

## **ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF GARCINIA MANGOSTANA MEDIATED SELENIUM INDUCED NANOPARTICLES - An invitro study**

### **Abstract :**

The biosynthesis of selenium nanoparticles (SeNPs) has gained interest due to their unique chemical and biological properties, which are critical for their possible applications in a variety of fields. *Garcinia mangostana* (mangosteen) is a tropical fruit known for its edible pulp. The edible pulp accounts for just 30% of the total fruit weight, while the pericarp and seed are discarded. In response to rising public demand for naturally safe foods and items, we conducted a study to determine the antioxidant ability and antibacterial activity of mangosteen pericarp induced with selenium nano particles.

### **Methods :**

The antioxidant capacities of selenium nano particles induced mangosteen's pericarp were determined using 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay, whereas agar well – diffusion technique was used to assess the antibacterial activity on chosen oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*.

### **Results :**

In this study, the *G. mangostana* mediated selenium nanoparticles were synthesized using a green synthesis method. The maximum absorbance of the synthesized selenium nanoparticles was measured using a double beam UV visible spectrophotometer,

resulting in a maximum absorbance of 325 nm. The *Garcinia mangostana* pericarp extract mediated selenium nanoparticles shows potent antimicrobial and antioxidant activity.

**Conclusion:** The outcomes of our study could facilitate future application of mangosteen waste as a bio preservative in the food industry.

**Key words :**

selenium nanoparticles- *garcinia mangostana* extract- pericarp- antioxidant - antimicrobial.

**Introduction :**

The field of nanotechnology in the last three decades has changed the way we think about drug discovery and development by revealing many previously unknown pathways in disease pathophysiology and treatment options.<sup>(1–3)</sup> The adage "tiny is the new large" is a good way to describe nanotechnology's position in modern therapeutics<sup>(4)</sup>. Polymers, dendrimers, liposomes, metal nanoparticles (Ag, Au, Ce, Cu, Eu, Fe, Se, Ti, Y, and so on. ), silicon and carbon-based nano materials, and others have all been used as effective therapeutic agents and drug delivery carriers <sup>(5,6)(7,8)(9)</sup> Selenium is an important trace element for humans, as it aids in the movement of seleno-enzymes, glutathione peroxidase, and other oxidative compounds that prevent free radical damage to cells and tissues in vivo. Novel piezoelectricity, photoconductivity, thermoelectricity, and non-linear optical reactions are demonstrated. Heart disease, immune deficiency, male infertility, and cancer may all result from a lack of selenium in the diet. When combined with other nanoparticles, selenium nanoparticles exhibit improved biocompatibility, bio adequacy, lower toxicity, excellent cell reinforcement movement, and disease prevention effects.<sup>(10–12)</sup>

Physical and chemical methods of nanoparticle synthesis have a number of drawbacks, including being time consuming, costly, and producing dangerous by-products.(13)To meet this requirement, green nanoparticle synthesis was used to create a clear, less toxic, and environmentally friendly nano component ((14)

Mangosteen pericarp has been used as traditional medicine to heal several diseases such as flu, cystitis, diarrhoea, dysentery, eczema, fever, pruritus, gut and skin disease(15)Mangosteen has been shown to have cardioprotective, anti-inflammatory, anti-carcinogenic, and antimicrobial effects, which are all associated with anthocyanins and other antioxidants.(15,16).Antibacterial and antioxidant activities have been identified for extract and isolated compound from mangosteen pericarp.(17) Antibacterial agents are chemical compounds that can prevent microorganisms from multiplying by interfering with their metabolism.(18) Antibacterial agents are required in the pharmaceutical industry to prevent or treat infectious diseases. Besides that, the antioxidant agent may help slow down the oxidation of the substrate.(19)A free radical compound produced by an oxidation reaction may be capable of initiating other oxidation reactions. It has the potential to destroy cells and, in the worst-case scenario, to kill them. As a result, the antioxidant agent is needed to inhibit the other oxidation reaction and prevent damage by stopping the intermediate free radical compound.(20))

