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# Green Synthesis Of Selenium Nanoparticles Using Leaf Extract Of Mukia Maderasapatana And Deciphering Its Biomedicinal Properties

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## ABSTRACT

Selenium nanoparticles (SeNPs) are tiny particulates, which possess the ability for assimilation by biological systems, and have garnered attraction among researchers due to its exceptional biological properties and potential therapeutic functionalities. In the present study, SeNPs were synthesized via an effective and eco-friendly technique using leaf extract of *Mukia maderaspatana*. Then SeNPs were characterized by various analytical techniques. UV-Vis spectroscopy showed absorption peak at 265 nm. Biomolecules acts as stabilizers for synthesized SeNPs. Alcoholic group, nitro compounds, aromatic compounds in SeNPs is revealed by the O-H bond, N-O bond and C-C bond by FTIR analysis. SEM analysis showed dimensions of synthesized SeNPs ranging from 73.24 nm to 187.6 nm. EDX analysis highlights the elemental composition of SeNPs as (13.75%) of Selenium, (69.27%) of oxygen and (16.98%) of sodium, and XRD spectroscopy revealed the crystalline structure of SeNPs. The antioxidant potential of SeNPs via *in vitro* assays like NO, ABTS, FRAP and $H_2O_2$ showed higher inhibition as 86.71%, 89.18%, 87.54%, and 89.9% at a concentration of 50 µg/ml. Anti-inflammatory effects of SeNPs demonstrated a noteworthy inhibition of Bovine Serum Albumin, Egg Albumin denaturation as 78% and 77%, also in membrane stabilization with increase in the concentrations of SeNPs it stabilizes the cellular membranes. Brine shrimp lethality assay performed to assess the cytotoxicity effects of SeNPs and recorded the 50% of survival at 80 µg/mL of concentration, and thereby validated its biosafety profile and potential therapeutic index. Collectively, this investigation highlights the multifunctional capabilities of SeNPs as promising candidates for applications in the biomedical field.

***Keywords:*** *Selenium nanoparticles, Mukia maderaspatana, green synthesis, antioxidant, anti-inflammatory, Cytotoxicity*

## 1. INTRODUCTION

In recent years, nanotechnology has emerged as an innovative discipline with multifaceted applications across a spectrum of scientific fields, notably in the medicine domain [1]. Nanoparticles are defined as particles that exhibit dimensions in the range of 10^-9 meters, and those nanoparticles formed from metallic elements are referred as metallic nanoparticles. These nanoparticles exhibit a wide variety of applications in diverse domains, such as biotechnology, medicine, cosmetics and others. It also possess numerous beneficial properties relevant to health and diagnosis. Due to their distinctive characteristics and properties that are dependent upon their size, metallic nanoparticles have garnered substantial focus from scientists [2]. The synthesis of nanoparticles via the utilization of plant sources or their derivatives is known as green synthesis, and this method is recognized for its safety, economic feasibility, and environmental sustainability. It dispenses the obligation for toxic chemicals or reducing agents; instead the extract of plant serves as a reducing agent. The plant extract promotes the metal ion reduction; leading to the nanoparticle formation. Nanoparticles synthesized via sustainable eco-friendly

methodologies exhibits a diverse range of beneficial properties, including antioxidant, antimicrobial and antitumor activities [3]. Selenium (Se) is categorized as a metalloid, manifesting features to both metals and non-metals. It has been confirmed that selenium serves as an integral dietary micronutrient vital for the optimal physiological efficacy of human body. It includes diverse collection of important medical applications [2]. Previous studies have revealed that selenium plays an essential function in the diverse disease prevention, including cystic fibrosis, cardiovascular disorders, and stress-related conditions [4].

In biological systems, selenium is an essential trace element, exhibiting specific functions in numerous health-related processes. In various allotropic forms selenium exhibits which includes both amorphous and crystalline configurations, and it is considered as a remarkable material due to its chemical versatility for the synthesis of nanoparticles [1]. Selenium nanoparticles (SeNPs) exhibits enhanced bioactivity and lower in toxicity in contrast to various sources of selenium such as selenate and selenomethionine. Recently, SeNPs have drawn significant academic attention as a vital research area due to their tiny size and remarkable physical and chemical properties, with an enhanced focus on their synthesis methods, features, and potential uses in the life sciences stream [5]. Hence, the green synthesis of SeNPs has garnered considerable interests of the researchers about their potential uses in biomedical field. The biosynthetic process for SeNPs is recognized for its safe, non-toxic and environmentally friendly aspects. Moreover, owing to the organic compounds residing on their surfaces biosynthesized SeNPs shown greater stability which effectively deter the nanoparticles aggregation over time [6].

Oxidative stress refers to the presence of entities known as free radicals and reactive oxygen species (ROS), which are generated during standard physiological processes but can become harmful when not adequately neutralized by endogenous defense mechanisms. Indeed, oxidative stress arises from a dysregulation between the production of reactive oxygen species and the capacity of endogenous antioxidant systems to mitigate their effects. ROS serve as principal agents that trigger oxidation and induce oxidative stress, which is implicated in a myriad of diseases and pathological conditions. An excessive accumulation of ROS is detrimental, as it instigates biomolecular oxidation that culminates in cellular apoptosis and the manifestation of oxidative stress. Furthermore, oxidative stress incites inadvertent activation of enzymes and leads to oxidative damage within the cellular environment [7]. This ROS facilitates the oxidative impairment of macromolecules, leading to the onset of various diseases. Human possesses an intrinsic protective mechanism designed to inhibit the generation of free radicals. These protective systems may be compromised under certain pathological states, necessitating the introduction of antioxidant supplements to stave off the production of free radicals. Antioxidants engage with the oxidation process via radical scavenging and chelating activities, thereby mitigating the oxidative damage instigated by free radicals. A variety of synthetic compounds, including butylated hydroxylanisole and butylated hydroxyltoluene, are commercially accessible; however, these compounds are associated with numerous adverse effects in both humans and animals. Conversely, plant-derived constituents such as flavonoids, tannins, proanthocyanidins, and phenolic compounds exhibit robust antioxidant properties [8].

