

## ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF WHITE PEPPER OLEORESIN MEDIATED SELENIUM NANOPARTICLES

**Running title:** Activity of white pepper oleoresin mediated selenium nanoparticles

### ABSTRACT:

**Introduction:** Indian spices such as turmeric, ginger, and mint bring color and flavor to food and cure many ailments too.

**Aim:** To study the anti-inflammatory and antioxidant activity of white pepper oleoresin-mediated selenium nanoparticles.

**Materials and Method:** The white pepper oleoresin extract was collected from Synthite Industries Pvt Limited, with a product code - 4010000835. Selenium nanoparticles were initially prepared using white pepper oleoresin and DPPH assay was performed on the different concentrations of white pepper oleoresin (10, 20, 30, 40, 50 µg/ml) for antioxidant activity, and anti-inflammatory activity was tested by the following method proposed by Muzushima and Kabayashi with specific alterations.

**Results:** White pepper has shown a very good anti-inflammatory effect and antioxidant activity when compared to the standard used at higher concentrations. Selenium nanoparticles appeared to have 90 percent inhibition in 50µL, which was also higher than the standard. Selenium nanoparticles seemed to have 85 percent inhibition in 50µL, which was also lower than the standard.

**Conclusion:** The present study has demonstrated an eco-friendly and cost-effective synthesis of Selenium nanoparticles using White pepper oleoresin. White pepper oleoresin mediated Selenium nanoparticles were with good anti-inflammatory and antioxidant activity which may be utilized include for its use in inflammatory and other conditions where there is a requirement for antioxidant activity.

**Keywords:** Anti-inflammatory activity; Antioxidant activity; white pepper oleoresin; selenium nanoparticle, environment friendly, green synthesis

## INTRODUCTION:

Nanoparticle, an ultrafine unit with dimensions measured in nanometers (1). Due to their submicroscopic scale basic material characteristics, and engineered nanoparticles can find functional applications in several fields, including medicine, engineering, catalysis, and environmental remediation (2). A nanoparticle is a microscopic particle between 1 and 100 nanometers in size (3).

White pepper contains the seed of the pepper plant on its own and the darker-colored skin of the pepper fruit is separated. This is normally achieved by a method known as retting, where red pepper berries, which are entirely mature, are immersed in water for about a week, after which the pepper- flesh softens and decomposes (4). White pepper is also used in cream sauces, Chinese and Thai dishes, and salads. White Pepper Oleoresin (5) is produced by solvent extraction of the decorticated, ground dried berries of *Piper nigrum L.* The scent of the product is typical of white pepper, with a clear caustic note and pungency behind it. Its taste is initially mildly warm and friendly, accompanied by a pungent, biting feeling (6).

Inflammation, which is a pattern of response to injury involves the accumulation of cells and exudates in irritated tissues, which allows protection from further damage. Inflammation has been investigated for thousands of years to counteract its impact on the body. [In AD 30, Cel-is described the 4 classic signs of inflammation \(rubor, calor, dolor, and tumor, or redness, heat, pain, and the inflammatory\) basis of the disease becomes clear, anti-inflammatory food and food products become of greater interest \(7\).](#) The role of oxidation in the body and food has been generally recognized. Oxidative metabolism is important for cell survival. A side effect of this dependency is the development of free radicals and other reactive oxygen species which cause oxidative changes. There is growing evidence of the presence of such organisms in several natural *in vivo* regulatory systems (8). When excess free radicals are produced, they will bypass

defensive enzymes such as superoxide dismutase, catalase, and peroxidase and trigger destructive and lethal cellular effects (e.g. apoptosis) by oxidizing membrane lipids, cellular proteins, DNA, and enzymes, thereby shutting down cellular respiration. Besides, reactive oxygen species seem to affect cell signaling processes in currently unraveled ways (9). Oxidation can also affect food, where it is one of the main causes of chemical spoilage, resulting in rancidity and/or degradation in the nutritional consistency, color, taste, texture, and protection of food. It is predicted that half of the world's fruit and vegetable crops are lost due to deteriorating post-harvest reactions. Defense mechanisms against the effects of excessive oxidation are given by the action of different antioxidants and the need to quantify antioxidant activity is well documented (10).