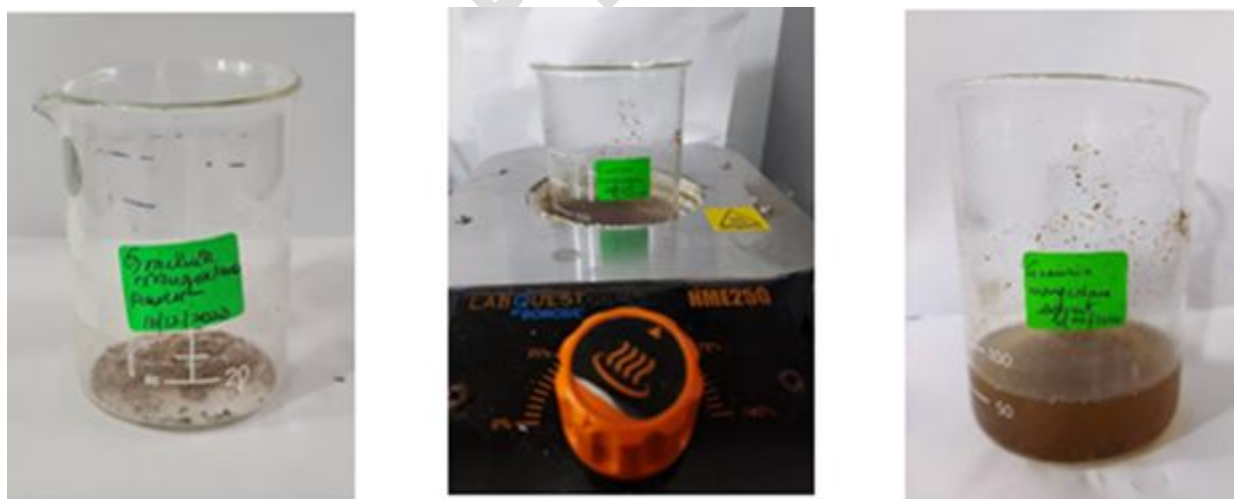
A number of therapeutic benefits that are associated with anthocyanins and other antioxidants have been extracted from mangosteen. Facing the increasing public demand for natural and microbiologically safe foods and products, our present study therefore aimed to elucidate the antioxidant capacity and antibacterial activity of selenium nanoparticle induced Garcinia mangostana pericarp extract

## Materials and methods:

Hi-media laboratories Pvt. Ltd, India, provided the chemicals used in this analysis, such as sodium selenite, Mueller Hinton agar, and ascorbic acid. Saveetha dental college and hospital, SIMATS, Poonamallee, Tamilnadu, India, provided DPPH and bacterial cultures such as *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*.

### Preparation Of plant extract :

*Garcinia mangostana* pericarp dried packet was bought from amazon online shopping application. Prior to extraction, the pericarp was dried for 24 hours at 60°C in an oven. With the aid of liquid nitrogen and a mortar and pestle, 5g of dried pericarp were ground into fine powder, was dissolved in 100ml distilled water and boiled for 10 minutes at 60-80°C using a heating mantle to prepare the extract. Whatman No.1 filter paper was used to filter the boiled extract. For further experiments, the filtrates were held at 5°C.



*Fig 1 : indicates the preparation of g.mangostana extract*

### **Synthesis of selenium nanoparticles using garcinia mangostana extract :**



***Fig 2 : indicated SeNP particles-solution centrifuged at 8000rpm for 10 minutes***

The bio reduction process was carried out using an aqueous extract of *G.mangostana*. SeNP, 0.2M of sodium selenite was dissolved in 60ml of distilled water and kept in a magnetic stirrer for a few minutes. To that, 40 mL filtered *g.mangostana* extract was added .For 72 hours, the solution mixture was stirred at 650-800 rpm in a magnetic stirrer. Using a double beam uv visible spectrophotometer with a wavelength range of 25.-650nm, the color changes in the reaction mixture were observed continuously. The mangostana extract-mediated selenium nanoparticles were centrifuged for 10 minutes at

8000rpm shown in fig. The selenium nanoparticle pellet was calcined for 2 hours in a hot air oven at 70°C and stored in airtight vials for later use.

