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Thymus Vulgaris Mediated Selenium Nanoparticles, Characterization and its Antimicrobial Activity - An In Vitro Study

Research Article

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Abstract

Background: Among all other nanoparticles, selenium nanoparticles (SeNP) is one of the most extensively studied nanoparticle which has zero oxidation state, nontoxic and biologically inert nature. This is the reason why Selenium is considered as a major nanoparticle. In this study SeNPs were synthesized using leaf extract of Thymus vulgaris.
Aim: The aim of the present study was to characterize and to assess the antimicrobial efficacy of selenium nanoparticles reinforced with the herb Thymus vulgaris against oral pathogens.
Methods: The organisms used were Streptococcus mutans, Staphylococcus aureus, Lactobacillus sp and Candida albicans. Agar well diffusion method was used to assess the antimicrobial efficacy of the nanoparticles at various concentration that ranges from 25 µL, 50 µL and 100 µL. The synthesized nanoparticles were characterized by using ultraviolet double beam spectrophotometer in the wave length range of 250-750nm.
Results: Zone of inhibition was found to be highest at 100 µL concentration against Staphylococcus aureus and Candida albicans. The mean zone of inhibition was found to be increased as the concentrations of Se NPs increased.
Conclusion: Findings from this study suggest that selenium nanoparticles reinforced with Thymus vulgaris extracts act as a potential antimicrobial agent and can be used as an alternative to commercially available antimicrobial drugs.

Keywords: Anti-Microbial; Oral Pathogens; Selenium Nanoparticles; Thymus Vulgaris.

Introduction

There has been increasing concern in the era of antibiotic resistance as bacteria rapidly continue to develop adaptive counter measures against conventional antibiotics [1]. Bacteria are potentially life-threatening agents, capable of promoting infectious diseases. The history of bacteria acting as causative agents for infection goes back to the 14th century. Salvarsan was the first antimicrobial agent introduced in 1910. Soon after that, other antimicrobial agents such as chloramphenicol, nalidixic acid, and macrolides were used worldwide. The 20th century experienced temporary relief to infectious bacterial pathogens. Nevertheless, overexposure to antibiotics and evolution of effective countermeasures against antibacterial agents led to the emergence of antibiotic-resistant bacteria [2]. The economic impact of antimicrobial-drug resistance is escalating at an alarming rate due to the mounting medical and human cost [3].

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Antibiotic resistance currently spans all known classes of natural and synthetic compounds leading to an urgent requirement for new drugs and alternatives. But very few novel antibiotics have been discovered in the past 40 years due to the cost and complexities associated with drug discovery and development [4]. The search for new antimicrobial agents or modifications in already existing ones to improve their antimicrobial activity becomes indispensable. Nanotechnology provides a good platform to alter physico-chemical properties of different materials compared to their bulk counterpart that can be harnessed for bio applications [5-7].

Generally, metallic NPs are synthesized from inorganic metals by several chemical methods including gas condensation, attrition, precipitation, implantation, pyrolysis and hydrothermal treatment [8]. These methods employ many chemical reagents or consume excessive energy to progress the NP synthesis reactions by a multistep process. Chemical precursors and toxic solvents in the reaction pots are ultimately considered as hazardous wastes, threatening the living environment and human health [9].

Currently, an alternative method has developed based on the usage of biological reagents, which have more advantages over the chemical synthesis. These methods are known as green synthesis in which a living system such as bacteria, fungi, algae and plant extracts are often responsible for the reduction of metals for NPs formation [10].

Besides, synthesis of various types of metal NPs using plant extracts is found to be more efficient, easily controllable due to the existence of various metabolites like emulsifying, capping and stabilizing agents [11]. Reports of the reduction of precious metals by inactivated plant materials date back almost 100 years. Molisch [12] and Iwase [13] studied the reduction of Ag by plant chlorophyll and the reduction of Au by extract of fresh leaves, respectively. About 66 years ago, Nagai [14] reported the reduction of $Ag(NO)_3$ by plant cells. A decade ago, Armendariz et al. [15] reported the bio reduction of $KAuCl_4$ by powdered wheat (Triticumaestivum) and oat (Avena sativa) biomasses and the subsequent formation of Au NPs. These researchers reported that the solution pH is a key variable to control the size and to reduce the polydispersity of the biogenic Au NPs.