Methods for evaluating antioxidant behavior fell into two broad categories representing the emphasis on food activity or human bioactivity. In the case of food systems, it is essential to determine the effectiveness of the antioxidant(s) in providing food protection against oxidative spoilage. The subcategory includes measuring food activity, in particular fruit, vegetables, and drinks, but to predict dietary pressure and *in vivo* activity. Oxidative stress in humans is caused by imbalances in antioxidant status (reactive oxygen species versus defense and repair mechanisms). Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, plus vitamin E, uric acid, and serum albumins, are among the endogenous defenses. In addition to these defenses, the intake of dietary antioxidants is also essential. The lack of a single, definable substrate in several instances *in vivo* is a significant difference from food-based systems (11).

The antioxidant agent may be defined as 'any material that significantly slows or prevents oxidation of the substrate when present at low concentrations compared to those of the oxidizing substrate. Antioxidants are basically classified into two groups, primary or chain-breaking antioxidants, and secondary or preventive antioxidants (12). Secondary or preventive antioxidants are substances that slow down the oxidation rate. This can be done in various ways, including reducing or singlet oxygen. Primary antioxidants, AH, when present in trace quantities, can either postpone or inhibit the initiation of lipid radical reactions or inhibit the spread by reacting with peroxy or alkoxy radicals (13).

Previously, our team had conducted numerous studies on oleoresins and nanoparticles, (14- 16), gold nanoparticles (17,18), and other nanoparticles (19 - 20). Our team has extensive knowledge and research experience that has translated into high quality publications (21–43) The aim of the present study was to evaluate the anti-inflammatory and antioxidant activity of white pepper oleoresin-mediated selenium nanoparticles.

## **MATERIALS AND METHODS:**

**Study setting:** The study was conducted in the nanomedicine laboratory, Dept of Pharmacology, Saveetha Dental college after obtaining approval from the Scientific Review Board (IHEC/SDC/UG-1921/21/130 )

### **Preparation of plant:**

The white pepper oleoresin extract was collected from Synthite Industries Pvt Limited, Kerala, with a product code - 4010000835. The preparation was obtained by solvent extraction of the white pepper prepared from ripe pepper berries by the disintegration of the skin, decortication, and drying. The product claims to have 23 - 25 ml/100g of volatile oil content.

### **Synthesis of selenium nanoparticles:**

Selenium nanoparticles were synthesized using white pepper oleoresin by adding 0.2 mL of white pepper oleoresin to 100 mL of distilled water and 20 millimolar of sodium selenite was added and kept in an orbital shaker. The nanoparticles were then centrifuged with the aid of a Lark refrigerator centrifuge for 10 minutes at 8000 rpm. The pellets were then separated from the supernatant and transferred into an Eppendorf tube. The color changes were observed at different periods by a double-beam UV-Vis spectrophotometer at wavelengths ranging from 250 nm to 750 nm. The selenium nanoparticles, thus obtained, were purified by repeated centrifugation at 8000 rpm for 10 minutes. The pellet was collected in a cuvette and the peak value was measured.

### **Anti-inflammatory activity:**

The anti-inflammatory activity for white pepper oleoresin-mediated selenium nanoparticles was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (44). 0.05 mL of white pepper oleoresin of various fixation (10 $\mu$ L,20 $\mu$ L,30 $\mu$ L,40 $\mu$ L,50 $\mu$ L) was added to 0.45 mL bovine serum albumin (1% aqueous

solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control (45).

Percentage of protein denaturation was determined utilizing the following equation,

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

### **Antioxidant activity:**

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay was used to test the antioxidant activity of selenium nanoparticles. Diverse concentrations (2-10µg/ml) of white pepper oleoresin 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. 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,

