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SYNTHESIS OF PLANT-DERIVED SELENIUM NANOPARTICLES FROM LANKARAN-ASTARA TEA (*Camellia sinensis* L.) PLANT AND EVALUATION OF THEIR ACTIVITIES ON DIFFERENT ENZYMES

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Abstract. In this study, selenium nanoparticles (SeNPs) were produced utilizing a green technique with *Camellia sinensis* (green tea) extract, which is high in polyphenols, flavonoids and catechins, all of which are known antioxidants and bioactive. For this reason, the tea plant can be used in the treatment and prevention of many diseases. Selenium nanoparticles (SeNPs) produced by green synthesis method with plant extracts provide more advantages compared to traditional physical and chemical methods. In this study, *Camellia sinensis* L. (tea) plant was used as a reducing agent in the synthesis of selenium nanoparticles (SeNPs). The obtained tea extract selenium nanoparticles (CS-SeNPs) were tested on various enzymatic activities such as anticholinesterase (AChE), butyrylcholinesterase (BChE), urease, α-amylase, α-glucosidase and tyrosinase inhibitor activities. The results revealed that CS-SeNPs exhibited strong inhibitory effects especially on AChE and BChE activities. (AChE IC50: 25.33 ± 0.78 µg/mL, BChE IC50: 17.53 ± 0.27 µg/mL). Additionally, CS-SeNPs exhibited the strongest inhibitory activity against urease enzyme with an IC50 value of 13.72 ± 0.18 µg/mL. CS-SeNPs also showed strong inhibition on α-amylase enzyme with an IC50 value of 25.98 ± 0.83 µg/mL, while the inhibitory effect on tyrosinase was determined as 31.40 ± 0.51 µg/mL. In compared to conventional inhibitors such as galantamine, acarbose, thiourea and kojic acid, the CS-SeNPs showed weaker but still significant inhibitory action in all enzyme models. The current study is significant because it investigates the environmentally friendly synthesis of selenium nanoparticles using Camellia sinensis and assesses their potential as multifunctional enzyme inhibitors, which could help to develop alternative therapeutic agents for neurodegenerative and metabolic diseases.

Keywords: *Green synthesize, tea, selenium nanoparticles, bioactivity.*

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1. Introduction

Tea (Camellia sinensis) is one of the most widely consumed beverages globally and its consumption is associated with numerous health benefits (da Silva, 2013). The tea plant has been widely used for centuries by ancient cultures for its medicinal properties. Tea is popularly consumed in unfermented (green tea) , semifermented (oolong tea) and fermented (black and pu-erh or red) forms (Zhu *et al.,* 2002) Herbal drinks are typically made from natural ingredients derived from various plant parts, such as leaves, stems, roots, fruits, buds and flowers, which have been consumed for generations. The type of tea and the methods used to prepare tea beverages are believed to significantly affect their physicochemical, phytochemical and pharmacological properties. Furthermore, the phytochemical compounds found in tea are thought to influence the pharmacological effects of herbal drinks. Ultimately, tea drinks have the potential to be commercialized as healthy beverage products, offering numerous health benefits and attracting consumers. It is well-known as a rich source of bioactive compounds such as vitamins, polyphenols and polysaccharides (Lorenzo & Munekata, 2016). Among these, polysaccharides and polyphenols are the primary bioactive components. Both green and black tea contain a group of polyphenols known as tea flavanols, which include epicatechin (EC), epigallocatechin (EGC), EC gallate (ECG) and EGC gallate (EGCG) (Yang *et al*., 2001). Tea polysaccharides have been shown to exhibit various biological activities, including antioxidant, antibacterial, immunomodulatory and glycosidase inhibitory effects (Chen *et al*., 2009). Many of these compounds possess strong antioxidant properties, enabling them to neutralize reactive free radicals, lessen oxidative stress and regulate inflammatory processes. Numerous studies have suggested that regularly consuming tea (including black, green, oolong, etc.) can aid in preventing lifestyle-related diseases such as cardiovascular diseases, certain cancers and type 2 diabetes. In recent decades, the effects of tea on health have been extensively explored through animal studies and human epidemiological and clinical research. Tea is a globally cherished beverage valued for its taste and health benefits, largely attributed to its rich content of polyphenols and amino acids. Selenium (Se), an essential trace element for both plants and humans, plays a dual role in promoting plant growth and enhancing human health, yet its effect on tea quality remains underexplored (Liao *et al*., 2025). Recent advances in nanotechnology have brought selenium nanoparticles (SeNPs) into focus, as they offer enhanced bioavailability, reduced toxicity and improved therapeutic potential compared to conventional selenium compounds (Zhang *et al*., 2005; 2008).