Inflammation constitutes a cellular and tissue response to injury triggered by a multitude of factors, including infectious agents, chemical substances, thermal exposure, and mechanical trauma [9]. The management of inflammation predominantly involves the utilization of both steroidal and nonsteroidal anti-inflammatory pharmacological agents. Within the domain of biomedicine, especially concerning anti-inflammatory therapies, the integration of nanotechnology has been extensively explored and thoroughly established [10]. A significant proportion of anti-inflammatory pharmaceuticals function as potential inhibitors of the cyclooxygenase (COX) pathway, which is integral to the metabolism of arachidonic acid, subsequently leading to the synthesis of prostaglandins, acknowledged as the key mediators of the inflammatory process. The suppression of prostaglandin biosynthesis is critical for the effective management of inflammation. Consequently, the administration of analgesic and anti-inflammatory compounds is deemed necessary for therapeutic intervention. The presently accessible anti-inflammatory pharmacological agents pose significant hazards to human health, particularly in terms of their toxicity and the probability of relapse of the disorder after the discontinuation of treatment. The traditional system of plant-based medicine plays a crucial role in healthcare, with numerous plant extracts and their isolated constituents recognized as potent anti-inflammatory agents [9]. Recently, the utilization of medicinal plants has been investigated to formulate nanoparticles (NPs), aimed at enhancing therapeutic efficacy while mitigating adverse effects [10].

One of the most extensive and heterogenous groups of flora, comprising both domesticated and wild species is Cucurbitaceae family. The species belonging to this family are generally regarded as “Cucurbits” and are valued for their significant contributions of vital dietary macromolecules. Furthermore, it is extensively used in Ayurvedic systems and in modern medical practices to solve multitude of health-related issues [11]. *Mukia maderaspatana* is commonly referred as Madras pea pumpkin, a medicinal species generally found in tropical and subtropical zones [12]. Leaves are generally utilized for various applications among the different parts of plant. Within the Indian cultural milieu, traditional practitioners employ various plant materials in several forms such as decoctions, extracts, juices and salads to tackle a wide range of health challenges [13]. Nonetheless, existing literature regarding the antioxidant and cytotoxic properties of *M. maderaspatana* remains notably sparse. Taking into account the previously highlighted insights and reinforced by the scientific corpus, the current research marks the pioneering systematic investigation into the cytotoxic potential of selenium nanoparticles synthesized from *M. maderaspatana*, alongside a comprehensive exploration of its antioxidant and anti-inflammatory

activities.

## 2. MATERIALS AND METHODS

### *2.1 Chemicals and Reagents*

All chemicals and reagents employed in the study exhibit a high degree of purity and adhere to analytical grade specifications.

### *2.2 Preparation of Extract*

*M. maderaspatana* plant specimens were procured from the Chengalpattu district, Tamil Nadu, India. The leaves were harvested and thoroughly rinsed with tap water. Thereafter, they were subjected to sun-drying and subsequently processed using a mechanical grinder. Approximately 10 grams of the pulverized leaves were solubilized in 100 mL of distilled water and maintained under agitation for 15 minutes, followed by thermal treatment at 60°C for 30 minutes. The resultant solution underwent centrifugation for 15 minutes at a speed of 10,000 revolutions per minute. Thereafter, the supernatant was collected and filtered using Whatman filter paper.

### *2.3 Synthesis of selenium nanoparticles*

In the initial phase, a 50 mL of 0.1 M sodium selenate was added to the plant extract. Then, at 37°C of temperature with a stirring speed of 120 rpm the reaction mixture was incubated for 24 hours. A significant change of colour is observed at the end of the incubation duration, which signifies the successful synthesis of selenium nanoparticles (SeNPs) [14]. The resulting mixture was then centrifuged at 10000 rpm for 20 mins to enhance the SeNPs separation. The supernatant was removed, then the SeNPs were washed multiple times with distilled water to remove any contaminants. Consequently, the SeNPs underwent drying process in an oven for overnight at a temperature of 60°C [15].

### *2.4 Characterization of selenium nanoparticles*

The ultraviolet-visible spectrum was recorded within the wavelength range of 200–800 nm utilizing the UV–Vis 1601 spectrophotometer manufactured by Shimadzu. Fourier Transform Infrared (FTIR) spectroscopy was employed to elucidate the stabilization of selenium nanoparticles (SeNPs). The analysis of the selenium nanoparticles was conducted with the Shimadzu IRTracer-100 (Shimadzu Corporation, Kyoto, Japan), and the corresponding spectrum was obtained across a frequency range of 400 – 4000 cm−1. Scanning electron microscopy (SEM) analysis was used to investigate the surface morphology of the synthesized selenium nanoparticles. Moreover, energy dispersive X-ray spectroscopy (EDX) was utilized to identify the chemical composition of the SeNPs. X-ray diffraction (XRD) analysis was executed to examine the crystalline characteristics of the sample by using Siefert X-ray diffractometer.

### *2.5 In vitro antioxidant activity*

*In vitro* antioxidant assays, like ABTS antioxidant assay and Hydrogen peroxide ($H_2O_2$) activity, are executed following the protocols established by Kalaiselvi Krishnamoorthy et al., 2023 [16]. The FRAP assay is performed according to the methodology of Jessica Rumpf et al., 2023 [17]. The nitric oxide scavenging activity is carried out accordingly to the procedure of Karempudi Venkata Krishna et al., 2023 [18].

### *2.6 In vitro Anti-inflammatory activity*

The assay for the denaturation of egg albumin is conducted in accordance with the protocol established by Daniel Anokwah et al., 2022 [19], with some slight modifications. The assay for the stabilization of human red blood cell (HRBC) membranes is executed following the methodology of Magdum et al., 2024 [20]. The denaturation assay for Bovine Serum Albumin (BSA) is carried out in alignment with the procedures outlined by Ameena et al., 2023, with some modifications [21].

### *2.7 Brine Shrimp lethality bioassay for cytotoxicity*

The cytotoxicity of the selenium nanoparticles (SeNPs) was assessed accordingly to the methodology reported by Md. Ashaduzzaman Nur et al., 2023, with some modifications [22]. In an aquarium setting, brine shrimp eggs were incubated in 1 L of a 1 M sodium chloride brine solution (pH 8.5) to facilitate hatching. The incubation period lasted for 48 hours under fluorescent illumination, after which the newly hatched nauplii were transferred into designated experimental test tubes. Each test tube received ten pairs of nauplii, to which 1.5 mL of NaCl solution was subsequently added. In this experimental assay, five distinct concentrations of SeNPs (5, 10, 20, 40, and 80 µg/mL) were employed. Following this, an additional 1 mL of NaCl solution was introduced to each test tube. Vincristine sulfate served as a positive control for lethality, while DMSO (1% solvent) was utilized as a negative control. After a 12-hour incubation period, the total number of surviving nauplii was enumerated, and the percentage of mortality was calculated. The lethality percentage of nauplii for each concentration and the control group was subsequently computed. The live and deceased nauplii were quantified in each tube, allowing for the determination of the percentage (%) of mortality.