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

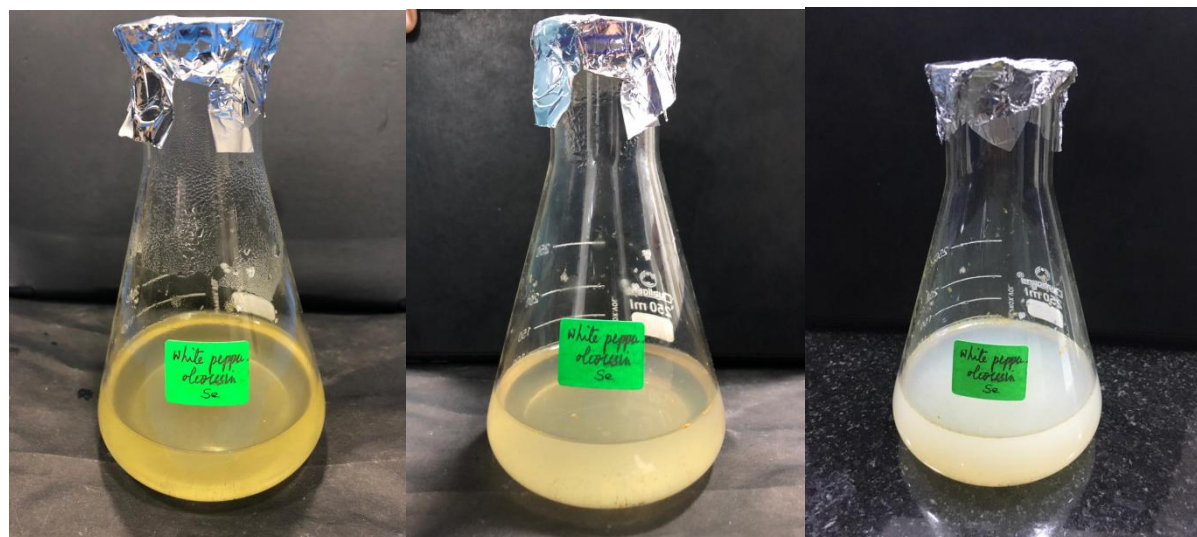
## **RESULTS AND DISCUSSION:**

White pepper is considered to possess a good anti-inflammatory and antioxidant activity effect at higher concentrations (50µL) compared to the standard used. Over the most recent years, research on medicinal, antimutagenic, antioxidant, and anticarcinogenic properties of spices has been conducted. In this study, the anti-inflammatory and antioxidant activity was conducted by convention proposed by Muzushima and Kabayashi with specific alterations and DPPH assay respectively.

### **Visual Identification**

The color changes in the reaction mixture were observed at regular interval. The sodium Selenite is reduced into selenium nanoparticles and it is identified by color change (Figure 1). The color

changed from light yellow to cream viscous liquid indicating the formation of selenium nanoparticles. The intensity of viscous liquid rises with an increase in incubation time. After 24hr cream viscous liquid was observed. No color change was seen further which indicated that synthesis of selenium nanoparticles was completed.



