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Green Synthesis of Selenium Nanoparticles Using Black Tea (Camellia Sinensis) And Its Antioxidant and Antimicrobial Activity

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ABSTRACT
Selenium is an essential trace element that is more important for normal functioning of human biological system. Traditional supplements of selenium based medications have a low level of absorption and high toxicity. Hence, it is important to create an alternative and innovative method to transport selenium compounds, which would raise the bioavailability of this component and also permit its controlled discharge in the life form. Nanoscale selenium has pulled in an extraordinary enthusiasm as a nourishment added substance particularly in people with selenium inadequacy, yet additionally as a therapeutic agent without leading to any harmful side effects. In this present investigation, the black tea mediated selenium nanoparticles were synthesized by green synthesis method. The synthesized selenium nanoparticles were characterized using double beam UV visible spectrophotometer and it results in maximum absorbance at 380nm. Agar well – diffusion technique was used to assess the antibacterial activity on chosen oral pathogens such as Streptococcus mutans, Staphylococcus aureus, Enterococcus feacalis. DPPH assay was assigned to determine the free radical scavenging activity. The black tea mediated selenium nanoparticles shows potent antimicrobial and antioxidant activity.

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BACKGROUND

The rise of nanotechnology over the most recent three decades has changed the view of medication revelation and advancement by opening many hidden entryways in disease pathophysiology and treatment choices [1-3].The adage, "small is the new big" appropriately fits to portray the role of nanotechnology in modern-day therapeutics [4]. An assortment of nanostructures, including polymers, dendrimers, liposomes, metal nanoparticles (Ag, Au, Ce, Cu, Eu, Fe, Se, Ti, Y, and so on.), silicon and carbon based nanomaterials have been utilized as effective therapeutic agents and drug delivery carriers [5-10]. Selenium being one of the essential trace component for humans, it plays a major role in the movement of seleno-enzymes, glutathione peroxidase and being a significant piece of oxidative compounds keep free radicals from harming cells and tissues in vivo. It shows novel piezoelectricity, photoconductivity, thermoelectricity, non-linear optical reactions. Dietary selenium insufficiency will lead to major health problems like heart sickness, immune dysfunction, male infertility and cancer. Selenium nanoparticles display better biocompatibility, bio-adequacy, lower harmfulness, superb cell reinforcement movement and disease prevention effects when compared to that of other inorganic and organic seleno compounds [11-13].

Synthesis of nanoparticles by physical and chemical methods leads to various cons such as time consuming, expensive and result in hazardous by-products [13]. To overcome this criteria, green synthesis of nanoparticles has been adopted to obtain simple, less toxic, and eco-friendly nano component [14]. Camellia sinensis is a species of evergreen shrub classified in the family Theaceae found throughout India. Black tea (Camellia sinensis) has multiple health benefits. The chemical composition of black tea contains polyphenols, amino acids, vitamins, proteins, carbohydrates, trace elements. Natural compounds such as caffeine (1,3,7-trimethylxanthine), theobromine and theophyllineare also present in tea [15]. Random surveys shows that black tea lessens the occurrence of dental cavities and also it possess potent antimicrobial activity against oral pathogens [16].Through its anti-inflammatory, antioxidant, antiviral properties black tea reduces the occurrence of chronic diseases [17, 18]. The current study was focused on the black tea extract (Camellia sinensis) intervened selenium nanoparticles and to assess their antimicrobial and antioxidant activity.

MATERIALS AND METHODS

The chemicals used in this study such as sodium selenite, Mueller Hinton agar, Ascorbic acid were purchased from Hi-media laboratories Pvt.Ltd, India. DPPH from Sigma Aldrich and the bacterial cultures such as Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis were isolated and collected from Saveetha dental college and hospital, SIMATS, Poonamallee, Tamilnadu, India.

Preparation of plant extract

Black tea powder (Camellia sinensis) was bought at a supermarket near Poonamallee. To set up the extract, 1g of black tea powder was dissolved in 100ml of distilled water and boiled at 60-80°C for 10 minutes using a heating mantle. The boiled extract was filtered using Whatmann No.1 filter paper. The filtrates were stored in 5°C for further experiments.