Percentage (%) of Death = number of dead nauplii / [number of dead nauplii + number of live nauplii] × 100

### *2.8 Statistical analysis*

In this study, all the obtained results are presented as mean ± SD where 'n' equal to the number of replicates used (n=3).

## 3. RESULTS AND DISCUSSION

### *3.1 Synthesis of selenium nanoparticles*

The synthesis of selenium nanoparticles was effectively attained via the employment of an aqueous extract extracted from *M. maderaspatana*, which operated as a bioreductant in the reduction of sodium selenite (Fig. 1). The optimal concentration for the synthesis of highly stable selenium nanoparticles was established to be within a 10 mM precursor solution supplemented with a 1% aqueous extract, upholding a ratio of 10:1 between the precursor and the aqueous extract, respectively. It is suggested that the biomolecules present in the aqueous extract play a pivotal role in the conversion of the inorganic form of sodium selenite ($Se^{IV}$) to elemental selenium ($Se^{0}$), while at the same time stabilizing the selenium nanoparticles through capping by different biomolecular agents. To elucidate the characteristics of the green synthesized selenium nanoparticles, purification was performed through centrifugation (10000 g for 15 minutes) of the reaction mixture, followed by meticulous washing three times with ethanol and subsequently with milli-Q water. Current research has extensively detailed the green synthesis of selenium nanoparticles (SeNPs) via the use of diverse plant species, microorganisms and by-products, thereby accentuating their economic feasibility and the simplicity of synthesis technique. In response to these findings, the current study highlights the effortless synthesis of selenium nanoparticles (SeNPs) using *M. maderaspatana*, a plant that has been highly honored in ancient literature and acknowledged for its therapeutic properties in Ayurvedic system. The eco-friendly synthesis method by using plant extracts delivers a wide range of beneficial properties, such as safety, stability, environmental sustainability and economic efficiency. After the utilization of extract of *M. maderaspatana* leaves, selenium nanoparticles were synthesized through the conversion of selenate to elemental selenium, which was accompanied by a distinct colour change. Furthermore, the synthesized nanoparticles using this technique exhibits significant physicochemical properties, which are linked with wide range of bioactive phytochemical compounds [23,24]. *M. maderaspatana* possess a wide variety of phytochemicals, consisting antioxidants and reducing agents like glycosides, triterpenoids and vitamin C, which effectively plays an important role as reducing agents in the SeNPs synthesis process [25]. The observed colorimetric alteration of the solution served as an marker for the surface plasmin resonance phenomenon associated to the nanoparticles, thus signifying the synthesis of selenium nanoparticles. Among various nanoparticles, SeNPs have garnered significant interest due to their broad-range of biological and pharmaceutical applications from various research organizations. Owing to their remarkable physiochemical properties, such as chemical stability and electron transfer properties, SeNPs are being recognized as promising options for various applications [26].


**Fig. 1. Biosynthesis of Selenium Nanoparticles**

### *3.2 Characterization of biosynthesized SeNPs*

#### *3.2.1 UV-visible spectroscopy*

The synthesized selenium nanoparticles have acquired considerable research attention via extensive investigations employing vast analytical techniques for their characterization. This procedure is initiated with UV–visible spectroscopy. After the incubation duration, the transformation in the color of the reaction mixture indicates selenite reduction and the later development of selenium nanoparticles. Upon conducting an analysis across a wavelength spectrum of 200–800 nm through the application of UV–Vis spectroscopy, the synthesized SeNPs demonstrated a significant absorption peak around 265 nm,

which can be attributed to the surface plasmon resonance (SPR) effect related to the conduction electrons present on the SeNPs' surface, as depicted in Fig. 2. Additionally, Siddharth Satpathy et al. (2024) [14] have correspondingly identified an absorption peak at 279 nm. Although there is a plethora of chemical and physical techniques employed for the production of bioactive selenium nanoparticles, many of these strategies are viewed as inadequate for biocompatible applications owing to concerns regarding aggregation and instability. Alternatively, the biosynthesis of SeNPs using plant, algal, and microbial resources indicates increased stability owing to the presence of biologically active metabolites that operate as stabilizers and capping agents. Furthermore, this biosynthetic mechanism is distinguished by its sustainability and dependability [27].

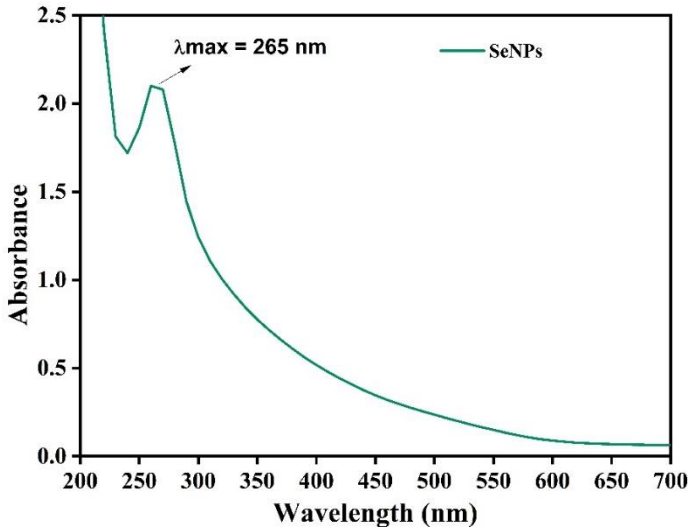


**Fig.2. UV-visible spectrum of SeNPs**

### *3.2.2 FT-IR analysis of SeNPs*

FT-IR is a common technique for analyzing the surface chemistry of particulate matter [27]. In this study, the purified selenium nanoparticles were subjected to FT-IR spectroscopy to detect the presence of biomolecules attached on their surface, which are vital for the synthesis and stabilization of the nanoparticles. As illustrated in Fig. 3, the absorption peak observed at approximately 3208.41 $cm^{-1}$ in SeNPs indicates the presence of O-H bonds that are observed in alcohols and phenolic substances. Similarly, the absorption peak measured at 1637.01 $cm^{-1}$ validates the existence of N-O bonds related with nitro compounds, while the peak at 1399.41 $cm^{-1}$ is associated with the C-C stretching vibrations of aromatic compounds. The subsequent absorption peak detected at 1079.48 $cm^{-1}$ validates the presence of C-N bonds which are emblematic of aliphatic amines, and the peaks recorded at 719.75 $cm^{-1}$ indicates C-H bending vibrations. The presence of various functional groups in the biomolecules may be integral to the reduction and stabilization of the synthesized SeNPs [15]. The outcomes of the current study are consistent with previous study published by Siddharth Satpathy et al., 2024 & Ayonposi Bukola Olaoye et al., 2024 [14,15].

