



Green synthesis of selenium nanoparticles using *Annona muricata* fruit extract and its free radical scavenging activities

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ABSTRACT

Background: Most people agree that medicinal plants form the basis for health care prevention and therapy. *Annona muricata* Most people agree that medicinal plants form the basis for health care prevention and therapy. *A. muricata* is a member of the Annonaceae family of plants, and a number of medicinal uses for its leaves, bark, stems, fruits, and seeds have been documented throughout the world. The main components of *A. muricata*, which contains more than 212 bioactive chemicals, include acetogenins, alkaloids, and phenols.

Aim: To evaluate the free radical scavenging activity of selenium nanoparticles using *Annona muricata* fruit extract.

Materials and methods: The fruit extract was prepared and selenium nanoparticle characterisation was done. The antioxidant activity through DPPH and H₂O₂ assays were done at different levels of concentration.

Results: At greater concentrations, *A. muricata* exhibited remarkable antioxidant activities equivalent to conventional agents.

Keywords: *Annona muricata*, *graviola*, *soursop*, *acetogenins*, *antioxidant*, *selenium nanoparticles*

INTRODUCTION

Natural substances originating from plants and their derivatives have long been utilised to treat a variety of illnesses, including cancer(1). Recent research has concentrated on the development, management, and prevention of cancer using these substances, although there is still opportunity for development (2). Directly or indirectly, a number of popular

chemotherapeutic drugs are derived from botanical natural compounds. However, in addition to these significant medications, a number of unrefined herbal or botanical formulations have also demonstrated promise efficacy for cancer and other diseases. *Graviola* is one such wonder plant that belongs to Annonaceae family,

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Annona genus and *muricata* species(3). Alternate names for this evergreen plant, which is primarily found in tropical and subtropical regions of the world, include graviola, soursop, guanabana, guanavana, guanaba, corossol épineux, huanaba, toge-banreisi, durian benggala, nangka blanda, and cachiman épineux. Graviola's beneficial effects against anticonvulsant, antiparasitic, anti-arthritic, antimalarial, antidiabetic, hepatoprotective, and anticancer activities have been observed by a variety of laboratories (4,5). According to biological and chemical characterization investigations, the primary components of Graviola are annonaceous acetogenins.(6)

On *A. muricata*, various antioxidant screens have been carried out. Natural antioxidants derived from plant species have gained popularity because of their capacity to defend against oxygen-derived free radicals, which are linked to the onset of a variety of diseases, including cancer, cardiovascular disease, arthritis, and degenerative conditions like Parkinson's and Alzheimer's(7). For instance, the antioxidant activity of leaf extracts in methanolic, ethanolic, n-butanolic, and aqueous forms was assessed using DPPH. For instance, the commercial antioxidant butylated hydroxytoluene is 1000 times more powerful than aqueous extracts of fresh *A. muricata* fruit(8). According to tests for ABTS, FRAP, and ORAC, the pulp's antioxidant activity indicated that the antioxidant chemicals from *A. muricata* are primarily lipophilic, with hydrogen donation serving as the mechanism of action.(9)

Because of their unique physicochemical,

optical, and biological characteristics, nanoparticles can attach many therapies, enabling the integration of multifunctional capabilities that improves treatment. It is simple to engineer the desired size, surface charge, gene and drug loading capacity, and regulated release.(11) Their optical qualities have been utilised as diagnostic tools in MRI and ultrasound imaging. These nonviral vectors are preferable to viral vectors in that they can transport larger nucleic acid molecules and deliver combination medicines with fewer immunogenic reactions.(12)

Selenium is a metalloid that exists in selenite (SeO_2), selenate (SeO_3), selenide (Se^{2-}), and elemental selenium (Se^0), among other oxidation states. While plants obtain the majority of their selenium from the environment and can be found in the air, soil, and in the presence of organic selenium, inorganic selenium is more common in soil. There are claims that selenium, an important vitamin derived from dietary sources, has both therapeutic and chemopreventive effects. Inorganic selenium from the soil is transformed by plants into selenomethionine, which is then substituted for methionine in their proteins. The body needs selenium, a trace element that can scavenge free radicals and protect DNA from oxidative damage.(13)

MATERIALS AND METHODS

Graviola Extract Preparation

100 ml of distilled water and 10 g of fruit extract were combined, heated for 10 to 20 minutes, and then filtered through whatman filter paper.



FIGURE 1: Graviola fruit pulp

Selenium Nanoparticles Characterisation

For Selenium nanoparticles 50 of plant extract was mixed with 50 ml of 20Mm of Sodium selenite and colour change was observed on 6

hourly basis. After nanoparticle formation, solution was centrifuged at 10000 rpm for 15mins. Pellet was collected and kept in a hot air oven and stored.

