., ... & Qiu, D. (2019). Role of saponins in plant defense against specialist herbivores. *Molecules*, *24*(11), 2067 doi.org/10.3390/molecules24112067

Kar, P., Oriola, A. O., & Oyedeji, A. O. (2024). Molecular docking approach for biological interaction of green synthesized nanoparticles. *Molecules*, *29*(11), 2428 doi.org/10.3390/molecules29112428

Liu, W., Worms, I., & Slaveykova, V. I. (2020). Interaction of silver nanoparticles with antioxidant enzymes. *Environmental Science: Nano*, *7*(5), 1507-1517 DOI: 10.1039/C9EN01284B

Namratha, N., & Monica, P. V. (2013). Synthesis of silver nanoparticles using Azadirachta indica (Neem) extract and usage in water purification. Asian J. Pharm. Tech, 3(4), 170–174. https://doi.org/10.51966/jvas.2023.54.3.647-655

Maleeva, Y. V., Neverov, K. V., Obukhov, Y. N., & Kritsky, M. S. (2019). Water

soluble chlorophyll-binding proteins of plants: structure, properties and functions. *Molecular Biology*, *53*(6), 876-888 doi.org/10.1134/S0026893319060128
Siddiqi, K. S., & Husen, A. (2022). Plant response to silver nanoparticles: a critical review. *Critical Reviews in Biotechnology*, *42*(7), 973-990 doi.org/10.1080/07388551.2021.1975091

Theiss, C., Trostmann, I., Andree, S., Schmitt, F. J., Renger, T., Eichler, H. J., ... & Renger, G. (2007). Pigment− pigment and pigment− protein interactions in recombinant water-soluble chlorophyll proteins (WSCP) from cauliflower. *The Journal of Physical Chemistry B*, *111*(46), 13325-13335. https://doi.org/10.1021/jp0723968

Wei, G., Wei, F., Yuan, C., Chen, Z., Wang, Y., Xu, J., ... & Chen, S. (2018). Integrated chemical and transcriptomic analysis reveals the distribution of protopanaxadiol- and protopanaxatriol-type saponins in Panax notoginseng. *Molecules*, *23*(7), 1773 doi.org/10.3390/molecules23071773

Yan, A., & Chen, Z. (2019). Impacts of silver nanoparticles on plants: a focus on the phytotoxicity and underlying mechanism. *International journal of molecular sciences*, *20*(5), 1003 doi.org/10.3390/ijms20051003

Zhao, L., Lu, L., Wang, A., Zhang, H., Huang, M., Wu, H., ... & Ji, R. (2020). Nano-biotechnology in agriculture: use of nanomaterials to promote plant growth and stress tolerance. *Journal of agricultural and food chemistry*, *68*(7), 1935-1947 https://doi.org/10.1021/acs.jafc.9b06615

Zhou, Q. L., Zhu, D. N., Yang, X. W., Xu, W., & Wang, Y. P. (2018). Development and validation of a UFLC–MS/MS method for simultaneous quantification of sixty-six saponins and their six aglycones: Application to comparative analysis of red ginseng and white ginseng. *Journal of Pharmaceutical and Biomedical Analysis*, *159*, 153-165 doi.org/10.1016/j.jpba.2018.06.048