In recent years, several studies have pointed out the ability of selenium nanoparticles to exhibit anticancer, antioxidant, antibacterial and anti-biofilm properties. Selenium, being an indispensable dietary trace element, has been recently introduced in biomedicine as a 'Drug nanocarrier'. A lot of research has reported that Selenium possesses strong antioxidant, antibacterial and anticancer properties [16].

Selenium plays a crucial part in boosting immunity, protecting tissues against oxidative stress, Reproduction, growth, development and modulation [17]. Between Selenium's bioactivity and toxicity, there exists a thin margin only. Selenium shows immense biological activity, bioavailability and less toxicity if synthesized biologically rather the physicochemical methods [18]. So far, remarkable antimicrobial activity of these nanoparticles have been evidenced against pathogenic bacteria, fungi and yeasts [19].

So, with this note, in traditional medicine, Thymus genus is employed for treating spasms, inflammation, and bloat in the ruminant digestive system [20]. Thyme is an Iranian flora, naturally distributed in the mountainous semi-grazing areas and grows as the perennial subshrubs [21]. In the current years, the therapeutic potential of thyme extract was approved by many studies such as the treatment of retinal neovascularization, immunodeficiency syndrome, infection and cancer [22-24].

The rationale of this study is that no study has been conducted so far in which the antimicrobial properties of Selenium nanoparticles reinforced with Thymus vulgaris have been assessed. Hence the aim of the study was to assess the antimicrobial efficacy of Selenium nanoparticles reinforced with Thymus vulgaris against oral pathogens.

Materials and Methods

Collection and preparation of plant extract (fig 1)

Powdered Thymus leaves were purchased from the market of South India and identified and authenticated by Botanist. The obtained powder of *Thymus vulgaris* is stored in an airtight container. One gram of *Thymus vulgaris* powder was dissolved in 40 ml of distilled water and boiled for 20 minutes using heating mantle at 60-70oC. The extract was filtered using Whatman No 1 filter paper and allowed to stand undisturbed for 20 minutes. Then the filtered plant extract was transferred to an airtight container and used for green synthesis and further studies.

Preparation of Selenium nanoparticles (fig 2 and 3)

30mMof sodium selenite was weighed and mixed with distilled water of 60 ml. The sodium selenite solution was mixed with 40ml of filtered plant extract and kept in a magnetic stirrer for 1 hour and kept in a shaker for 72 hours to obtain green synthesized selenium nanoparticles. UV spectrophotometer periodically monitored the reduction of sodium selenite to selenium nanoparticles. The color change was visually noted and photographed. Using Lark refrigerated centrifuge, the selenium nanoparticles solution is centrifuged at 8000 rpm for 10 min, and the pellet is collected and washed with distilled water twice. The final purified pellet was collected and dried at 100-1500C for 24 h, and finally, the nanoparticles was stored in an airtight Eppendorf tube.

Characterization of Selenium Nanoparticles

The synthesized selenium nanoparticles solution is primarily characterized by using Ultraviolet(UV) Visible spectroscopy. 3ml of the solution was taken in the cuvette and scanned in double-beam UV- visible spectrophotometer from 250-750 nm wavelength.The results were recorded for the graphical analysis. The shape and size of the copper nanoparticles were analyzed by using Transmission Electron Microscope (JEOL JEM3100F). The crystalline nature of the nanoparticles was characterized by X-ray diffraction analysis and the Fourier transform infrared spectroscopy was used to detect the functional and chemical group in the range of 4000–400 cm.

Antimicrobial Activity

Media Preparation: Mueller Hinton agar for Streptococcus mu-

Figure 1. Plant (*Thymus vulgaris*) extract.

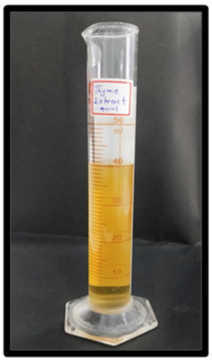
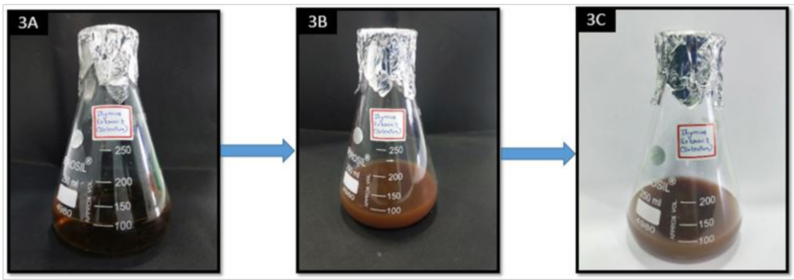


Figure 2. Sodium Selenite Solution.