The scientific community is actively investigating the use of nanoparticles in nanomedicine and biomedical fields. In biomaterials and bio-nanotechnology, selenium nanoparticles (SeNPs) are crucial due to their wide range of physical, chemical and biological properties, including antibacterial, antiviral, antifungal and anticancer activities. Green synthesis technology has become a preferred method because it is cost-effective, eco-friendly and biologically safe. The extract from green tea leaves can reduce selenium to SeNPs.

The main objective of this study is to synthesize plant-derived selenium nanoparticles using tea plant (*Camella Sinensis* L.) extract as a reductant. To determine the effect of the obtained plant-derived selenium nanoparticles on different enzyme inhibition (AChE and BChE) and their anti-diabetic activity.

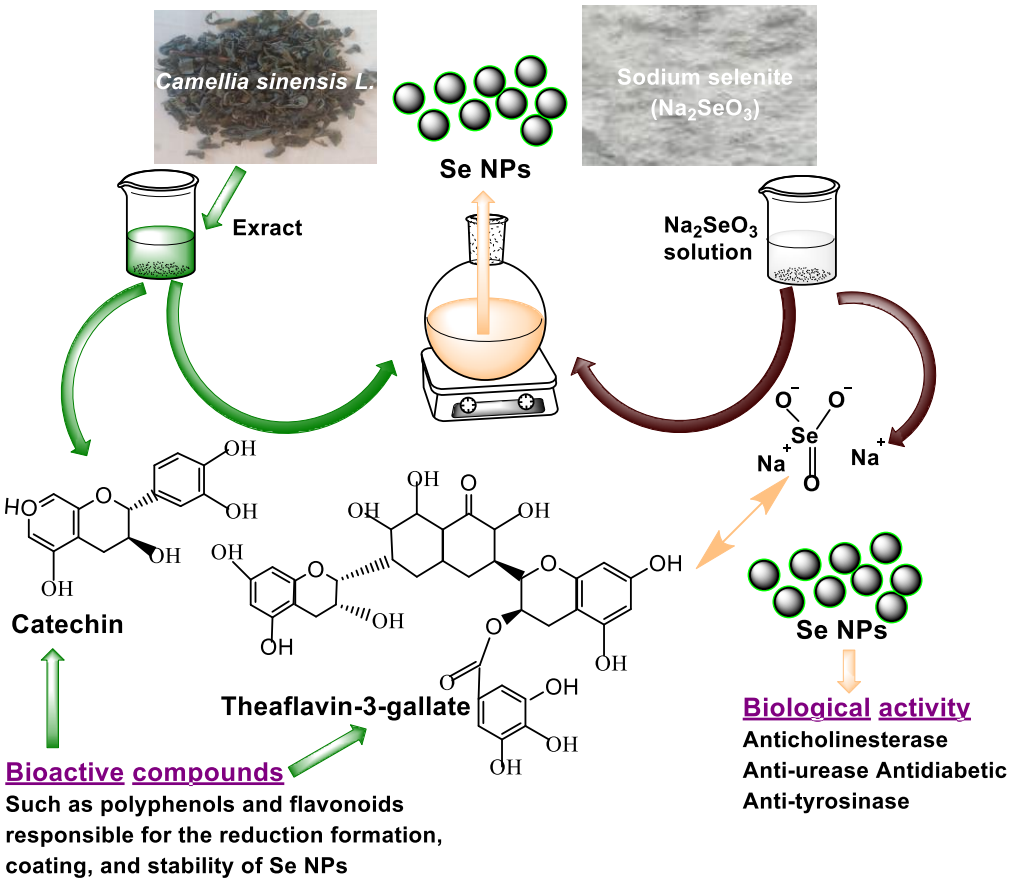
2. Material and Methods

2.1. Camellia sinensis L. Plant Materials

Tea plant (*Camellia sinensis* L.) Kuntze) grown in the experimental area of the Lankaran Tea Branch of the Scientific Research Fruit and Tea Cultivation Institute was provided. *Camellia sinensis* L. plant was dried. Then, 50 g of the dried tea plant was taken and boiled with pure water. The filtrate was removed. The dark red tea plant obtained was left to diffuse at +4°C to be used in experimental studies.

2.2. Synthesis of Selenium Nanoparticles from Tea (Camellia sinensis L.) plant

400 mL of previously prepared *Camellia sinensis* L. plant extract was taken and placed in a beaker. Then, 750 mL of 0.2 Molar selenium salt (Sodium selenite, Sigma) solution was prepared and slowly placed on the plant extract. The reaction conditions were kept at 75 °C on a magnetic stirrer for 8 hours. Then, it was seen that the dark solution started to precipitate. After the precipitation process, it was subjected to centrifugation. The precipitated part was dried on a glass pellet at 105 °C for 2 days (Sheme 1).



Sheme 1. Representative scheme of green synthesis of Se NPs from *Camellia sinensis* L. plant

2.3. Anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase enzyme inhibition activities were determined spectrophotometrically according to Ellman's method with modifications (Ellman *et al*., 1961; Tel *et al*., 2012). Galantamine was used as the reference compound.