Synthesis of selenium nanoparticles using black tea extract

The aqueous extract of Black tea (Camellia sinensis) was used for the bioreduction process. For biosynthesis of SeNP, 0.2M of sodium selenite was dissolved in 60ml of distilled water and kept in magnetic stirrer for few minutes. To that, 40ml of filtered black tea extract (Camellia sinensis) was added. The solution mixture was kept in magnetic stirrer at 650-800rpm for 72 hours. The colour changes in the reaction mixture were noted continuously using double beam uv visible spectrophotometer at different wavelength range from 25.-650nm.The synthesized black tea extract mediated selenium nanoparticles were centrifuged at 8000rpm for 10 mins. The obtained selenium nanoparticle pellet was calcined using a hot air oven at 70°C for 2 hours and preserved in air tight vials for further use.

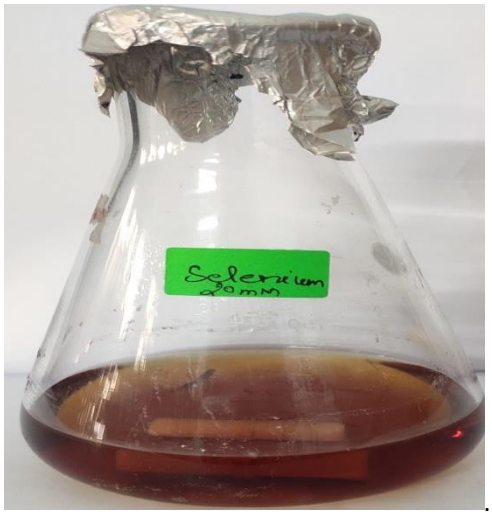

Fig 1: Synthesis of Black tea (Camellia sinensis) mediated selenium nanoparticles.

Characterization of selenium nanoparticles

Double Beam UV-vis spectrophotometer (uv-2450, Shimadzu) was used to decide the optical property of black tea intervened selenium nanoparticles in the frequency scope of 300-600nm.

Antioxidant activity of selenium nanoparticle

The DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical searching movement of black tea intervened Selenium nanoparticle was resolved by the technique reported in (Rajeshkumar, 2017) .Different concentrations (2-10 µg/ml) of black tea extract interceded selenium 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. 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= Absorbance of control- Absorbance of test sample × 100

Absorbance of control

Determination of antimicrobial activity of selenium nanoparticles

The antimicrobial activity was determined by agar well diffusion method.Mueller Hinton Agar was prepared, sterilized using autoclave at 121°C for 15-20minutes. The sterile MHA media was poured on the surface of the sterile Petri plates and allowed for solidification. After solidification, the pathogens such as Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis were swabbed using sterile cotton swabs. The wells were made using a T – shaped well cutter. Among four wells per plate 3 wells were loaded with black tea extract (Camellia sinensis) Se pellet solution in the concentration range of 25µL,50µL,100µL (100µg/ml) and the fourth well loaded with a standard antibiotic (Amoxyrite) in the concentration of 10µg/mL. 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 AND DISCUSSION

Visual observation

The visual identification of colour change is a preliminary tool that confirms the ability of plant extract in nanoparticle synthesis (Rajeshkumar,2016). Formation of brown colour in the reaction mixture could confirm the presence of selenium nanoparticles. Initially there was no colour change observed. The reaction mixture is again incubated in the magnetic stirrer at 650-800rpm at 60°C. The intensity of the brown colour increased after 24hours that confirms the ability of the black tea extract to reduce sodium selenite into selenium nanoparticles.

UV visible spectroscopy

The selenium nanoparticle formation was confirmed by the positioning of surface plasmon resonance in the uv spectroscopic analysis. Fig. 2 shows the UV spectra of the selenium nanoparticle synthesized by black tea extract that were exposed at different time intervals such as 1hr,2hr,6hr,18hr.The maximum absorption peaks were observed at 380nm which indicates the formation of selenium nanoparticles [19, 20].