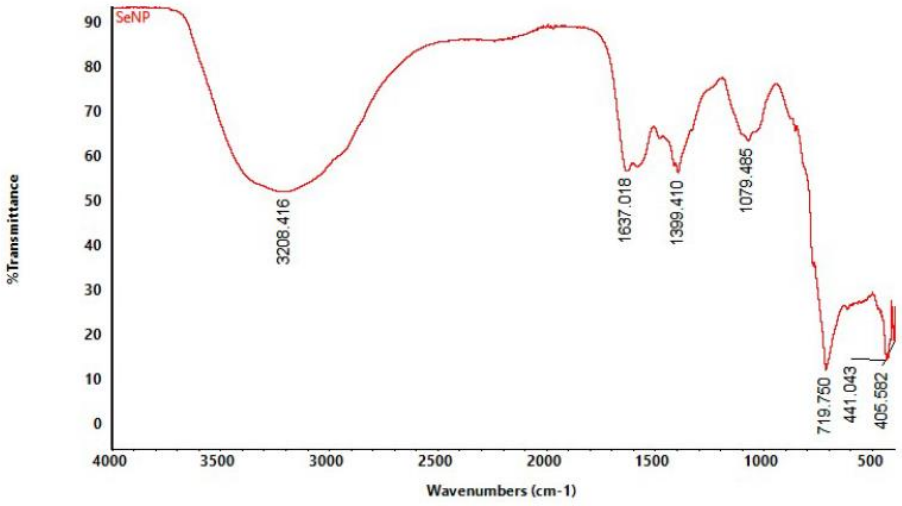


**Fig.3. FT-IR spectrum of SeNPs**

### *3.2.3 SEM and EDX analysis of SeNPs*

The image acquired via SEM of the synthesized SeNPs is displayed in Fig. 4. It is clear that the SeNPs revealed a distinctly uniform grain structure, which is evenly dispersed throughout the matrix, possessing dimensions ranging from 73.24 nm to 187.6 nm. This inference can be related to the potential of the bioactive compounds present in the extract to inhibit agglomeration. The nanoparticles were seen to be uniformly dispersed, notwithstanding a degree of aggregation. This phenomenon of agglomeration has been correlated with the functional groups that enhance the surfaces of the particles, which in turn constrains the majority of available metal ions to engage in a reduced quantity of nucleation events, ultimately resulting in metal agglomeration [15]. The biomolecules present in the leaf extract are instrumental in preserving the morphology and dimensions of the SeNPs through the mechanisms of steric hindrance and electrostatic repulsion. Synthesized SeNPs exhibits spherical morphology, a property that can be linked to the existence of capping agents within the extract and such capping agents are involved in the regulation of the selenium nanoparticle formation [14]. Energy dispersive X-ray (EDX) spectroscopy detailed the elemental composition and quantitative distribution of biosynthesized SeNPs, thereby it confirms the successful synthesis of SeNPS [6]. The existence of selenium atoms at a concentration of 13.75% within the SeNPs is confirmed by EDX spectrum and it is detected at an energy level of 1.5 KeV, as shown in Fig. 5. Moreover, the oxygen (O) (69.27%) and sodium (Na) (16.98%) were also confirmed by the complementary signals (Table 1). Moreover, across different physiological buffers SeNPs showed significant stability, thereby confirming their potential in biomedical applications [6]. The findings of the current study aligns with the study reported by Leila Asadpour et al., 2025 [28].