2.4. Anti-urease activity

Anti-urease activity was determined by measuring ammonia production by the indophenol method (Weatherburn, 1967). Canavalia ensiformis (Jack bean, Type III) urease enzyme was used and the enzyme solution was prepared with 100 mM sodium phosphate buffer (pH 8.2). Then, urease enzyme (25 µL) and urea (100 mM, 50 µL) solutions were mixed and incubated at 30 °C for 15 min after the addition of extracts (10 µL). After incubation, 45 µL of 1% (w/v) phenol reactant and 70 µL of 0.005% (w/v) alkali reactant were added to each well. After 50 min of incubation, absorbance was measured at 630 nm with a microtiter plate reader. Thiourea was used as the reference compound (Tamfu *et al*., 2020).

2.5. Antidiabetic activity

2.5.1. α-Amylase and α-glucosidase inhibition assay

α-amylase inhibition activity was measured using the starch-iodine method with slight modifications (Quan & Han, 2019; Deveci *et al*., 2021). α-amylase (from porcine pancreas) solution (0.1 unit/mL) was prepared with phosphate buffer (20 mM, pH = 6.9, phosphate buffer prepared with 6 mM NaCl). Then, 50 µL of α-amylase solution and 25 µL of sample solution were mixed into a 96-well microtiter plate. The mixture was pre-incubated at 37 °C for 10 min. After pre-incubation, 50 µL of starch solution (0.05%) was added and incubated at 37 °C for 10 min. HCl (0.1 M, 25 µL) and Lugol (100 µL) solutions were added to complete the reaction and the absorbance was measured at 565 nm in a 96-well microtiter plate. Acarbose was used as a standard. α-glucosidase inhibition activity was determined according to the method of Kim et al. (2010), with some minor modifications (Deveci *et al*., 2021). 50 µL of α-glucosidase (Saccharomyces cerevisiae) (0.1 unit/mL) phosphate buffer (0.01 M, pH = 6.0), 25 µL of PNPG (4-N-nitrophenyl-α-D-glucopyranoside) phosphate buffer (0.01 M, pH = 6.9), 50 µL of phosphate buffer (0.01 M, pH = 6.9) and 10 µL of sample solution were mixed in a 96-well microtiter plate. The solution was incubated for 20 min at 37 °C. The reaction was completed by adding 90 µL of sodium carbonate (0.1 M) and the absorbance was read at 400 nm. Acarbose was used as the reference compound. A 96-well microtiter plate reader was used to measure the absorbance at 400 nm. Acarbose was used as the reference compound.