### **Characterisation of selenium nanoparticles:**

In the frequency range of 300-600nm, a double beam UV-vis spectrophotometer (uv-2450, Shimadzu) was used to determine the optical property of g.mangostana intervened selenium nanoparticles.

### **Antioxidant activity of selenium nanoparticles:**

The technique mentioned in was used to overcome the DPPH (1,1-diphenyl-2-picrylhydrazil) free radical searching movement of the g.mangostana interfered Selenium nanoparticle (Rajeshkumar, 2017).Selenium nanoparticles were combined with 1 ml of 0.1 mM DPPH in methanol and 450 l of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes with different concentrations (2-10 g/ml) of g.mangostana extract interceded. After incubation, the decrease in the quantity of DPPH free radicals was estimated dependent on the absorbance at 517 nm. BHT was utilized as control. The rate restraint was determined from the accompanying equation,

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

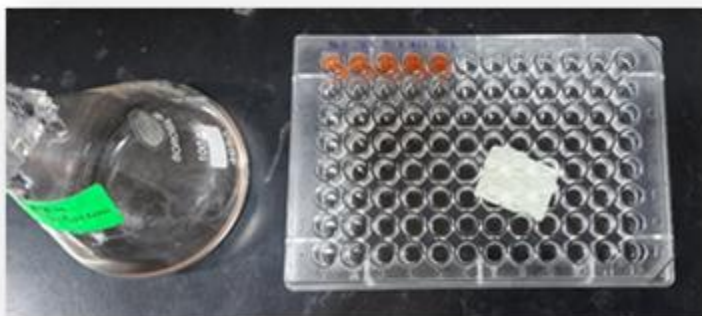


Fig 3 : DPPH solution added to SeNP extract

### **Determination of antimicrobial activity of selenium nanoparticles :**

Fresh bacterial cultures were prepared, in Hi-Veg broth medium, where 10ul cultures of *Staphylococcus aureus*, *Streptococcus* mutants, *Enterococcus faecalis* were inoculated, and incubated for 18 h, in a shaker. Mueller Hinton Agar was prepared and sterilized in an autoclave for 15-20 minutes at 121°C. The sterile MHA media was poured on the sterile Petri plates' surfaces and allowed to solidify. After solidification, the pathogens such as *Staphylococcus aureus*, *Streptococcus* mutants, *Enterococcus faecalis* were swabbed using sterile cotton swabs. A T-shaped well cutter was used to create the wells. Each plate has four wells. Three wells were filled with *G. mangostana* selenium pellet solution in concentrations of 25L, 50L, and 100L (100g/ml), while the fourth well was filled with a standard antibiotic (Amoxicillin ) in a concentration of 10g/mL.

*C. albicans* were cultured at 25°C in potato dextrose agar and Sabouraud's dextrose agar medium, respectively. Preparations of  $0.5 - 2.5 \times 10^3$  conidia/mL (final concentration) were obtained from *C. albicans* by using a standard neobar slide with the tubes containing 3 mL of sterile saline solution. Then 5 mL of diluted SeNPs of mangosteen extract was added to 45 mL of PDA medium at about 55°C, making the final concentration of SeNPs as 25, 50, 100 µg/mL separately. The control contained with a standard antifungal (Amphotericin B

) in a concentration of 10g/mL. A fungus block was inoculated in the center of each PDA plates. Then the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and measured for Zone of inhibition around the nanoparticle and antibiotic loaded wells.