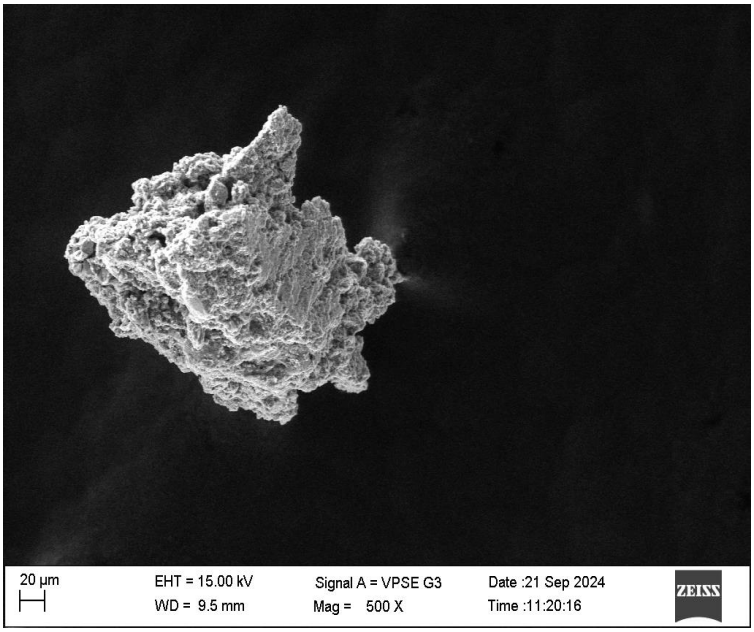


**Fig.4. SEM analysis of SeNPs**

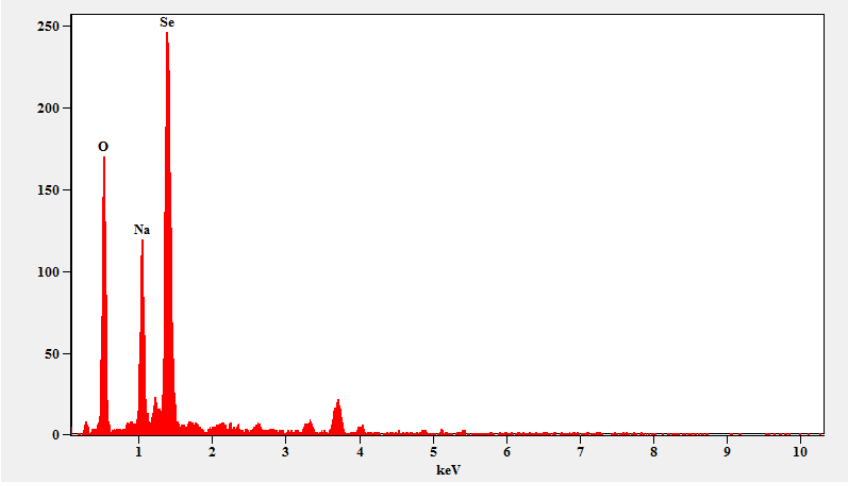


**Fig.5. EDX spectrum of SeNPs**

**Table.1. Elemental composition of synthesized SeNPs**

| Element Line | Weight % | Weight Error % | Atom % |
|---|---|---|---|
| **O K** | 42.88 | ± 1.33 | 69.27 |
| **Na K** | 15.10 | ± 0.90 | 16.98 |
| **Se K** | --- | --- | --- |
| **Se L** | 42.02 | ± 1.26 | 13.75 |
| **Total** | 100.00 | | 100.00 |

### *3.2.4 XRD analysis of SeNPs*

The X-ray diffraction (XRD) analysis exhibited notable peaks at specific diffraction 2θ angle values of 24.21°, 28.22°, 32.53°, 36.84°, 37.75°, 42.59°, and 43.65° for SeNPs, corresponding to the significant indices (100), (101), (110), (102), (111), (003) and (112) respectively as depicted in Fig. 6. In accordance with the reference code 06-0362 by JCPDS (Joint Committee on Powder Diffraction Standards) the XRD indices further confirms the crystalline properties of SeNPs. The current XRD findings align with the results reported by Siddharth Satpathy et al., 2024. The absence of significant peak is related to the organic components present in the leaves [14]. The intensity and specific properties of these diffraction peaks are closely in alignment with the research carried out by Kannan Kamla et a., 2024, which depicts the trigonal crystal structure of SeNPs synthesized using a mangrove species *Rhizophora mucronate* [29].

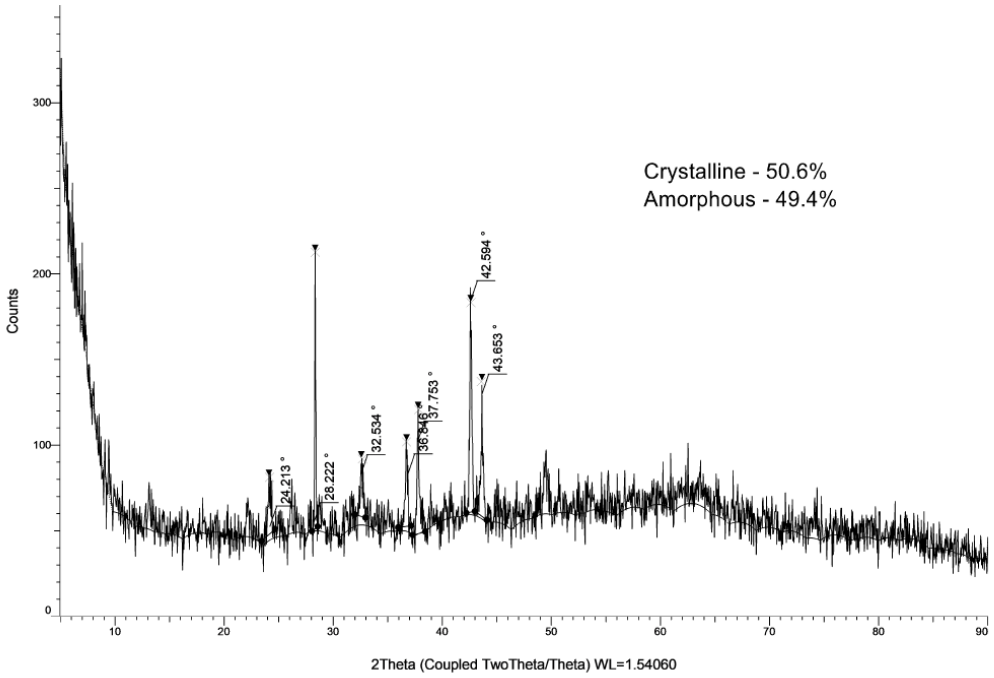


**Fig. 6. XRD pattern of SeNPs**

### *3.3 In vitro Antioxidant activity of SeNPs*

The generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been associated with the oxidative degradation of food products as well as with the pathogenesis of a multitude of human diseases. The hypothesized protective properties of antioxidants against these harmful oxidative-induced processes have garnered heightened scrutiny recently, particularly within the domains of biology, medicine, nutrition, and agro-chemistry. Although the terms oxidant and reductant are utilized in a chemical context, they are conventionally referred to as pro-oxidant and antioxidant, respectively, in biological settings. A pro-oxidant is defined as a compound that can instigate oxidative damage to a range of biological targets, including nucleic acids, lipids, and proteins. An antioxidant is characterized as a compound that can effectively neutralize a pro-oxidant, leading to the concomitant production of byproducts that possess negligible or low toxicity [30]. Antioxidants play a pivotal role in human health, as they possess the capacity to inhibit or postpone undesirable oxidation