2.6. Anti-tyrosinase activity

Anti-tyrosinase activity was determined by spectrophotometric method using mushroom tyrosinase enzyme (Masuda *et al*., 2005). Kojic acid was used as a reference compound.

2.7. Statistical analysis

SPSS (22.0) software package was used to perform one-way analysis of variance (ANOVA) in the study. Least Significant Difference (LSD) test was used to determine the significance of any difference between the groups found to be statistically significant ($p < 0.05$). All data were analyzed in triplicate. Results were recorded as mean ± standard error (SE).

3. Results and discussion

Enzyme inhibitors are an important family of drugs and dietary supplements that have applications in the treatment and prevention of cancer, cardiovascular diseases, aging and neurological problems (Rauf & Jehan, 2017). Recently, scientists have focused on finding new cholinesterase inhibitors from natural sources because these compounds have the potential to prevent and treat Alzheimer's disease. In addition, AChE and BChE are therapeutic targets for the improvement of cholinergic deficiency (Arantes *et al*., 2017; Nordberg & Amin, 2013). Anticholinesterase activities of CS-SeNPs synthesized from tea plant were determined against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes using the Ellman method (Table 1).

Table 1. Antidiabetic, cholinesterase, α-glucosidase, α-amylase, urease and tyrosinase inhibitory activities of the CS-SeNPS

Nanoparticles/ Standards	Cholinesterase inhibitory activity				Anti-diabetic activity				Urease inhibitory		Tyrosinase inhibitory	
	AChE		BChE		α-glucosidase		α-amylase					
	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)	IC_{50} (µg/mL)	Inhibition (%) (at 200 µg/mL)
SeNPS	>200	25.33±0.78	>200	17.53±0.27	>200	40.19±0.44	>200	25.98±0.83	>200	13.72±0.18	>200	31.40±0.51
Galantamine	5.50±0.20	89.25±0.48	42.20±0.35	79.43±0.60	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*
Acarbose	*NT*	*NT*	*NT*	*NT*	128.5±0.62	56.70±0.75	32.50±0.45	82.10±0.27	*NT*	*NT*	*NT*	*NT*
Thiourea	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	8.20±0.36	87.37±0.52	*NT*	*NT*
Kojic acid	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	*NT*	23.50±0.44	83.54±0.56

[a]Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$)
NT: not tested

The tested *Camellia sinensis* L. based selenium nanoparticles (CS-SeNPs) demonstrated high inhibitory activity against AChE. (IC50: 25.33 ± 0.78 µg/mL) and showed the highest activity against BChE (IC50: 17.53 ± 0.27 µg/mL) (Table 1). Against AChE and BChE, *Camella sinensis* L. extract showed moderate activity with IC50 values of 135.8 ± 0.88 and 87.23 ± 1.05 µg/mL, respectively (Tamfu *et al*., 2020) and the cholinesterase inhibitory effect of the methanol extract of *thymus nummularius* was evaluated using AChE and BChE enzymes and it was suggested that the inhibitory values were 13.31% and 47.18% at 200 µg/mL, respectively (Ertas *et al*., 2015).Previous studies have linked frequent tea consumption to improved cognitive abilities in older adults and a reduced risk of Alzheimer's disease and other types of neurodegenerative disorders (Polito *et al*., 2018).