## Results:

### Visual observation:

The visual recognition of color change is a preliminary method for confirming plant extract's ability to synthesise nanoparticles (Rajeshkumar,2016).The existence of selenium nanoparticles could be confirmed by the formation of a brown color in the reaction mixture. Initially, no color change was noticed. At 60°C, the reaction mixture is stirred again with a magnetic stirrer at 650-800rpm. After 24 hours, the brown color intensity increased, indicating that the g.mangostana extract would reduce sodium selenite to selenium nanoparticles as shown in figure

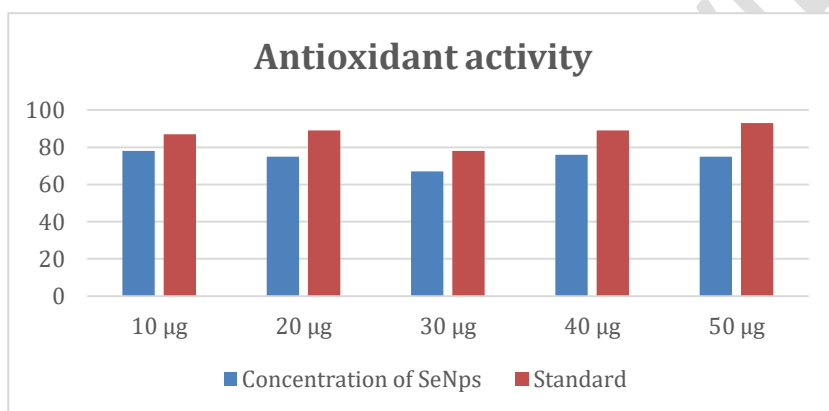


*Fig 4 : Visual observation of synthesized SeNP nanoparticles – color change observed*



## UV spectroscopy:

The selenium nanoparticle formation was confirmed by the positioning of surface plasmon resonance in the uv spectroscopic analysis. The UV spectra of selenium nanoparticles synthesized by g.mangostana extract exposed at various time intervals such as 1hr, 2hr, 6hr, and 18hr, are shown in Fig. The formation of selenium nanoparticles was indicated by the maximum absorption peaks at 325nm ((21)(22))



## Antioxidant activity:

Fig 5 : Antioxidant activity of SeNP biosynthesised using g.mangostana extract when compared to standard ascorbic acid.

Sample concentration (µg/ml)	SeNPs	Ascorbic acid
25 µg	18.99±4.2	23.2±0.3

50 µg	12±1.2	20.1±0.8
100 µg	15±2.4	18.2±2.2
Ab	19.2±2.3	21±0.2

**Table 1:** Antioxidant activity of SeNP biosynthesised using g.mangostana extract when compared to standard ascorbic acid.  $\pm$ standard deviation, pvalue >0.05

The DPPH assay was used to assess the free radical scavenging activity of selenium nanoparticles induced by g. mangostana extract. DPPH is a free radical that is stable. Any molecule that donates an electron or hydrogen to DPPH interacts with it and produces a color change(23). The absorbance values gradually decrease as the concentration increases. As a consequence, this study supported the antioxidant ability of selenium nanoparticles induced by g.mangostana

### **Antibacterial activity of SeNPs:**

As shown in the Fig 6 , the zone of inhibition (ZOI) of SeNPs is demonstrated against bacterial cultures. The zone of inhibition increased with the concentration of SeNPs , therefore at 100 µl /ml , the ZOI with highest of 25 ±1.3mm for c.albicans ,while the lowest ZOI for all the four organism with 9±1 mm at 25 µl/ ml as shown in the table.