reactions, thereby averting oxidative stress-related ailments such as hypertension, neurodegenerative diseases, and cancer [17]. Nitric oxide, a free radical, is generated from sodium nitroprusside and subsequently reacts with oxygen to yield nitrite. The assessment of antioxidant activity was conducted through the measurement of nitrite formation inhibition, accomplished by the application of plant extracts that directly interact with oxygen, nitric oxide, and other nitrogenous compounds [8]. Human beings are subjected to indirect exposure to $H_2O_2$ through environmental pathways. Hydrogen peroxide infiltrates the human system via the inhalation of vapor or mist and through contact with the eyes or skin. Within the organism, $H_2O_2$ undergoes rapid decomposition into diatomic oxygen and water, which may lead to the formation of hydroxyl radicals ($OH^{\cdot}$) capable of initiating lipid peroxidation and inflicting damage to DNA [31]. The reducing power antioxidant mechanism serves as a quantitative method to evaluate the electron-donating capability of an antioxidant, thereby reflecting its efficacy in neutralizing free radicals and mitigating oxidized intermediates. In this assay, the test compound (antioxidant) facilitates the reduction of ferric ions ($Fe^{3+}$) to ferrous ions ($Fe^{2+}$) through the donation of electrons. Typically, this reaction transpires in the presence of potassium ferricyanide, which is subsequently reduced to potassium ferrocyanide. The introduction of ferric chloride yields a Prussian blue complex ($Fe^{2+}$), which can be quantitatively analyzed using spectrophotometry, generally at a wavelength of 700 nm. The resultant colour intensity is directly correlated with the sample's reducing capacity. Antioxidant potential of compounds is exhibited by its enhanced reducing ability, thus illuminating their role as electron donors and their effectiveness in alleviating oxidative stress [32]. The ABTS assay offers a reliable methodological approach for evaluating the antioxidant potential of numerous compounds based on the ability to neutralize the ABTS radical cation ($ABTS^{\bullet+}$). Due to its effectiveness, sensitivity and significance to hydrophilic and lipophilic compounds this assay is extensively implemented. $ABTS^{\bullet+}$ is reverted back to its colourless neutral form via the transfer of hydrogen atoms and electrons in the presence of antioxidants. At a wavelength of 734 nm the reduction in the absorbance of $ABTS^{\bullet+}$ is analyzed quantitatively by using spectrophotometric techniques [32]. SeNPs antioxidant capacity is determined at concentration varying from 10 to 50 µg/mL by using methodologies like NO, ABTS, FRAP and $H_2O_2$. Enhanced scavenging efficacy of SeNPs is exhibited against the NO, ABTS, FRAP and $H_2O_2$ radicals. The results indicated that the antioxidant capacity of SeNPs increased in a concentration-dependent fashion. Ascorbic acid, serving as the standard, demonstrated the highest scavenging activity towards the NO, ABTS, FRAP, and $H_2O_2$ radicals (88.67%, 91.39%, 90.89%, and 89.9%, respectively). The concentration of 50 µg/ml exhibited significantly heightened inhibition of the NO, ABTS, FRAP, and $H_2O_2$ radicals (86.71%, 89.18%, 87.54%, and 89.9%, respectively) in comparison with the other concentrations examined. In this study, SeNPs disclosed a remarkable level of free radical inhibition, with the inhibition percentage closely paralleling with that of the standard reference (Fig. 7). Our study aligns with the findings of S.M. Bokhtiar et al., 2024, which explored the antioxidant potential of the crude extract of *Ulva Lactuca* [32].

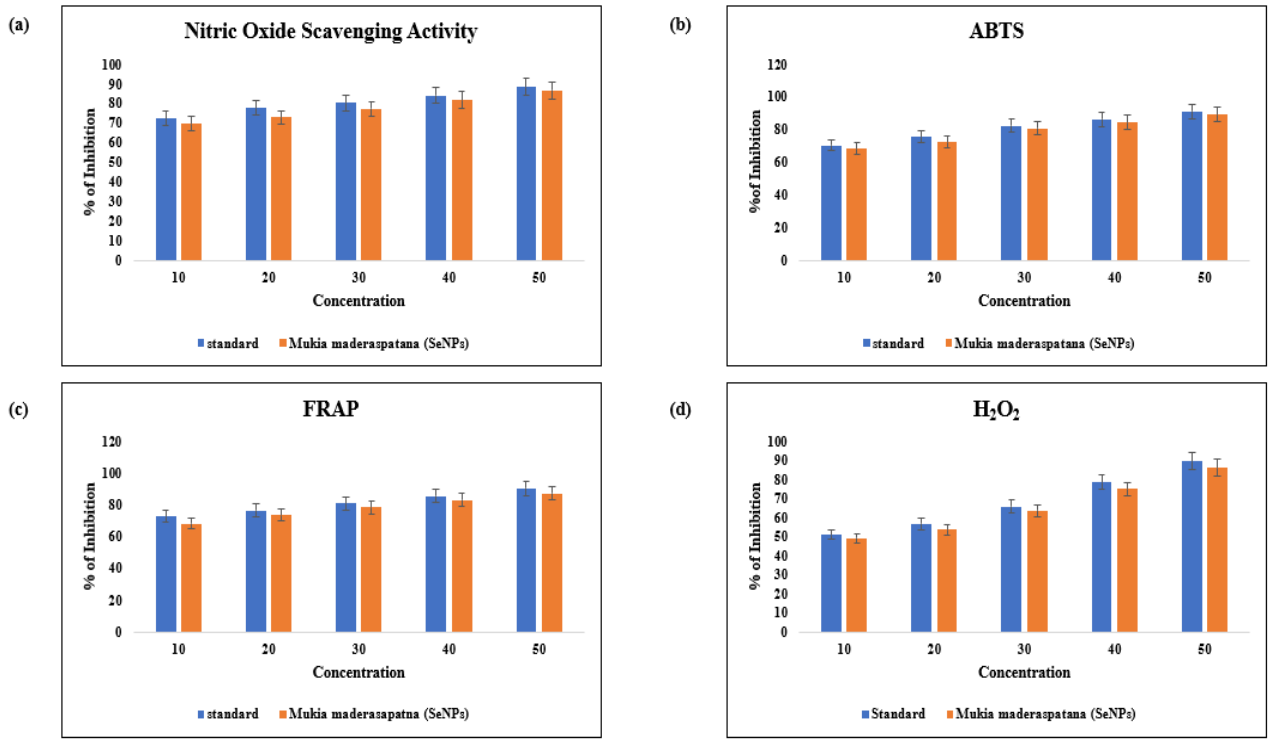


**Fig. 7. *In vitro* Antioxidant assays – (a) Nitric Oxide Scavenging Activity, (b) ABTS scavenging activity, (c) Ferric reducing antioxidant power assay, (d) Hydrogen peroxide scavenging activity**