A

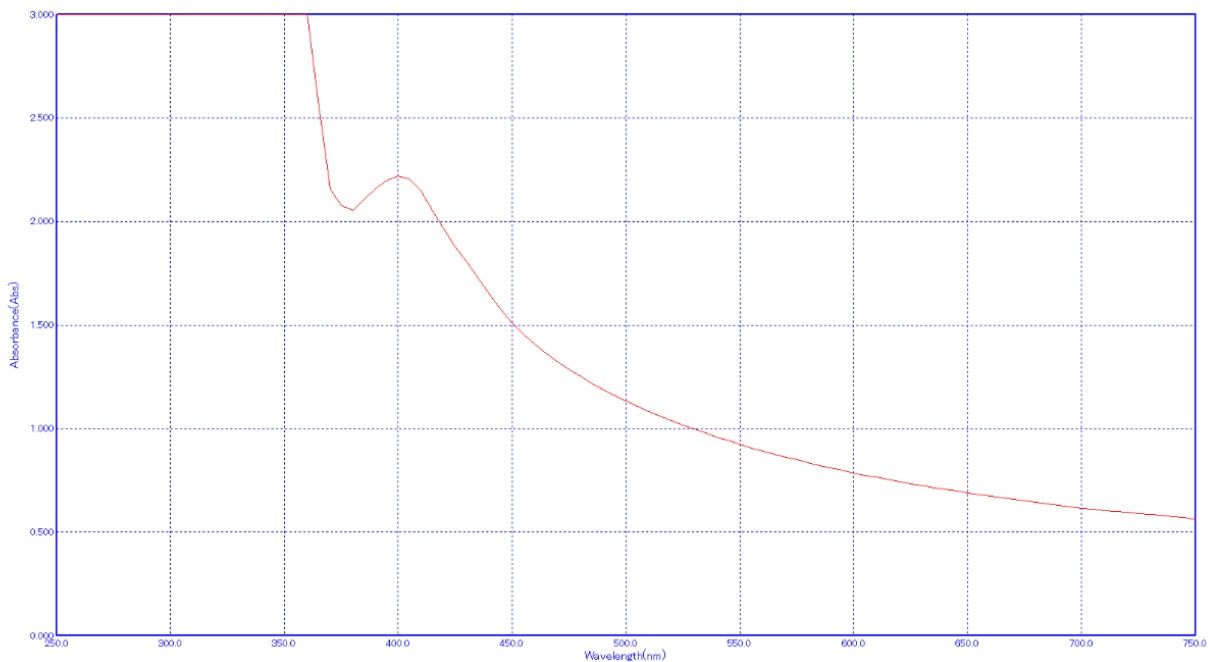
B

C

**Figure 1:** Visual observation of color changes at various periods of incubation time (A just after mixing ; B after 6 hours C after 24 hours) indicating the reduction of selenite ions to selenium nanoparticles.

#### **UV-Visible absorption spectrophotometer activity:**

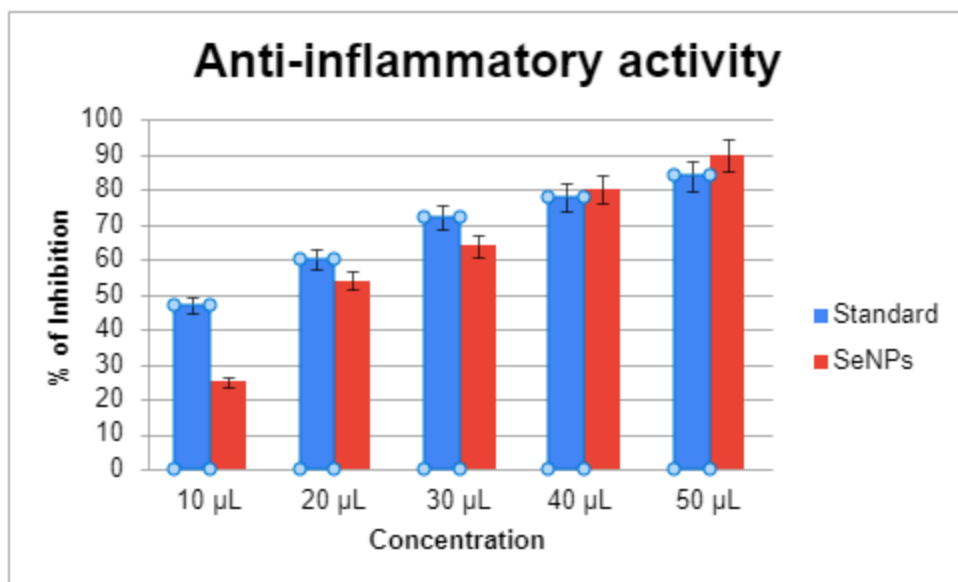
UV-Visible analysis depends on the color in the reaction mixture. This occurs due to the excitation of the surface plasmon resonance band in a reaction mixture at different wavelength regions from 250 nm to 750 nm and was observed at various time intervals. Figure 2 shows the UV absorption spectra of the synthesized selenium nanoparticles using white pepper oleoresin. With an increase in incubation time, the spectrum clearly shows that the absorbance steadily increases. The plasmon resonance band for selenium nanoparticles was peak positioned at 350 nm. The iron nanoparticles synthesized using an aqueous *Musa ornate* flower have been shown to produce a maximum absorption peak around 310 nm (46).



**Figure 2:** UV- Visible spectrophotometer analysis of White pepper oleoresin mediated selenium nanoparticles recorded as a function of time (‘X-axis’ represents ‘Absorbance’ [Absorbance] and Y-axis represents ‘Wavelength’ [nm] ).