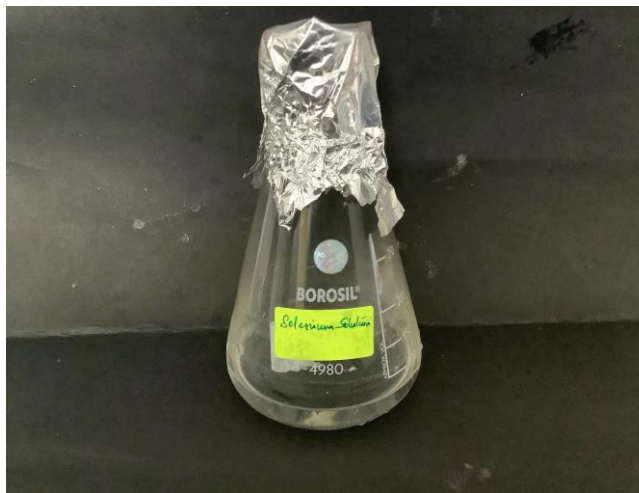


FIGURE 2: selenium nano particle characterization

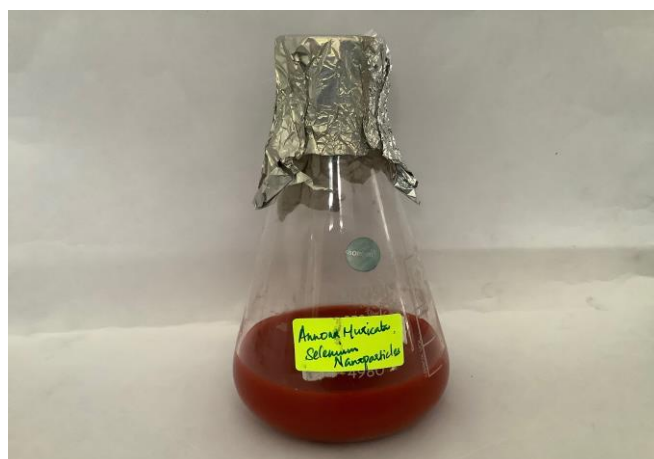


FIGURE 3: Visual observation

Antioxidant Activity

Dpph Method

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (2-10 µg/ml) of *Annona muricata* leaf extract interceded zinc oxide nanoparticle was mixed with 1 ml of 0.1 mM 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. BHT was employed as control. The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

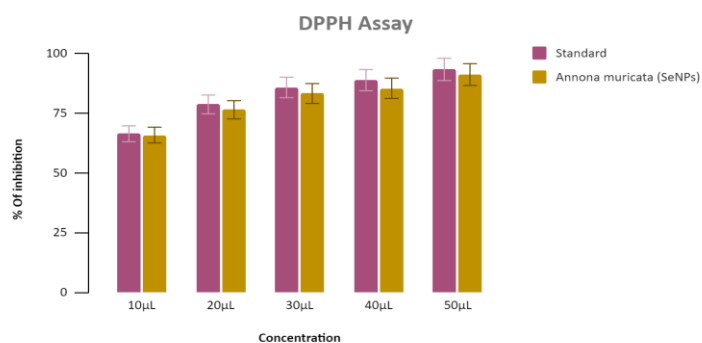


FIGURE 4: Antioxidant activity using DPPH assay

Hydroxyl Radical Scavenging Assay

Minor modifications were made to the Halliwell method [Halliwell et al., 1987] in order to carry out the assay. Every solution was made from scratch. In 1.0 mL of the reaction mixture, there were the following ingredients: 500 mL of a solution of different concentrations of Carcia papaya (10 to 80 g), 200 mL of 200 mM FeCl₃

and 1.04 mM EDTA (1:1 v/v), 100 mL of H₂O₂ (1.0 mM), and 100 mL of ascorbic acid (1.0 mM). The TBA reaction was used to gauge the degree of deoxyribose breakdown following an hour-long incubation time at 37°C. Using a blank solution as a reference, calculate the absorbance at roughly 532 nm. A positive control was utilised, which was vitamin E.



FIGURE 5: Antioxidant activity using H₂O₂ assay

RESULTS AND DISCUSSION

One of the most significant traditional medicinal plants, *Annona muricata*, contains a wide range of chemicals with different pharmacological properties. In this study, antioxidant *A. muricata* fruit extract was used to make selenium nanoparticles. The effect was increased by *A. muricata*-like pharmaceutical activity [13]. The DPPH reagent was used to test the antioxidant activity. After 30 minutes of incubation, absorption measurements were performed to allow for a reaction to take place between the test samples and DPPH free radicals. This antioxidant

test is based on the measurement of DPPH discoloration after reacting with samples. Proton donations to radicals are the mechanism by which antioxidants capture DPPH radicals. The colours that change from the originally concentrated violet to pale yellow can be seen as evidence of this. This modification demonstrates the sample's antioxidant activity [14]. The colour change in DPPH that corresponds to the energy of free radicals would be more yellow the higher the concentration of the sample containing antioxidant compounds. When DPPH exhibited a propensity for radical state instability.