### *3.4 In vitro Anti-inflammatory activity*

#### *3.4.1 Bovine Serum Albumin (BSA) Denaturation Assay*

Inflammation has been recognized as a critical factor in the pathogenesis of various diseases, including arthritis, stroke, and malignancies. It constitutes the inherent response of living tissues triggered by stimuli such as physical trauma, elevated temperatures, microbial invasions, and harmful chemical irritants, leading to clinical manifestations characterized by erythema, increased temperature, edema, nociception, along with enhanced vascular permeability, protein denaturation, and alterations in membrane integrity. Non-steroidal anti-inflammatory drugs (NSAIDs), exemplified by ibuprofen and diclofenac sodium, are routinely employed in clinical practice for the mitigation of inflammatory responses [33]. The capacity of a compound to inhibit protein denaturation may also contribute to the attenuation of an inflammatory process, given that the loss of biological activity in proteins has been associated with the development of inflammatory conditions such as rheumatoid arthritis, diabetes, and cancer. Serum albumins constitute the predominant proteins found in plasma and play a pivotal role in the transport of pharmaceuticals within the circulatory system. Bovine serum albumin (BSA) exhibits properties analogous to those of human serum albumin, thereby rendering it a widely utilized model protein in experimental studies. In the present investigation, BSA was employed to assess the potential of selenium nanoparticles (SeNPs) to inhibit protein denaturation, thereby evaluating their anti-inflammatory capabilities [34]. Within the BSA denaturation assay, SeNPs exhibited a concentration-dependent inhibition of BSA denaturation (Fig. 8a). At the minimal concentration of 10 µg/mL, SeNPs achieved an inhibition rate of 42%, in contrast to 47% for Diclofenac sodium. This pattern persisted with ascending concentrations, where SeNPs displayed inhibition rates of 54%, 69%, 75%, and 78% at 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL, respectively, while Diclofenac sodium demonstrated marginally superior inhibition rates of 60%, 72%, 78%, and 84% at corresponding concentrations. These findings suggest that SeNPs are efficacious in inhibiting BSA denaturation, albeit their effectiveness is slightly inferior to that of Diclofenac sodium. The current investigation corroborates the anti-inflammatory properties of *Hylocereus* spp. as reported by Md. Ashaduzzaman Nur et al., 2023 [22].

#### *3.4.2 Egg Albumin Denaturation Assay*

On the process of conversion of primary structure of the proteins into their secondary, tertiary structures and due to the external stressors impact proteins experience denaturation, with phenomenon of protein denaturation being influenced by changes in parameters such as hydrophobic forces, hydrogen bonding, electrostatic interactions and disulfide linkages [35]. Nanoparticles have garnered considerable academic interest over the past few decades as a promising therapeutic agent for anti-inflammatory treatments. In comparison to their bulk forms, nanoparticles show enhanced effectiveness owing to their increased surface area-to-volume ratio in reducing inflammation-inducing factors, including cytokines and enzymes that trigger inflammatory mechanisms [36]. The biosynthesized SeNPs anti-inflammatory potential was studied by Egg Albumin (EA) assay at different concentrations. The obtained results demonstrated a diminution in albumin denaturation in relation with SeNPs in concentration-dependent manner. SeNPs showed a 53% inhibition of albumin denaturation at 10 µg/mL of concentration, which is slightly lower than the 55% of inhibition exhibited by Diclofenac sodium. As the concentration escalated to 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL, SeNPs exhibited inhibition rates of 61%, 64%, 68%, and 77%, respectively. In contrast, Diclofenac sodium displayed greater inhibition percentages of 64%, 69%, 72%, and 81% at the corresponding concentrations (Fig. 8b). The experimental findings indicates that SeNPs show significant anti-inflammatory properties; however, their effectiveness seems to be moderately lesser than that of Diclofenac sodium in the egg albumin denaturation assay. To the best of our knowledge, this investigation represents the inaugural study on the anti-inflammatory potential of SeNPs derived from *M. maderaspatana* extract. The outcomes of the present study align with previously published findings on the anti-inflammatory properties of Ag@MnO2 synthesized using *Martynia annua* as reported by V. Thangapushbam et al., 2024 [36].

#### *3.4.3 Membrane Stabilization Assay*

Denaturation constitutes a biological phenomenon wherein proteins undergo a loss of their structural conformation along with their functional capabilities. This process may be expedited by an array of physical and chemical agents. The phenomenon of denaturation plays an essential role in the inflammatory response. Inflammation represents a physiological response through which the immune system of the organism reacts to a noxious stimulus. The innate immune system of the organism engages with the inflammatory process. Favourably, numerous anti-inflammatory agents mitigate the clinical manifestations associated with inflammation. The inflammatory process initiates various significant alterations, including tissue necrosis, edema, metabolic stress, and vasodilatation. These disturbances arise as a consequence of the synthesis of pro-inflammatory components such as inducible nitric oxide synthase (iNOS), Cyclooxygenase (COX-2), PG E synthase, and cytokines including TNF-α, INF-γ, IL-1α, IL-1β, IL-6, and IL-12, among others. The aforementioned components catalyze the primary inflammatory processes in various pathological conditions, namely cancer, atherosclerosis, cardiovascular diseases, arthritis, and asthma [37]. The membrane stabilization assay demonstrated the capacity of Selenium Nanoparticles (SeNPs) to reinforce cellular membranes and avert lysis. At an initial concentration of 10 µg/mL, SeNPs exhibited a 55% inhibition of membrane lysis, in comparison to a 58% inhibition observed for Diclofenac sodium. With an increase in concentration to 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL, SeNPs achieved inhibition rates of 66%, 73%,

78%, and 86%, respectively. Conversely, Diclofenac sodium exhibited marginally higher inhibition rates of 70%, 77%, 82%, and 89% at the corresponding concentrations (Fig. 8c). These findings elucidate that SeNPs are remarkably effective in stabilizing cellular membranes; however, their efficacy is slightly inferior to that of Diclofenac sodium. It is unequivocal from the present investigation that SeNPs have demonstrated anti-inflammatory properties. SeNPs derived from *M. maderaspatana* have exhibited notable anti-inflammatory capabilities and may mitigate tissue damage and injury resultant from inflammation. The findings of the current study are congruent with the anti-inflammatory potential of zinc oxide nanoparticles as reported by G. Joesna et al., 2025 [37].