### **Anti-inflammatory activity:**

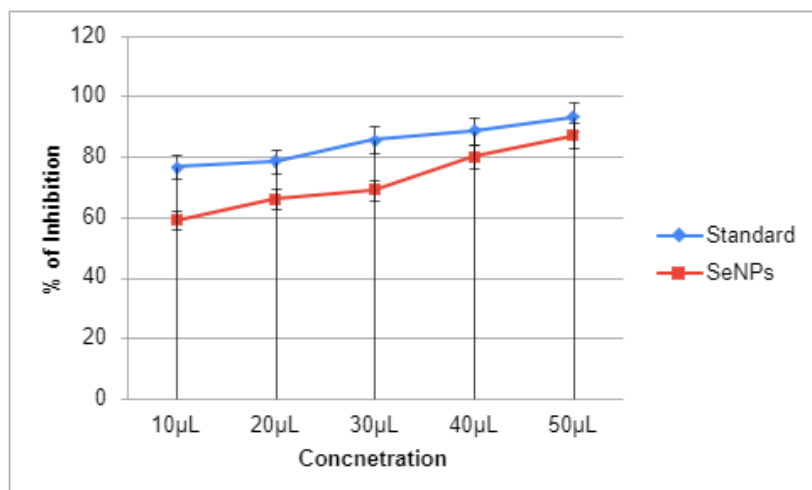
The anti-inflammatory activity for white pepper oleoresin-mediated selenium nanoparticles was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (44). The various fixations (47) of white pepper oleoresin from 10 $\mu$ L-50 $\mu$ L obtained various results in comparison to the standard Diclofenac sodium. In 10 $\mu$ L, selenium nanoparticles appeared to have 25% inhibition which was lower than the standard. In 20 $\mu$ L, the nanoparticle showed 55% inhibition which was more or less similar to the standard. In 30 $\mu$ L, selenium nanoparticles appeared to have 65% inhibition which was also similar to the standard. In 40 $\mu$ L, the nanoparticle showed 80% inhibition which was higher than the standard. In 50 $\mu$ L, selenium nanoparticles appeared to have 90% inhibition which was also higher than the standard. As a result, when the concentration of the selenium nanoparticles increased, the inflammatory activity also increased.



**Figure 3:** Anti-inflammatory activity at various concentrations from 10µL-50µL and the percentage of inhibition. The X-axis shows the concentration of the nanoparticle and the Y-axis shows the percentage inhibition. Values are done in triplicate n= 3, Mean +/- SD.

#### Antioxidant activity:

The antioxidant activity for white pepper oleoresin-mediated selenium nanoparticles was tested by DPPH Assay (Figure 4). The various fixations of white pepper oleoresin from 10µL-50µL obtained various results in comparison to the standard control butylated hydroxytoluene (BHT). In 10µL, selenium nanoparticles appeared to have 60% inhibition which was lower than the standard. In 20µL, the nanoparticle showed 65% inhibition which was significantly lower than the standard. In 30µL, selenium nanoparticles appeared to have 70% inhibition which was also lower than the standard. In 40µL, the nanoparticle showed 80% inhibition which was lower but the range was near the standard. In 50µL, selenium nanoparticles appeared to have 85% inhibition which was also lower than the standard whereas it increased and reached the standard. As a result, when the concentration of the selenium nanoparticles was raised, the antioxidant activity also increased. There were many studies which was motivation to carry out the present study (48-56).The limitation of the study was that the activity was evaluated only by *in vitro* techniques and can be extended in animal models in the future.



**Figure 4:** Antioxidant activity at various concentrations from 10µL-50µL and the percentage of inhibition. The X-axis shows the concentration of the nanoparticle and the Y-axis shows the percentage inhibition. Values are done in triplicate n= 3, Mean +/- SD.

## CONCLUSION:

The present study has demonstrated an eco-friendly and cost-effective synthesis of Selenium nanoparticles using White pepper oleoresin. The white pepper oleoresin-mediated selenium nanoparticles have showed good anti-inflammatory and antioxidant activity at higher concentrations based on the results obtained. White pepper is a natural product with its strong aroma and good properties that can be used in treating inflammation and can be used to prevent oxidation and prevent the detrimental effects of other medications. Further invasive studies can support its use in the future.

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