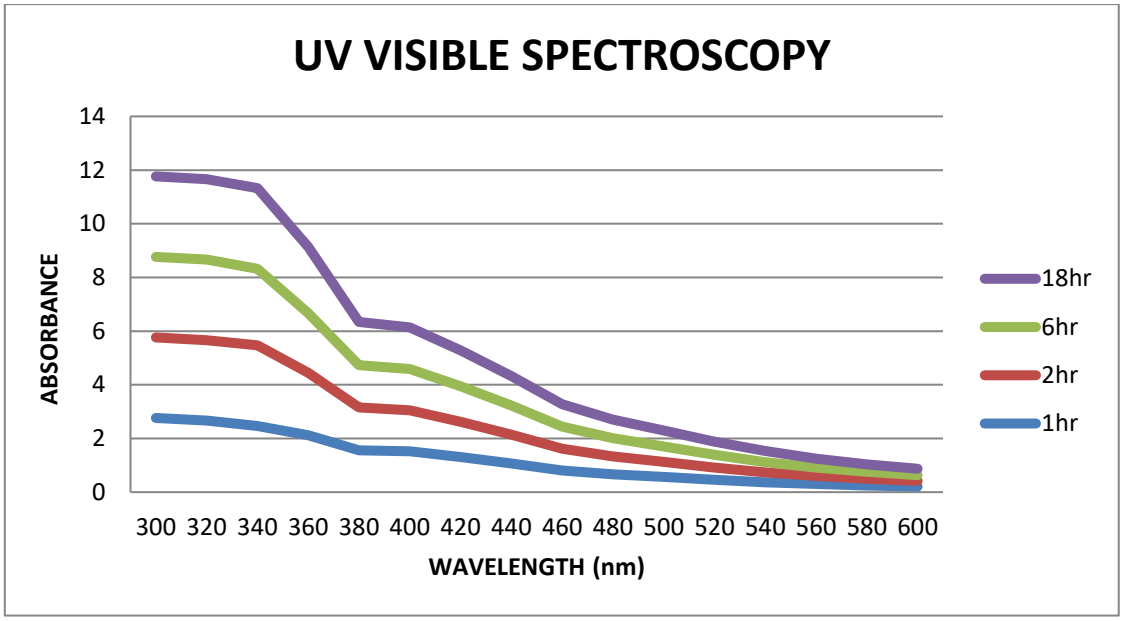

Fig 2: UV visible spectroscopy of black tea mediated selenium nanoparticles

Antioxidant activity

The free radical scavenging activity of black tea extract mediated selenium nanoparticles was determined by using DPPH assay. DPPH is a stable free radicle. Any molecule which donates an electron or hydrogen to DPPH, it reacts and results in change of colour [21]. As concentration increases there is a gradual decrease in the absorbance values. Hence this result confirms the potential antioxidant effect of the black tea mediated selenium nanoparticles.