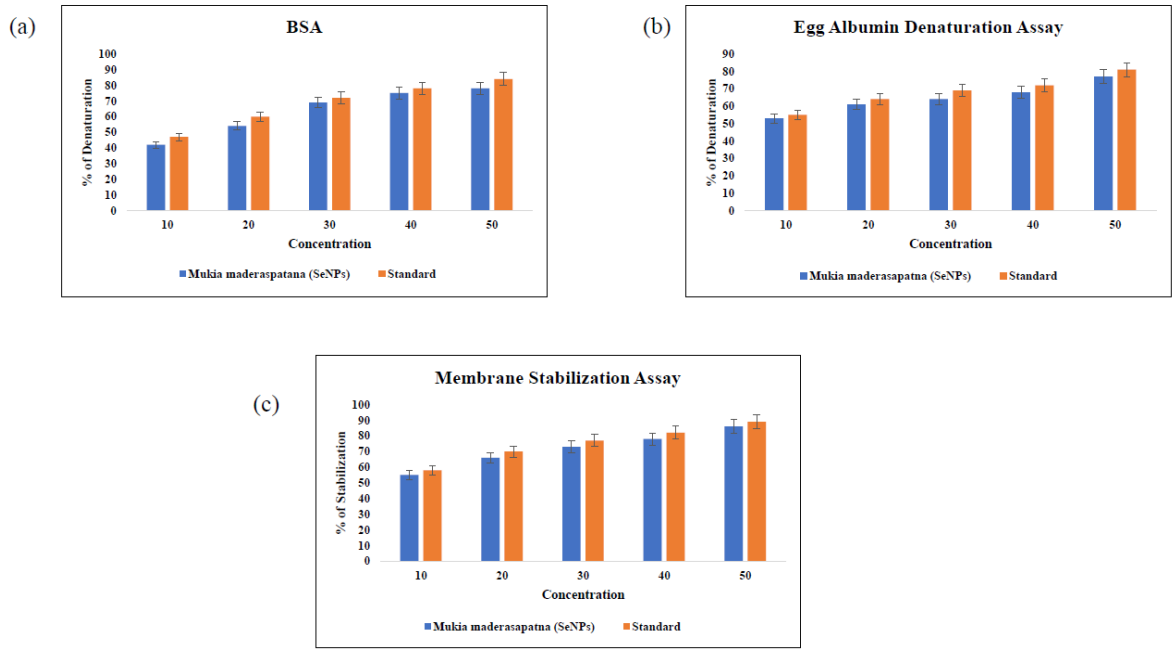


**Fig.8. *In vitro* Anti-inflammatory assays – (a) Bovine Serum Albumin (BSA) Denaturation Assay, (b) Egg Albumin Denaturation Assay, (c) Membrane Stabilization Assay**

### *3.5 Brine shrimp lethality assay*

Brine shrimp is an small crustacean which displays extraordinary adaptability to various thermal and salinity conditions, and further characterized by relatively short lifespan and enhanced reproductive capability. The assay is based on the test substances capacity to induce mortality in nauplii, signifying the larval stages of microcrustaceans [38]. Using Brine Shrimp lethality assay, the cytotoxic potential of synthesized SeNPs were analyzed, at different concentrations ranging from (5 µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, and 80 µg/mL) in an duration of 48 hrs and in relation to the control the survival rate is analyzed. The nauplii's survival rate was 100% in the initial stage of evaluation in all experimental conditions, showing the absence of instant toxic consequences from SeNPs, and the survival rate observed in the subsequent day was significantly concentration dependable. Survival rate minorly declined to 90% at the lower treatment concentration of 5 µg/mL, and a declination of survival rate to 80% is observed at the elevated concentration level to 10 µg/mL. As the concentration increases as 20 µg/mL, 40 µg/mL the survival rate gradually decreases as 70%, 60% and the 50% of survival has been observed in the maximum concentration of 80 µg/mL. Conversely, the 100% of survival rate is observed in control group throughout the experimental duration, indicating that SeNPs are the main factors for the results observed (Fig. 9). These results suggest that SeNPs did not cause immediate cytotoxicity in Brine shrimps, but prolonged exposure to SeNPs in concentration dependent manner causes significant lethality. This observation details the importance of analyzing both the concentration and exposure duration in the determination of safety profile of SeNPs for potential therapeutic applications. The current research findings are in agreement with the lethality assay performed on *Artemia salina* via utilizing monothiooxalamides reported by Maria M. Romero-Chavez (2024) [38].

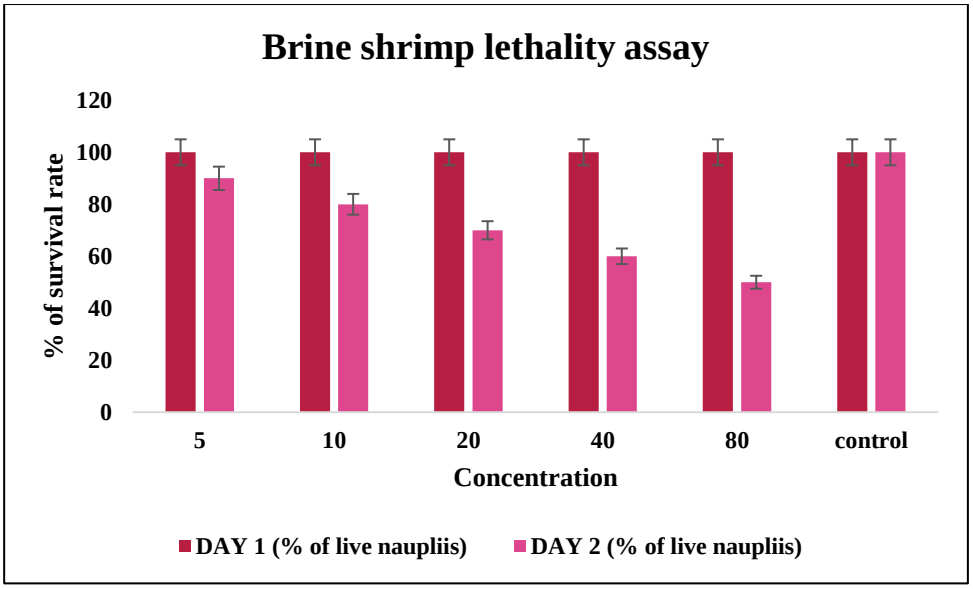


**Fig. 9. Brine shrimp lethality assay**

## 4. CONCLUSION

The current research highlights the fabrication of Selenium nanoparticle (SeNPs) using *M. maderaspatana* extract by an environmentally friendly method. In the synthesis of SeNPs the extract of *M. maderaspatana* serves as an capping and reducing agent. Characterization techniques like UV, FT-IR, SEM, EDX and XRD extensively detailed the structural and morphological properties of synthesized SeNPs. Further, SeNPs shows good antioxidant potential by scavenging the free radicals, and also possess significant anti-inflammatory and cytotoxic potential. Thus, the outcomes of the study shows that *M. maderaspatana* possess rich antioxidant potential and biological properties. To understand the mechanism of action in treating cancer the future research will be focusing on *in vitro* and *in vivo* study and further leads to the development of therapeutics.

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**Declaration of Competing Interest**

The authors declare that they have no known competing interests.

**Funding Declaration**

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**Ethics declaration:** Not applicable

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