Figure 3. 3A- Plant extract with sodium selenite, day 1 color change; 3B- Day 2 color change; 3C- Day 3 color change.



tans, Staphylococcus aureus, Lactobacillussp and SDA agar forCandida albicans and was prepared, sterilized and poured onto the Petriplates. The plates were allowed for solidification. Agar Well Diffusion method was used to assess the antimicrobial efficacy.

Swabbing: After solidification, the respective plates were swabbed with the oral pathogens-Streptococcus mutans, Staphylococcus aureus, Lactobacillusspand Candida albicans.

Well Formation: After swabbing, three wells on each plate were formed using a gel puncher. To those three wells, selenium nanoparticles with Thymus vulgaris solution were loaded in the concentration range of 25 μL, 50 μL and 100 μL. The plates were then incubated at 37oC for 24 h for Streptococcus mutans, Staphylococcus aureus, Lactobacillus spand 48 h for Candida albicans. After incubation, the zone of inhibition was measured and calculated.

Results

UV Visible Spectroscopy

UV-Vis absorption spectra of the green synthesized selenium nanoparticles were recorded at a different wavelength from 250-750nm. The selenium nanoparticles are synthesized using sodium selenite and Thymus vulgaris leaf extract as a reducing agent which shows absorption peak at 340 nm. Broadened SPR peak observed in this UV-Vis spectrum confirmed that polydispersenano sized particles. The peak found in the spectroscopy indicates the formation of Thymus vulgaris selenium nanoparticle.

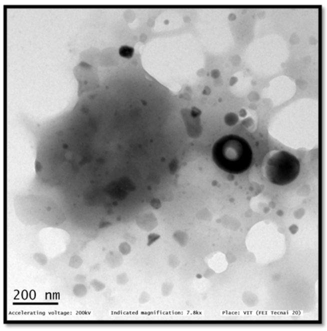
Transmission electron microscopy (Figure 4)

TEM is the most common tool to convict the structure, size, morphology, dispersion, and orientation of biological and physical samples. Figure 4 shows the typical TEM images of nanoselenium shape which was found to be spherical and has the broad size distribution between 5–70 nm.

Antimicrobial activity

The diameter of the inhibition zone for all tested concentrations of SeNPs achieved for bacterial strains is presented in Table 1. Figure 5 depicts the antimicrobial activity of seleniumnanoparticles reinforced with thymus vulgaris extract against Streptococcus mutans and Staphylococcus aureus. Zone of inhibition against Streptococcus mutans was found to be almost equal for all concentrations (9 mm). Zone of inhibition against Staphylococcus aureusat 25 μL was 10 mm, at 50 μL was 12 mm and at 100 μL was 18 mm. Figure 6 depicts the antimicrobial activity of selenium nanoparticles reinforced with thymus vulgaris extract against Lactobacillus spand Candida albicans. Zone of inhibition against Lactobacillus sp at 25 μL was 9 mm, 50 μL was 9 mm and at 100

Figure 4. TEM image of *Thymus vulgaris* mediated selenium nanoparticles.



Graph 1. Spectroscopic analysis of selenium.

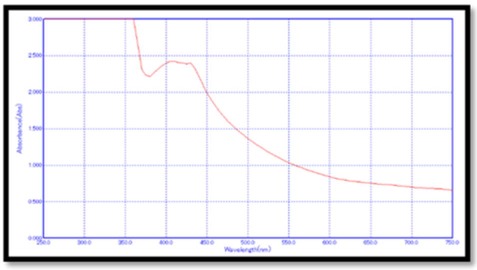


Table 1. Bacterial growth inhibition of biosynthesis SeNPs based on disc diffusion method.

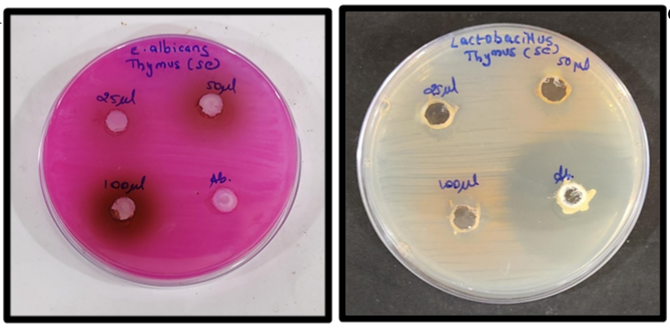
SeNPs (thymus) µg/ml	Zone of Inhibition, mm			
	S. mutans	*S. aureus*	*Lactobacillus sp*	C. albicans
25 µl	9	10	9	9
50 µl	9	12	9	10
100 µl	9	18	10	20
Ab	34	33	19	12