Followed by C.albicans , the gram positive organism Staphylococcus aureus shows its maximum zone of inhibition with zone diameter 20 ±1 mm at 100 µl concentration. Streptococcus mutans, gram positive organism and E.faecalis gram negative organism showed resistance to the selenium induced garcinia mangostana extract as compared to the other two organism. In this study , it is clear that both gram positive and gram

negative organism show effective antibacterial activity to the garcinia mangostana induced selenium nanoparticles similar to the synthetic antibiotic drug (24)

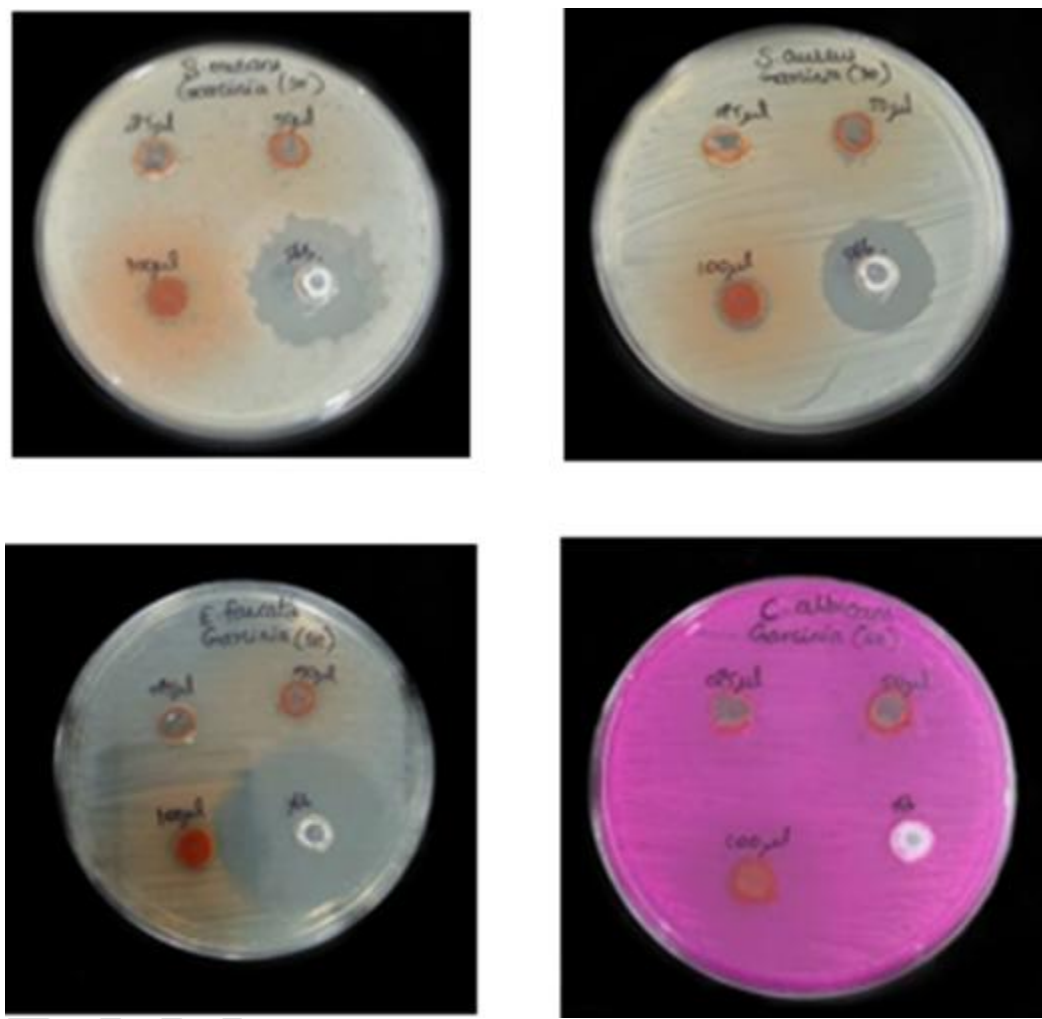
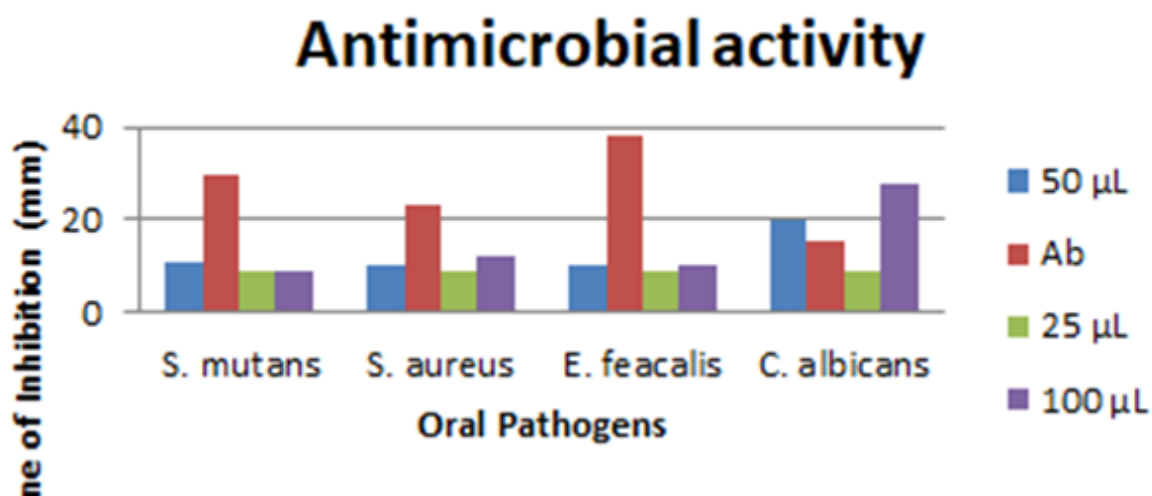