Diphenylpicrylhydrazyl (DPPH) has one N atom with an unpaired electron, which can bind to an atom that can donate electrons (the H atom) to form stable diphenyl picrylhydrazine when it reacts with substances that can reduce free radicals, as shown by the presence of purple fading from DPPH solutions (DPPH-H) [15].

In the present study, a visible color change was observed between different concentrations. Thus from the visual observation, it can be identified that the antioxidant activity is higher in SeNP *A. muricata* flower extract. Due to their ability to donate electrons, all concentrations of extracts significantly and dose-dependently scavenged free radicals, according to quantitative analysis. The findings also suggest that the extracts may shield biomolecules in vulnerable biological and food systems from reactive radical species damage. One method does not adequately assess the antioxidant capacity of plant-based extracts because different antioxidant compounds may act via various mechanisms. Therefore, the current study used two different tests to gauge the extract's antioxidant potential. The results of the DPPH and H₂O₂ assays showed that the antioxidant potentials increased dosage-dependently over a range of values with distinct extract-specific efficiencies. Baskar et al in 2007 identified that *A. muricata* leaves have antioxidant activity. But none of the studies have used Selenium nanoparticle-coated *A. muricata* flowers [16]. This is the first study to combine *A. muricata* flower extract and SeNP. The antioxidant property shows the pharmaceutical effect of the extract.

Moreover, Jabir et al in 2021 identified silver nanoparticle-coated *A. muricata* extract (AgNPs) to have an apoptosis-inducing capacity in cancer cells. The study showed that the AgNPs prevented the proliferation of THP-1 and AMJ-13 cells. In the meantime, in both in vivo and in vitro models, the AgNPs markedly increased autophagy and decreased IL-1 β and NLRP3 levels. While NLRP3 inflammasome degradation was increased, IL-1 secretion was reduced. These results suggest that AgNPs exert an anti-proliferative activity against THP-1 and AMJ-13 cells by inducing the p53 protein pathway and stimulating apoptosis through mitochondrial damage. In addition, IL-1 and NLRP3 inflammasome activation were decreased by AgNP-induced autophagy. This showed that the AgNPs increase autophagy regulated by the IL-1

pathway through two unique novel mechanisms. The first one controls IL-1, caspase-1, and ASC activation, and the second one targets NLRP3 for lysosomal degradation [17]. Thus this previous evidence proves the importance of nanoparticle-coated *A. muricata* extracts.

Interestingly, Hasmila et al 2019 analyzed the antioxidant activity of *A. muricata* extracts and proved that the plant indeed has a high antioxidant capacity and could be beneficial [18]. Accumulating shreds of evidence suggest that *A. muricata* is of high value and being a naturally occurring edible plant, *A. muricata* has a wide range of therapeutic potentials. It has a long history of being used traditionally in India to treat a variety of illnesses. The phytochemicals in *A. muricata*'s methanolic and aqueous leaf extracts were identified in earlier studies, which also confirmed the plant's capacity to scavenge free radicals and protect DNA. The present study added to evidence that SeNP-coated *A. muricata* flower extracts also possess antioxidant activity and on further analysis could be used as a pharmaceutical agent. Hence further analysis is required to validate the pharmaceutical ability of SeNP-coated *A. muricata* fruit extracts.

CONCLUSION

The study concludes that, the aqueous extract of *A. muricata* has promising therapeutic compounds which possess significant radical scavenging in a dose-dependent manner thereby providing an evidence for the development of a potent ethano medicine.

REFERENCES

1. Paulraj J., Nagar P., Antimicrobial efficacy of triphala and propolis-modified glass ionomer cement: An in vitro study, 2020
2. Neppala G., Maiti S., Rajeshkumar S., Ganapathy D., Antimicrobial efficacy of temporary and permanent denture soft lining material modified by titanium-dioxide nanoparticles-an in-vitro study 2020 International Journal of Dentistry and Oral Science
3. Jacob B., Malli Sureshbabu N., Ranjan M., Ranganath A., Siddique R. The Antimicrobial Effect of Pomegranate Peel Extract versus Chlorhexidine in High Caries Risk Individuals Using Quantitative Real-Time Polymerase Chain Reaction: A Randomized Triple-Blind Controlled Clinical Trial 2021 International Journal of