Figure 5. Antibacterial activity of selenium nanoparticles reinforced with thymus vulgaris extract against Streptococcus mutans(5A)and Streptococcus aureus(5B). Against S.mutans the zone of inhibition at 25µl, 50µl, 100µl was 9mm at all concentration. Against S.aureus the zone of inhibition at 25µl, 50µl, 100µl was 10mm, 12mm, 18mm respectively.

5A 5B

Figure 6. Antibacterial activity of selenium nanoparticles reinforced with thymus vulgaris extract against Candida albicans (6A)and *Lactobacillus* (6B). Against *C. albicans* the zone of inhibition at 25µl, 50µl, 100µl was 9mm, 9mm, 10mm respectively. Against Lactobacillus the zone of inhibition at 25µl, 50µl, 100µl was 9mm, 10mm, 20mm respectively.

6A 6B



μL was 10 mm. Zone of inhibition against Candida albicans at 25 μL was 9 mm, 50 μL was 10 mm and at 100 μL was 20 mm. So, it was seen that as the concentration of the selenium nanoparticles reinforced with Thymus vulgaris extract increased, the antimicrobial activity increased.

Discussion

Now microorganisms have become resistant to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents. Nanotechnology is an emerging technology and has led to a new revolution in every field of science. Among the various inorganic nanoparticles available, selenium has easy processing methods, is inexpensive, has a wide range of applications in dentistry and is a safe material [25].

The studies by author Chudobovaet al, Webster et al, Wang et alfound that Se NPs may inhibit the growth of S. aureus by up to 60 times compared with the control [26-28]. Webster et alused Se NPs (80-220 nm) in an antibacterial coating to inhibit the growth of this pathogen. Inhibitory effects were observed at early time points (up to 5 h) using Se NPs-coated biofilms as compared to the uncoated counterpart [27]. Although our results aren't agreement with these findings, showing the antimicrobial effect of Se NPs to *S. aureus*, we show that Se NPs are a potent antibacterial agent to *S. aureus*.

The different types of analysis such as HR-TEM and UV visible spectroscopy are carried out to study the characteristics of the selenium nanoparticles. The nanoparticles synthesized are of 100–250 nm with identical sphere structure. The selenium NPs synthesized from actinobacteria has activity against dengue virus [30]. Enterococcus faecalis are able to synthesize selenium NPs from sodium selenite. The characteristics of the NPs are analyzed using TEM and UV-spectroscopy. TEM reveals that the size of the NPs is 29-195 nm and is spherical in shape [30]. The bacterial protein of Zooglearamigera is responsible for the reduction of selenium nanoparticles. The prepared nanoparticles are hexagonal phase nanocrystals. XRD, TEM, SEM, SAED, and DLS are carried out to test the characteristics of the nanoparticles which reveals that the SeNPs is 30-150 nm and spherical in shape [31].

In a study done by El-Ghazalyet al., regarding the anti-inflammatory effect of selenium nanoparticles on the inflammation induced on irradiated rats, Nano-Se were administered orally in a dose of 2.55 mg/kg. It has been found that Nano-Se lessened the elevating inflammation in both irradiated and non-irradiated rats [32]. Melatonin-SeNPs treatment decreased pathological abnormalities of the liver, proinflammatory cytokines and splenocyte proliferation. The combination of silymarin and selenium nanoparticle at Low concentration is an excellent candidate possessing anti-inflammatory as well as antioxidant properties [33]. Nanoparticles are endorsed as sustainable antimicrobial agents having remarkable perspective to resolve the microbial multidrug resistance problem [34-35]. From the present results, it is indicated that the biosynthesized SeNPs mediated by thymus extract has the efficient antimicrobial activity against pathogenic organisms.

Conclusion

In this study, *Thymus vulgaris* mediated selenium nanoparticles have been synthesized. This bioengineered nanoparticle has proved to exhibit significant anti-microbial properties with higher concentrations. It is non-toxic, without any side effects as that of steroidal and non-steroidal anti-inflammatory drugs. Future studies will be carried out to identify other properties present in selenium nanoparticles synthesized using *Thymus vulgaris*.

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