Diabetes is characterized by high blood sugar levels, which can lead to serious complications such as kidneys, eyes and cardiovascular system and can cause organ function deterioration (King, 1999). *The species Camellia sinensis* L. has been reported to have a beneficial effect in the treatment of diabetes mellitus. The positive effect of *Camellia sinensis* L. against type-2 diabetes depends on its biologically active

phytochemicals (Vinholes & Vizzotto, 2017; Matsui *et al*., 2006). These secondary metabolites are distributed throughout the plant and extracted in solvents, then fractionated based on polarity. α-glucosidase is responsible for carbohydrate digestion and targets the modulation of postprandial hyperglycemia (Krentz & Bailey, 2005). Enzymes such as α-amylase and α-glucosidase are enzymes found in the gastrointestinal mucosa that play a role in starch digestion. Starch is hydrolyzed by α-amylase to maltose, maltotriose and dextrins, which are then digested by α-glucosidase in the small intestine. The main product of α-glucosidase digestion is glucose, which is actively transported into the blood and increases plasma glucose (Proença & Oliveira, 2017). CS-SeNPs α-glucosidase and α- amylase inhibition activities are given in Table 1. The enzyme activities of CS-SeNPs exhibited the strongest inhibitory activity of α-amylase considering the IC50 values of 40.19 ± 0.44 and 25.26 ± 0.83 mg/mL, respectively. Urease inhibitors have been considered as targets for the treatment of gastric and peptic ulcers (Amtul *et al*., 2012). New, safe and natural urease inhibitory agents from natural sources are needed to prevent important health problems. Urease inhibitory activity was evaluated by the indophenol method and the results are given as IC50 in Table 1. CS-SeNPs showed the strongest inhibitory activity against urease enzyme with an IC50 value of 13.72 ± 0.18 µg/mL. *C. sinensis* extract showed high urease inhibitory activity with an IC50 value of 103.4 ± 0.95 µg/mL, while C. longa extract was reported to exhibit moderate activity against urease (IC50: 192.8 ± 1.50 µg/mL) (Tamfu *et al*., 2020). Tyrosinase is a copper-containing monooxygenase found in animals, plants and microorganisms. It catalyzes the hydroxylation of monophenols to o-diphenols by monophenolase activity and the oxidation of o-diphenols to their corresponding o-quinones by diphenolase oxidase activity (Bayramoglu *et al*., 2013). The tested tea extract selenium nanoparticles (CS-SeNPs) inhibitory effects on tyrosinase were analyzed and showed an activity of 31.40 ± 0.51 µg/mL as IC50. In a study on the phytochemical profile, enzyme inhibition, antioxidant and antibacterial activity of Rosa pimpinellifolia L., bioactive properties of different parts of the fruit were investigated. It was suggested that fruit extract was found to be a moderate tyrosinase inhibitor (52.64%) compared to the positive control kojic acid (87.19%), whole fruit extract showed low inhibitory activity (35.66%) and no activity was observed for seed extract (Findik *et al*., 2024).

4. Conclusion

Camellia sinensis L. (green tea) is a plant that attracts attention with its known biological activity potential. In this study, CS-SeNPs synthesized from the tea plant were evaluated for various enzyme inhibition activities. Selenium nanoparticles based on *Camellia sinensis* L. (green tea) exhibited strong inhibitory activities especially against important enzymes such as AChE, BChE, α-amylase and urease, indicating the potential therapeutic uses of these compounds. Nanoparticles can increase their biological activity due to their high surface area, which can be especially useful in the treatment of neurological diseases and enzymatic disorders. In addition, due to its inhibition of enzymes such as α-amylase and urease, it can potentially be used in the management of diabetes and in the treatment of gastrointestinal disorders. In general, Camellia sinensis has significant biological activity, especially as enzyme inhibitors and these findings indicate that the plant offers potential treatment options in the pharmaceutical and health fields. Such studies may allow the integration of plant-derived compounds into modern treatment methods.

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