Fig 6 : Zone of inhibition with SeNP containing *S.mutans*, *S.aureus*, *E.coli*, *C.albicans*

	S. mutans	S. aureus	E. faecalis	C. albicans
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25 µl	9 ±1.21	9 ±0.21	9 ±1.21	9 ±0.67
50 µl	11 ±0.46	10 ±0.66	10 ±0	20 ±0.22
100 µl	12 ±0.56	20 ±1.23	10 ±0.54	25 ±0.22
Ab	30 ±0.34	23 ±0.12	38 ±0	15 ±0

*Table 2: indicates the measured zone of inhibition for the organism against the selenium induced garcinia mangostana pericarp extract. ±standard deviation, pvalue >0.05*



*Fig 7 : represent the bar graph of Anti microbial activity of Garcinia induced selenium Nano particles.*

## Discussion

Despite the availability of previous reports on SeNP biosynthesis mediated by plant extract (25) and microorganisms (26), there is a paucity of scientific literature on SeNP biosynthesis mediated by bacteria. As a result, this paper proposes a novel approach for biosynthesis of SeNPs induced by *G. mangostana* extract. The conversion of color from light brown to reddish brown confirmed the reduction of selenium ions into SeNPs induced by extracts. The most important property of nanoparticles is their color, which turns orange when acidic sodium selenite solution is added. The results are consistent with a previous report (27) on SeNP synthesis mediated by Aloe Vera leaf aqueous extract. The current findings also correlate to UV-visible light. The peak of SeNPs biosynthesised from an aqueous extract of *G. mangostana* is at 325 nm respectively.

In our study, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to examine the antioxidant capacities of mangosteen pericarp. The absorbance values gradually decrease as the concentration increases. As a consequence, this study supported the antioxidant ability of selenium nanoparticles induced by *G. mangostana*. Only a few studies have been reported on the antioxidant capacity of mangosteen pericarp ((27,28)(29)). To the best of our knowledge, there have been few studies on the antioxidant capacity of mangosteen pericarp. Although several studies on mangosteen pulp and seeds have been published, it is difficult to make a direct comparison with the current findings. This obstacle is mainly due to the variation in the extraction methods used, the antioxidant determination assays and the standard solutions used to quantify the antioxidant capacity. Development of a standard protocol to measure the antioxidant capacity of fruits is thus crucial. Nevertheless, the current findings have provided a clear overview of the antioxidant capacities of mangosteen, which might enhance the future utilisation of these fruit wastes.

The antibacterial activity of mangosteen extracts was tested using the disc diffusion method against two Gram-positive bacteria (*Staphylococcus aureus*) and one Gram-negative bacteria (*Escherichia coli*), as well as one fungal organism (*Candida albicans*). Antibacterial activity against *S. aureus* was found in all extracts (table I). The results indicated that the zone of inhibition of mangosteen extracts were dose-dependent. When the amount of the extract increased from 25  $\mu$ l to 100  $\mu$ l, a similar increment in the diameter of the inhibition zone was

observed. Among the microorganisms, the inhibitory effect of candida albican increased activity than the other gram positive and negative organism.

The current findings were inconsistent with several reports in which the Garcinia species contain naturally occurring compounds which have a very strong antimicrobial activity against *S. aureus*. Several studies have reported that the mangosteen, the semi-synthetic derivatives of  $\alpha$ -mangostin, showed higher anti-bacterial activity whereas showed the most significant anti-fungal activity.(30). Hence, the current findings are timely and significant, as the seed and pericarp extracts could be developed as an alternative treatment for fungal infection.(30,31))

Unlike *c.albicans*, only mangosteen's pericarp extract showed positive antibacterial activity against *S.aureus*.The mangosteen peel extract contains alpha-mangostin (one of the xanthone derivatives), which has been shown to be effective against MRSA in several studies.(32)

Only a few studies have been published on the antimicrobial activity of mangosteen extracts against *B. cereus*. Sundaram et al. found that mangosteen pericarp extract was effective against *Bacillus subillis*. Due to the presence of xanthones, Negi et al. reported that the pericarp extracts of *Garcinia cowa* and *Garcinia pedunculata* exhibited high inhibitory effects against *B. cereus* (33). There is a high possibility that the antibacterial activity of mangosteen peel extract in this study might be due to the xanthones. Nonetheless, further studies should be conducted to clarify and identify the antibacterial compounds present in the mangosteen extracts.

Our study result showed, Antibacterial activity against *E. coli* was found to be moderate in mangosteen extracts which is consistent with Sundaram et al., on the other hand, found that mangosteen extracts were moderately susceptible to *E. coli*(31). However, compared to the other Gram-positive bacteria tested, the level of susceptibility was significantly lower. Negi et al. also found that *G. cowa* and *G. pedunculata* extracts had higher antimicrobial activity in Gram-positive bacteria than in Gram-negative bacteria. (33)

According to the results of the present study, it is observed that the increasing quantity of SeNPs have comparatively higher antibacterial activity against *Staphylococcus aureus* probably due to thinner peptidoglycan layer and presence of porins. From the present results, it is indicated that the biosynthesized SeNPs mediated by *G.mangostana* extract has the efficient antioxidant and

antimicrobial activity against pathogenic organisms. The anti fungal activity of SeNPs extract was enhanced by increasing the concentration, confirming the inhibition activity of the extract was concentration dependent. Furthermore, much work is needed in the anti fungal activity of standard antifungal cycloheximide against the biosynthesized SeNPs.

## **Conclusion:**

The biosynthesis of selenium nanoparticles using black tea extract is a simple, environmentally friendly, and cost-effective procedure. The UV-visible spectrophotometer was used to characterise the synthesised selenium nanoparticles, which revealed a maximum absorption peak at 325 nm, confirming the reduction of sodium selenite into selenium nanoparticles by the garcinia mangostana extract. It also has strong antimicrobial and antioxidant properties, indicating that it may be used to treat a variety of degenerative diseases including atherosclerosis, diabetes, asthma, and cancer. As a result, the antimicrobial and antioxidant properties of Garcinia mangostana pericarp extract -mediated selenium nanoparticles can be used on a wide scale.

## **COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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