Antimicrobial activity

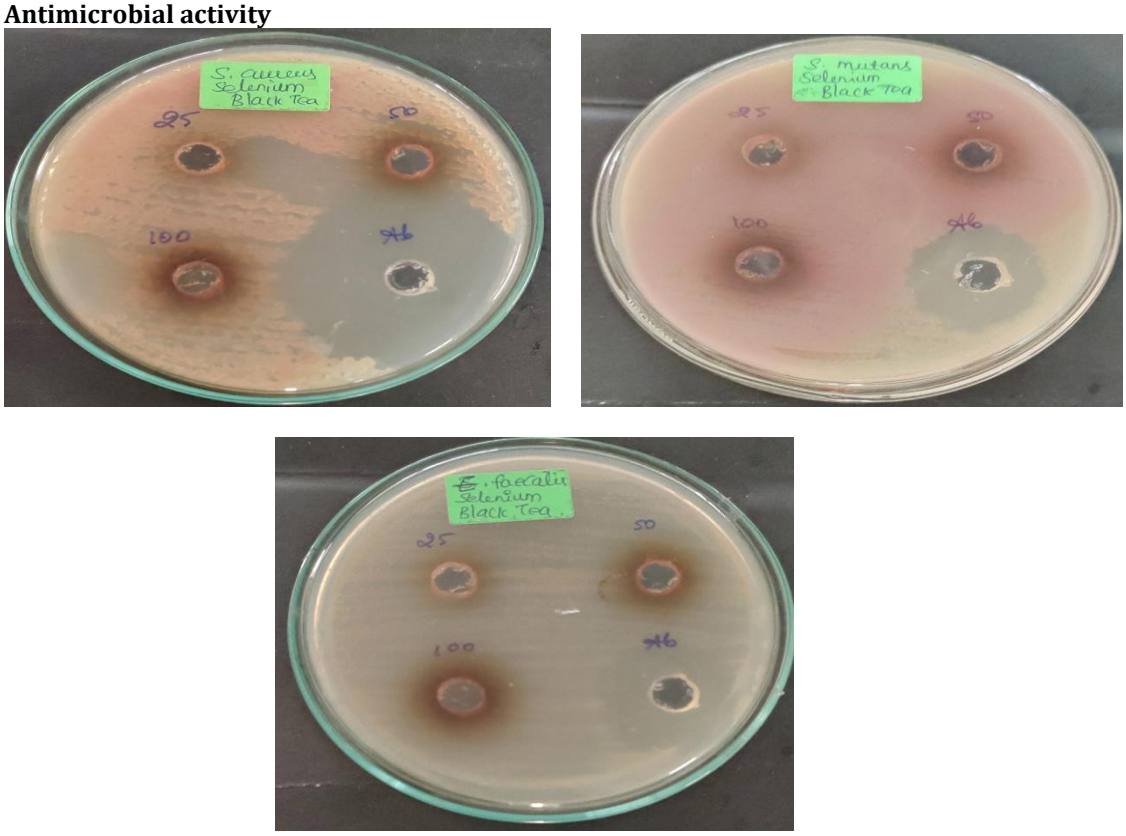

Fig 3: Antibacterial activity of black tea mediated selenium nanoparticles.

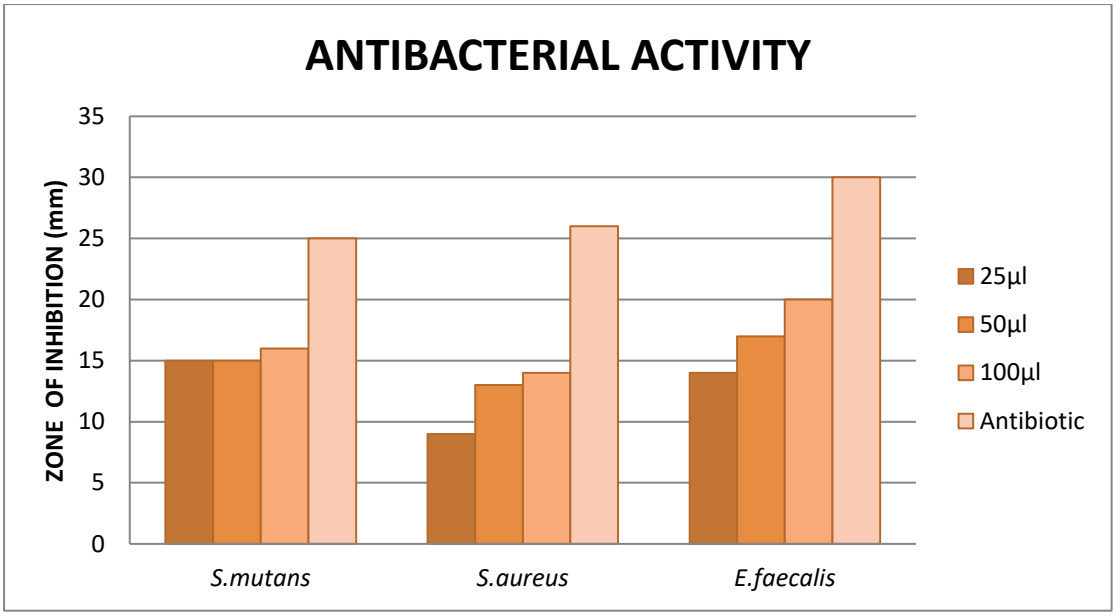

Fig 4: Histogram of antibacterial activity of black tea mediated selenium nanoparticles.

The Fig 4 represents that gram negative organism Enterococcus faecalis is more sensitive to selenium nanoparticles as it shows maximum zone of inhibition with zone diameter of 20±1mm at 100µL concentration. Followed by E. faecalis, the gram positive organism Staphylococcus aureus shows its maximum zone of inhibition with zone diameter of 16±1mm at 100µL concentration. Sterptococcus mutans, gram positive organism shows resistance to the black tea mediated selenium nanoparticles as compared to other two organisms. In this study, it is clearly revealed that both gram negative and gram positive organisms showed effective antibacterial activity to the black tea intervened selenium nanoparticles similar to the synthetic antibiotic drug [22].

CONCLUSION

The biosynthesis of selenium nanoparticles using black tea extract is a simple, eco-friendly and cost effective method. The synthesized selenium nanoparticles were characterized by UV-visible spectrophotometer and found that it shows maximum absorption peak at 380nm which confirms the reduction of sodium selenite into selenium nanoparticles by the black tea extract. It also shows potent antimicrobial and antioxidant activity which confirms its therapeutic application in various degenerative diseases such as atherosclerosis, diabetes,asthma and cancer. Thus, this synthesis of black tea mediated selenium nanoparticle can be used on large scale for their potent antimicrobial and antioxidant properties.

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