- Dentistry 2021 :
doi:10.1155/2021/5563945
4. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *Int J Mol Sci.* 2015;16(7):15625-15658. Published 2015 Jul 10. doi:10.3390/ijms160715625
 5. Mutakin M, Fauziati R, Fadhilah FN, Zuhrotun A, Amalia R, Hadisaputri YE. Pharmacological Activities of Soursop (*Annona muricata* Linn.). *Molecules.* 2022;27(4):1201. Published 2022 Feb 10. doi:10.3390/molecules27041201
 6. Abdul Wahab SM, Jantan I, Haque MA, Arshad L. Exploring the Leaves of *Annona muricata* L. as a Source of Potential Anti-inflammatory and Anticancer Agents. *Front Pharmacol.* 2018;9:661. Published 2018 Jun 20. doi:10.3389/fphar.2018.00661
 7. Prasad SK, Pradeep S, Shimavallu C, et al. Evaluation of *Annona muricata* Acetogenins as Potential Anti-SARS-CoV-2 Agents Through Computational Approaches. *Front Chem.* 2021;8:624716. Published 2021 Jan 27. doi:10.3389/fchem.2020.624716
 8. Onohuean H, Alagbonsi AI, Usman IM, et al. *Annona muricata* Linn and *Khaya grandifoliola* C.DC. Reduce Oxidative Stress In Vitro and Ameliorate Plasmodium berghei-Induced Parasitemia and Cytokines in BALB/c Mice. *J Evid Based Integr Med.* 2021;26:2515690X211036669. doi:10.1177/2515690X211036669
 9. Gavamukulya Y, Maina EN, El-Shemy HA, et al. *Annona muricata* silver nanoparticles exhibit strong anticancer activities against cervical and prostate adenocarcinomas through regulation of CASP9 and the CXCL1/CXCR2 genes axis. *Tumour Biol.* 2021;43(1):37-55. doi:10.3233/TUB-200058
 10. Neglo D, Tettey CO, Essuman EK, et al. Evaluation of the Modulatory Effect of *Annona muricata* Extracts on the Activity of Some Selected Antibiotics against Biofilm-Forming MRSA. *Evid Based Complement Alternat Med.* 2021;2021:9342110. Published 2021 Dec 20. doi:10.1155/2021/9342110
 11. Kim WS, Han JM, Song HY, Byun EH, Lim ST, Byun EB. *Annona muricata* L.-Derived Polysaccharides as a Potential Adjuvant to a Dendritic Cell-Based Vaccine in a Thymoma-Bearing Model. *Nutrients.* 2020;12(6):1602. Published 2020 May 29. doi:10.3390/nu12061602
 12. Son Y, Lee H, Son SY, Lee CH, Kim SY, Lim Y. Ameliorative Effect of *Annona muricata* (Graviola) Extract on Hyperglycemia Induced Hepatic Damage in Type 2 Diabetic Mice. *Antioxidants (Basel).* 2021;10(10):1546. Published 2021 Sep 29. doi:10.3390/antiox10101546
 13. Kim WS, Kim YE, Cho EJ, et al. Neuroprotective effect of *Annona muricata*-derived polysaccharides in neuronal HT22 cell damage induced by hydrogen peroxide. *Biosci Biotechnol Biochem.* 2020;84(5):1001-1012. doi:10.1080/09168451.2020.1715201
 14. Justino AB, Miranda NC, Franco RR, Martins MM, Silva NMD, Espindola FS. *Annona muricata* Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against α -amylase, α -glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. *Biomed Pharmacother.* 2018;100:83-92. doi:10.1016/j.biopha.2018.01.172
 15. Mitsuwan W, Sin C, Keo S, et al. Potential anti-Acanthamoeba and anti-adhesion activities of *Annona muricata* and *Combretum trifoliatum* extracts and their synergistic effects in combination with chlorhexidine against *Acanthamoeba triangularis* trophozoites and cysts. *Heliyon.* 2021;7(5):e06976. Published 2021 May 10. doi:10.1016/j.heliyon.2021.e06976
 16. Adewole SO, Ojewole JA. Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. *Afr J Tradit Complement.* 2009;6:30-41.
 17. Attaguile G, Russo A, Campisi A, Savoca F, Acquaviva R, Ragusa N, Vanella A. Antioxidant activity and protective effect on DNA cleavage of extracts from *Cistus incanus* L. and *Cistus monspeliensis* L. *Cell Biol Toxicol.* 2000;16:83-90. doi: 10.1023/A:1007633824948
 18. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181:1199-1200. doi: 10.1038/1811199a0
 19. Baskar R, Rajeswari V, Kumar TS. In vitro antioxidant studies in leaves of *Annona* species. *Indian J Exp Biol.* 2007;45:480-485.
 20. George VC, Kumar DR, Suresh PK, Kumar RA. Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. *J Food Sci Technol.* 2015;52(4):2328-2335. doi:10.1007/s13197-014-1289-7
 21. Hasmila I, Natsir H, Soekamto NH. Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.). *J Phy.* 2019; 032027. doi:10.1088/1742-6596/1341/3/032019. Ezhilarasan, D., Apoorva, V.S., Ashok Vardhan. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells