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Synthesis and characterization of silver nanoparticles from *Chryzopogon zizanioides* root for its anti-microbial, anti-inflammatory, and anti-cancer activities

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ABSTRACT

The study aims to synthesize silver nanoparticles from aqueous extract of *Chryzopogon zizanioides* plant using Soxhlet Extraction method, and to evaluate its anti-microbial, anti-inflammatory, and anti-cancer activity potential. *Chryzopogon zizanioides* mediated silver nanoparticles exhibited strong anti-cancer, anti-inflammatory, and anti-microbial activity. The percentage of inhibition of silver nanoparticles synthesised from Vetiveria zizanioides was found to be more effective at high concentration (67.85% for 80µL, and 72.85% for 100µL). The findings indicate that *Chryzopogon zizanioides* demonstrates encouraging pharmacological properties, and the synthesized silver nanoparticles was proven to have a strong antioxidant, anti-inflammatory, anti-bacterial, and anti-cancer properties. It would be useful in promoting research aiming at the development of new effective anti-cancer activity against breast cancer. Hence, the utilization of natural harmless materials like root extracts for nanoparticles synthesis offers various potential therapeutic uses in clinical settings for drug, and biomedical applications.

Keywords: Anti-cancer activity, *Chryzopogon zizanioides*, Nanotechnology, Silver nanoparticles.

1. INTRODUCTION

Vetiver (*Vetiveria zizanioides* (L.) Nash) is a plant that has been used traditionally to treat a variety of illnesses, including rheumatism, nerve disorders, arthritis, muscle aches, insomnia, irregular menstruation, and fertility problems as well. Vetiver belongs to the Gramineae family, having several properties that make it an ideal erosion control plant in tropical regions of Asia, Africa, Oceania, and Central, and South America (Martinez et al., 2004). Vetiver's roots reach a depth of 2-4 meters (7–13 feet), almost entirely below ground, deeper than the roots of certain trees (Fig. 1). Because of this, vetiver prevents soil erosion, and water conversion, and has a great stabilizing hedge for terraces, rice fields, and stream banks. It cannot be removed since the roots are anchored well in the ground. In an effort to stop mudslides, and rockfalls, vetiver has been employed to strengthen railway cuts, and embankments in

geologically difficult areas, such as the Konkan railway in western India. Compacted soils are also penetrated, and loosened by the plant. The late King Bhumibol of Thail, promoted the use of vetiver to prevent erosion (Chomchalow, 2003). The vetiver plant also has few other uses, e.g. as forage for livestock, ornamentals, and other miscellaneous uses (Gnansounou et al., 2017). A key characteristic of the vetiver root is the high content of essential oil, known for applications in markets such as perfumery, and pharmaceuticals. By 2022, the vetiver oil market is expected to reach USD 169.5 million globally according to Gr, and View Research. On industrial scale, the distillation of essential oils gives an essential oil content of 1–3 %, which is estimated to yield 10– 30 kg of essential oil per hectare of planting area (Chandra & Rustgi, 1998).

Figure 1. Vetiveria *Chryzopogon zizanioides*

The main vetiver oil producing countries include Haiti, Indonesia, and the Reunion Island, whose yearly production is estimated to about 140 tons. It helps to reduce the visibility of skin scars, and dark patches. In West Bengal and Bihar, the root is used to cure burns, scorpion stings, and snakebites and the root is used to make a decoction that strengthens the body (Lavania, 2003). The root paste is applied topically to treat sprains, headaches, and rheumatism. The most effective remedy for urinary tract infections is its stem decoction. The roots' diaphoretic, fragrant, antifungal, stimulant, cooling, and expectorant qualities aid in the treatment of a variety of skin conditions as well as gallstones, amentia, sleeplessness, amenorrhea, renal issues, asthma, and hysteria (Gligor et al., 2019). The anthelmintic characteristics of the plant are utilized in Ayurvedic treatment in Madhya Pradesh and Maharashtra and it is among the greatest natural, treatments for sprains, arthritis, and sore muscles (Cheng et al., 2015).

Throughout India's plains, and lower hills, *Chryzopogon zizanioides* is an evergreen perennial herb that resembles lemongrass. It is especially common along riverbanks, and in rich, swampy soil (Jackson, 2020) that helps to restore soil contaminated by met als, and can prevent soil erosion (Sreenivasagan et al., 2020). The plant has historically been used in aromatherapy to treat anxiety, tension, stress, and sleeplessness (Chen et al., 2009). *Chryzopogon zizanioides* has been grown for a variety of industrial uses, one of which being the extraction of the highly prized volatile oil from its root, which is used both medicinally, and commercially (Dankovich & Gray, 2016). In the perfumery, cosmetics, soap, and food industries, vetiver oil is frequently used as an odor contributor, and flavoring agent (Zhang et al., 2016). Vetiver roots have an extremely robust, and well-organized root system. It can reach a depth of 3.4–4 m (10–13 ft) in its first year. There are no rhizomes or stolons on vetiver plants.

The vetiver plant is extremely drought-tolerant, and can aid in preventing soil erosion due to all of these qualities. Nodes that are buried can sprout new roots in the event of sediment accumulation. It enhances fertility as well. It aids in lessening the visibility of skin scars, and dark patches.In West Bengal, and Bihar, the roots are used to cure burns, scorpion stings, and snakebites. The root is used to make a decoction that strengthens the body (Rao & Suseela, 2020). The root paste is applied topically to treat sprains, headaches, and rheumatism. The most effective remedy for urinary tract infections is its stem decoction.The roots' diaphoretic, fragrant, antifungal,

stimulant, cooling, and expectorant qualities aid in the treatment of a variety of skin conditions as well as gallstones, amentia, sleeplessness, amenorrhea, renal issues, asthma, and hysteria. The anthelmintic characteristics of the plant are utilized in Madhya Pradesh, and Maharashtra. The principal ingredient in Ayurvedic treatment is vetiver. It is among the greatest natural treatments for sprains, arthritis, and sore muscles (Chou et al., 2016).

Nanotechnology has so many different uses, it has become one of the most promising technologies of our time. Nanotechnology is the study of particles ranging in size from 1 to 100 nm as well as their manufacture, and manipulation (Varadharajan et al., 2010). Nanostructures have new or improved properties that make it suitable for a wide range of applications, depending on their size, distribution, and shape. Because of its numerous uses in biosensors, optoelectronics, bionanotechnology, cosmetics, the environment, biomedicines, pharmacology, food processing, and other fields, the production of nanoparticles is exp, anding exponentially (Wang & Wang, 2014; Rajeshkumar et al., 2021; Ali et al., 2020). Silver nanoparticles are among the most promising systems for these various applications. Nanoparticles can be produced using both chemical, and physical techniques. The potential uses of metal, and semiconductor nanoparticle production in biosensing, optoelectronics, recording media, and catalysis make it a significant area of study (Ganapathy, 2020; Murthy, 2007).

Techniques like electrochemistry, thermal, laser, microwave, polyol, radiolysis, and sonochemistry are examples of chemical processes. The necessity to create an environmentally acceptable method for synthesizing nanoparticles without using hazardous chemicals is currently increasing to prevent negative impacts in medical applications (Gupta & Xie, 2018; Lee & Jun, 2019). A variety of stabilizing, and capping chemicals have been used to alter the characteristics of silver nanoparticles, for a range of purposes. When it comes to cost, biological techniques of synthesizing nanoparticles will be superior to chemical approaches and the use of hazardous substances would increase the risks of carcinogenicity, and toxicity (Syafiuddin et al., 2017; Shathviha et al., 2021). The extensive usage of substances such as stabilizers, organic solvents, and reducing agents has given rise to toxicity problems.

Nanoparticle production requires a suitable method which should be safer for both human, and environment. Biological method of synthesizing the nanoparticles may be an appealing approach because of their biocompatibility, low toxicity, and eco-friendliness (Nasim et al., 2020). Silver nanoparticles (Ag NPs) has shown to be a viable antibacterial agent due to the high surface-to-volume ratio, which has increased interaction with microbes and has less side effects than drugs by reducing damage to healthy cells. The size-dependent physicochemical properties of nanoparticles promote their application in many products but the same unique properties also can lead to physiological responses in living systems by interaction with these materials (Peng et al., 2014; David et al., 2019). Since ancient times, people have utilized silver to cure, and prevent a wide range of bacterial diseases, including infections. This is because silver compounds have long been recognized for their antibacterial properties and have been shown to exhibit antifungal, anti-inflammatory, antiviral, antiangiogenesis, and antiplatelet activity (Luqman et al., 2009; Raj, 2020; Shifa et al., 2020).

1.1. Antioxidant Effect

Oxidative stress is a major component in the pathophysiology of many chronic diseases (Shunmugam et al., 2021). It is a condition that occurs when the balance between a cell's antioxidative defence and oxidants gets disturbed by the presence of excessive free radicals. Reactive oxygen species, including free radicals, are the significant factors that have a role in the aging process (Jiao et al., 2012; Morsy et al., 2021; Morsy, 2022) and as a result of the mitochondria's ATP production, free radicals are generated. In biological systems, antioxidants fundamentally inhibit the propagation of free radicals (Mohapatra et al., 2020; Kim et al., 2005). The antioxidant capacity can be measured in medicinal plants or other materials for characterisation of the trait (Moon et al., 2020; Aarthi & Murugan, 2012; Ali et al., 2021). It is unknown at this time if the silver nanoparticles synthesized from the root extract of the Chrysopogon zizanioides plant have antioxidant properties.

1.2. Anti-cancer effect

Cancer is the largest cause of death, accounting for more than one-third of the global population. With almost 20% of deaths worldwide attributed to it, it is the primary cause of death. Cancer is a major public health concern in both industrialized, and developing nations. This kind of cancer results in death because the body's cells grow improperly (Kannappan et al., 2017). Cancer cells typically infiltrate, and kill normal cells. The World Health Organization (WHO) reports that about 10 million new instances of cancer are examined annually, and statistical trends indicate that this number may treble in the coming decades. Breast cancer is a condition where malformed breast cells proliferate, and develop into tumors. Tumors have the potential to grow throughout the body, and become lethal if ignored. The milk ducts, and/or the breast's milk-producing lobules are where breast cancer cells first proliferate. Early

detection is possible for the first form, known as in situ, which is not life-threatening. Cancer cells have the ability to invade the neighboring breast tissue and tumors produced due to it can results in thickening or lumps formation in breasts (Paillat et al., 2012).

Metastasis is the process by which invasive tumors move to neighboring lymph nodes or other organs. Metastasis can be lethal, and perhaps fatal. 2.3 million women worldwide had a breast cancer diagnosis in 2022, and 670,000 people died from the disease. All across the world, breast cancer affects women at any age after adolescence, however its prevalence rises with age. Global estimations show stark differences in the incidence of breast cancer based on Human Development Index (HDI). For example, in nations with a very high HDI, 1 in 12 women may receive a breast cancer diagnosis during their lifetime, and 1 in 71 will pass away from the disease (Ai et al., 2022; David et al., 2023). The significant use of *Chrysopogon zizanioides* to treat human diseases, is known well from the ancient times. The root extracts of vetiver are projected to be widely employed in traditional therapy as 80–85% of the world's population and is estimated to rely on traditional medicines for their primary health care needs. *Chryzopogon zizanioides* have a broad spectrum of antioxidant, and immuno-modulatory properties in addition to anti-cancer properties. These compounds promote both specific, and non-specific immunity. It strengthens the host's defenses against infection, and restoring physiological homeostasis. The components that can prevent the progression of cancer are being found by utilizing the anti-cancer qualities of vetiver (Tyagi et al., 2024).

1.3. Anti-inflammatory Effect

Plants continue to be an important source of medicinal chemicals and traditionally used in drugs industries directly or indirectly for the products produced from it (Kumar et al., 2023). The benefits of medicinal plant-based medications are their simplicity, efficacy, and broad spectrum of action compared to the synthetic ones (Saikia et al., 2012). Many powerful anti-inflammatory medications have been found as a result of the screening of natural products. This makes looking for additional options appear important, and helpful. medicinal plants with a diverse range of compounds that may yield new anti-inflammatory drugs.

In India, Vetiveria zizanioides is referred to as Khus grass or Khas Khas. The plant's root decoction has been used as an antioxidant, anthelmintic, and analgesic for rheumatism since ancient times. It is used to cure ailments like mouth ulcers, acid reflux, headaches, toothaches, sprains, urinary tract infections, malaria, and a variety of bacterial, and fungal illness. The current study shows the use of vetiver root's for its anti-inflammatory, anti-bacterial, and anti-cancer activities to support the folklore claim (Seshadri et al., 2023).

Scope of study

Vetiver has been traditionally used to cure a wide range of ailments, including rheumatism, nerve problems, arthritis, muscular pains, insomnia, and painless, regular menstruation. The scope of the study includes

➢ Extraction, and analysis of plant, and vetiver root extracts.

➢ To perform quantitative, and qualitative analysis to determine the total phenol, and flavonoid content from plant extract, and the synthesized nanoparticles

➢ To study the anti-microbial activity, anti-inflammatory activity, and anti-cancer activity of plant, and synthesized nanoparticles from vetiver root.

2. MATERIALS AND METHODS

2.1. Collection of plants

Chryzopogon zizanioides roots (Fig.2) are purchased from villupuram district, and the analysis work are performed in the Bionyme Laboratory, Chromepet, Chennai, Tamil Nadu.

2.2. Organoleptic evaluation

Organoleptic evaluation done for the identified plants according to the color, size, odor, taste parameters, and texture of *chryzopogon zizanioides* were performed, and reported in Table 1.

2.3. Soxhlet Extraction

The soxhlet extractor, a Round-bottom, extraction chamber, syphon tube, and condenser at the top make up this apparatus. 80g of coarsely powdered vetiver root was soaked with 1 litre of acetone for 24 hours. After the solvent gets heated from the bottom flask, it evaporates, and travels through the condenser, condenses, and flows downward to the extraction chamber, where it interacts with the medication to extract it. As a result the solvent, and the extracted plant material flow back to the flask when the amount of solvent in

the extraction chamber reaches the top of the syphon up until the moment at which a solvent flowing from the extraction chamber leaves no trace behind, the entire procedure is repeated until the plant is completely extracted (Fig.3). However, thermolabile plant materials cannot be processed using this method.

Figure 2. Vetiver Sample for Analysis

Table 1. Organoleptic Evaluation

Root of Plant	Color	Odor	Taste	Texture
Chryzopogon zizanioides	Brown	Earthy	Woody	Fibrous & Stiff

Figure 3. Extraction of Vetiver root using Soxhlet apparatus

2.4. Qualitative analysis (Phytochemical study)

2.4.1. Test for alkaloids

Dragendroff's test: 1ml of extract was taken, and placed into a test tube. Then 1ml of potassium bismuth iodide solution (Dragendroff's reagent) was added, and shaken. An orange -red precipitate formed indicates the presence of alkaloids.

Test for carbohydrate: Take 1ml of sample in a clean, dry test tube. The concentration of the test samples should be 5% (w/v). Take control of 1ml of distilled water in another tube. Add 2-3 drops of Fehlings reagent to both the tubes, and mix them in a vortex. Keep the test tubes in a water bath for 1-2 minutes. Observe the colour for red colour indicates the presence of sugar.

Test for Glycosides: To 2ml of extract, 3ml of chloroform, and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

Test for Saponins: To 2ml of extract, 2ml of distilled water is added, and shaken in a graduated cylinder for 15 min. It results in the formation of 1cm layer of foam that shows the presence of saponins.

Test for Proteins: Biuret test: 5mg extract was added with few drops of Biuret reagent. The obtained mixture was shaken well, and allowed to warm for 1to 5 min. appearance of red or violet colour indicated presence of proteins.

Test for amino acids: Ninhydrin test: To 1ml of extract few drops of Ninhydrin reagent was added, and heated in a boiling water bath. A purple blue colour indicates the presence of proteins.

Test for phenol: Ferric chloride test: A small amount of the acetone extract was taken with 1ml of water in a test tube, and 1to 2 drops of Ferric chloride was added. A blue, green, red or purple color is a positive test.

Test for triterpenoids: Horizon test: Two mL of trichloroacetic acid was added to 1ml of extract. The presence of terpenoids was confirmed by the formation of a red precipitate.

2.5. Quantitative analysis

2.5.1. Determination of total phenol

2ml of extract made upto 3ml of distilled water. Then 1ml of Folin-Ciocalteu's phenol was added to the tube. The tubes were kept in incubation for 3 minutes. After 3min, 2ml of 20% sodium carbonate was added to the tubes, and kept for incubation after which, its absorbance measured at 620 nm. The total phenol content in the sample was calculated using this formula

C (GAE) = C*V/M

where,

C= concentration of sample from the curve obtained (mg/ml).

V=volume used during the assay (ml), and

M= mass of the sample used during the assay (g)

The absorbance reading of the sample as Y into the equation to obtain a value, let it be z in mg GAE/ml. Using the equation, calculate total phenolic content of the sample in mg GAE/g. C = z mg GAE/ml * vol. of chemical used in assay (ml)* 1/mass of sample or crude extract.

2.5.2. Determination of total flavonoids

2 ml of extract made upto 3 ml with distilled water. Then 1 ml of 5% sodium nitrate is added. The solution was vortexed, and allowed to st, and at room temperature for 5 minutes, and add 1ml of aluminium chloride. After 6 minutes, 2ml of 1M sodium hydroxide was added to the test tube. The solution was made up to 10ml with distilled water. The absorbance is read at 620 nm. The total flavonoid content was calculated as quercetin equation (mgQE/g) using the formula.

X= (A.M0/A0M)

Where,

A = absorption of sample

A0 = absorption of st, andard (quercetin)

M = weight of sample (mg/ml)

M0 =weight of quercetin in solution (mg/ml)

2.6. Synthesis of Silver nanoparticles

In the present study, 30 mg of plant extract was dissolved in 30 ml of acetone to make a stock solution. 100 mL of 1 mM sliver nitrate was prepared in 250 mL of a conical flask. The sample (vetiver extract) concentration (2 ml, 4 ml, 6 ml, 8 ml, 10 ml) is made up to 10 ml with distilled water. Add 5 ml of silver nitrate solution to all the tubes. The tubes are incubated for 24 to 48 hours, and observed for the color changes, Fig.4a &Fig.4b.

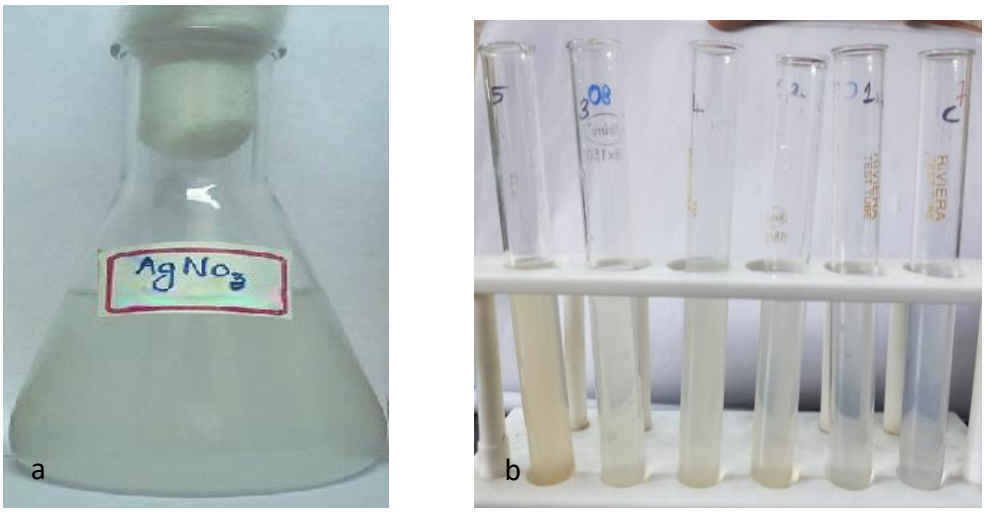

Figure 4. a. Plant extract for the Synthesis of sliver nanoparticles; **4b.** Test tubes incubated for the Synthesis of sliver nanoparticles

2.7. Antioxidant assay

2.7.1. DPPH radical scavenging activity assay

The free radical scavenging activity of the fractions is measured in vitro by 2,2-diphenylpicrylhydrazyl (DPPH) assay according to the method described earlier. The stock solution is prepared by dissolving 24 mg of the DPPH with 100 ml acetone, and stored at 20°C until required. The working solution is prepared by diluting the DPPHI solution with acetone to attain an absorbance of about 0.98+0.02 at 540 nm using the spectrophotometer. A 3 ml aliquot of this solution is mixed with 100µl of the sample at various concentrations (10-500 µg/ml). The reaction mixture is shaken well, and incubated in the dark for 15 minutes at room temperature. Then the absorbance is read at 540 nm. The control is prepared as above without any sample. The scavenging activity is estimated based on the percentage of DPPH radical scavenged as follows

Scavenging effect % = {[control absorbance-sample absorbance]/control absorbance}*100

2.7.2. Anti-inflammatory assay

The anti-inflammatory effect in vitro is estimated using the HRBC method. Using a sterile Alsevers solution in an identical volume, blood is drawn from healthy participants. Following the separation of the packed cells, this blood sample is centrifuged at 3,000 rpm. After making a 10% v/v suspension with isosaline, the packed cells were cleaned using isosaline solution.The anti-inflammatory property of the HRBC suspension is estimated using 1ml of phosphate buffer, 2ml of hyposaline, and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37°C for 30 minutes, and centrifuged at 3000 rpm. The supernatant liquid is decanted, and the hemoglobin content is estimated by spectrophotometer at 560 nm. The percentage hemolysis is estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100-(OD sample/OD control) *100

2.7.3. Anti-bacterial activity

Agar Well Diffusion Method: Muller-Hinton agar plates were used for the well diffusion method. Petri plates were made by adding 50 ml of Muller- Hinton agar, and allowing them to harden before being used in bacterial susceptibility testing. After the plates had dried,

0.1 mL of the inoculum is added, distributed, and left to dry. Following the cork borer, an agar well was created on an agar plate, extract, and synthesized nanparticles sample concentrations were added to the well (100 µl). The negative or blind control, while the positive control contained streptomycin. The gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, and gram positive bacteria (*Staphylococcus aureus*). The plates were incubated for twenty-four hours at 37°C. The zone of inhibition is assessed, and quantified (Rao & Suseela, 2000).

2.7.4. Anti-cancer activity

MCF-7 cells were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells are maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin. They were maintained at 37°C with 5% CO_2 in 95% air humidified incubator. The Cytotoxicity effect of the sample is tested against MCF-7 cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mossman, 1983). The cells were seeded in 96-well micro plates (1 x 106 cells/well), and incubated at 37°C for 48 h in 5% CO_2 incubator, and allowed to grow 70-80% confluence. Then the medium is replaced, and the cells are treated with different concentrations (100, 120, 140, 160, 180, 200 µg/mL) of sample, and incubated for 24 hrs. The morphological changes of untreated (control), and the treated cells were observed under inverted microscope (20Xmagnification) after 24 h, and photographed. The cells are then washed with phosphate-buffer saline (PBS, pH-7.4), and 20 µL of (MTT) solution (5 mg/mL in PBS) was added to each well. The plates were then incubated at 37ºC in the dark for 2 hrs. The formazan crystals were dissolved in 100 µL DMSO, and the absorbance was read spectrometrically at 570nm. Percentage of cell viability is calculated using the formula,

Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100.

3. RESULTS & DISCUSSION

3.1. Phytochemical analysis

The presence of phytochemical compounds, such as vitamins, phenolics, flavonoids, alkaloids, protein, saponin, triterpenoids, glycosides, carbohydrates, and other metabolites with strong antioxidant activity, are abundant in plants (Tiwari & Sarangi et al., 2017). These phytocompounds have been shown to have antiinflammatory, anti-cancer, and anti-microbial properties (Table 2). Secondary plant metabolites, or phytochemicals, have received a lot of attention lately as a potential source of therapeutic drugs. The screening analysis is performed in order to identify those secondary metabolites which is present in *Chryzopogon zizanioides* in both plant extract, and nanoparticles suspension (Fig 5a & Fig.5b).

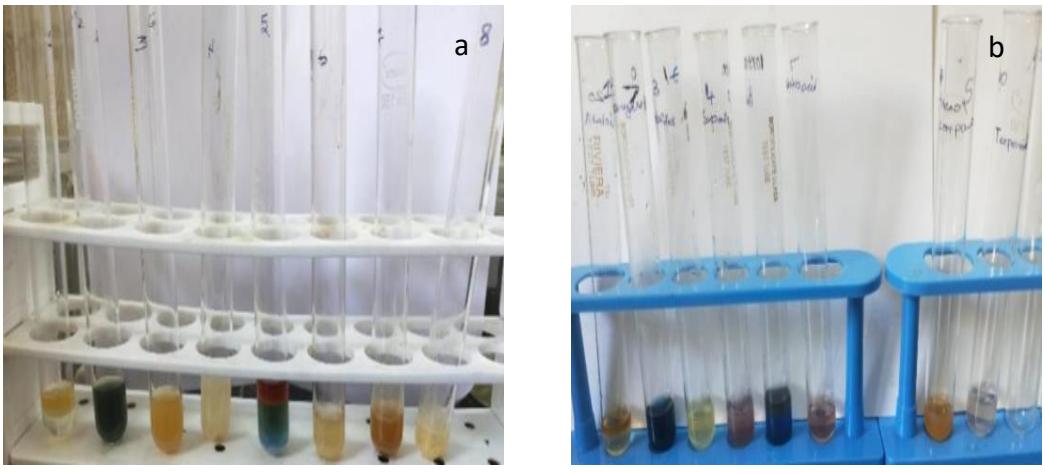

Figure 5. a) phytochemicals analysis of plant extract; **b)** Phytochemicals analysis of synthesized nanoparticles

All the assay mixtures were incubated at 370c for 30 minutes, and centrifuged at 3000 rpm. The supernatant liquid was decanted, and the hemoglobin content was estimated by spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the hemolysis produced in the control as 100%.

Percentage protection= 100-(OD sample/OD control) *100

Table 2. Phtochemical Analysis of plant extract, and synthesized nanoparticles from vetiver

phytochemicals	Plant extract (+, -)	Nanoparticles (+, -)
Alkaloids	+	+
Carbohydrates	-	-
Glycosides	-	-
Saponin	+	+
Protein	+	+
Amino acids	-	-
Phenol	-	-
Triterpenoids	-	-

3.2. Quantitative analysis of Total phenol and Flavonoid

The total phenolic content present in both plant extracts, and nanoparticles has been quantified based on the procedure mentioned in the methodology. The observations are recorded in the following Table 3.

Table 3. Quantitative analysis of total phenol and flavonoid

Phytocomponents	Plant extract	Nanoparticles
Phenol	13mg GAE/g	10.7mg GAE/g
Flavonoids	13.9mg QE/g	10.5mg QE/g

3.3. Antioxidant assay

3.3.1. DPPH antioxidant activity

The DPPH assay performed in both plant extracts, and nanoparticles has been quantified based on the procedure mentioned in the methodology. The observation recorded in the following table, 4, and Fig.6. The percentage of inhibition of silver nanoparticles synthesised from *Chryzopogon zizanioides*. was 35.71% for 20µL, 42.85% for 40µL, 60.75% for 60µL, 67.85% for 80µL, and 72.85% for 100µL. The percentage of inhibition of the standard was 10.10% for 20µL, 26.0% for 40µL, 56.3% for 60µL, 70.7% for 80µL, and 92.5% for 100µL. Hence maximum inhibition was observed at 100µL that is at higher concentration. The silver nanoparticles synthesised from *Chryzopogon zizanioides*, shows good antioxidant activity, and are comparable to the standard plant extract.

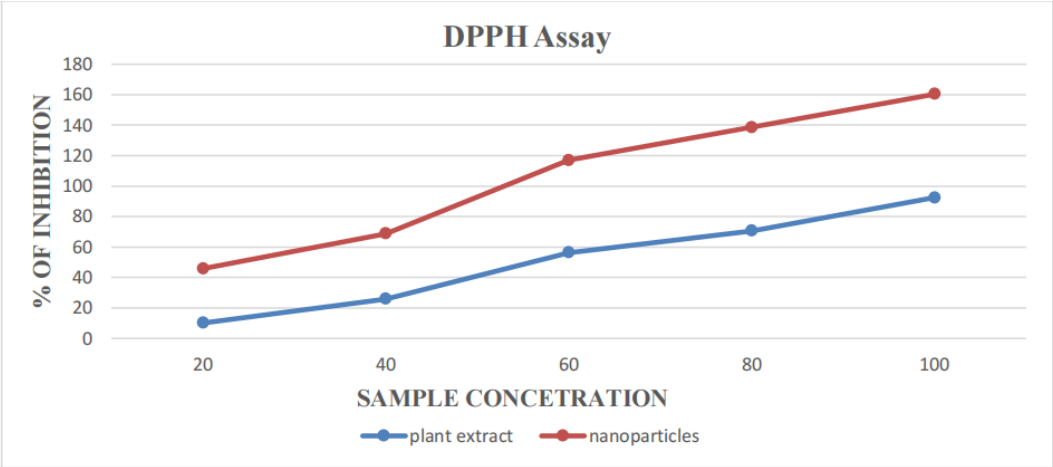

Figure 6. Antioxidant activity of plant and synthesized nanoparticles

Table 4. Antioxidant assay

Sample concentration	plant extract OD value	% of inhibition	Nanoparticle OD value	% of inhibition
20	1.69	10.10	0.18	35.71

40	1.39	26.0	0.16	42.85
60	0.82	56.3	0.11	60.71
80	0.55	70.7	0.09	67.85
100	0.14	92.5	0.09	67.85

3.3.2. Anti-inflammatory Assay

The HRBC assay performed in both plant extracts, and nanoparticles has been quantified based on the procedure mentioned in the methodology. The observation is recorded in following table 5, and Fig.7. The results shows that the maximum % of inhibition of silver nanoparticles at 100 µg/mL.

Table 5. Anti-inflammatory assay

Sample concentration µg/mL	Plant extract OD value	% of inhibition	Nanoparticle OD value	% of inhibition
20	1.69	8.15	0.45	32.83
40	1.69	8.15	0.42	37.83
60	1.39	24.45	0.39	41.79
80	1.00	45.65	0.35	47.76
100	0.50	72.82	0.29	56.71

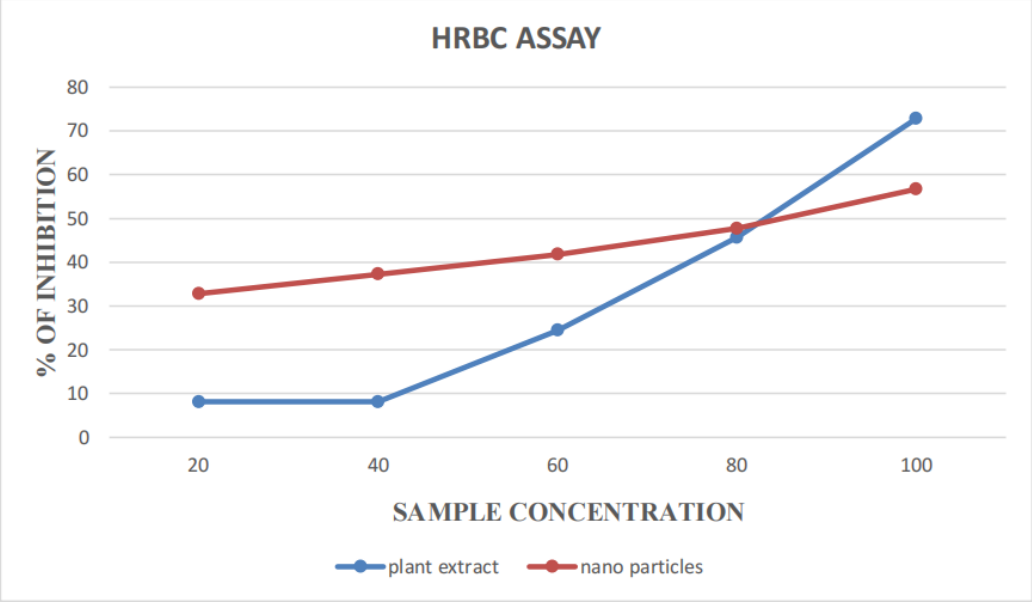

Figure 7. Anti-inflammatory Assay of plant, and synthesized nanoparticles

3.3.3. Anti-bacterial and Anti-cancer activity

In this study, silver nanoparticles synthesized from zizanioides root extracts were tested for its antioxidant, and antibacterial activities. The bacteriostatic, and bactericidal activity of silver nanoparticles against the gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, and gram positive bacteria *Staphylococcus aureus* is determined using bacterial growth inhibition method. The results confirmed that the silver nanoparticles have more antioxidant activity as compared to vitamin C. Antioxidant, and antibacterial activity of silver

nanoparticles is due to the presence of bioactive molecules on the surface (Xu et al., 2009; Ketaubon et al., 2024; Champagnat et al., 2008). The anti bacterial activity, and anti-cancer activity results were shown in Fig.8.

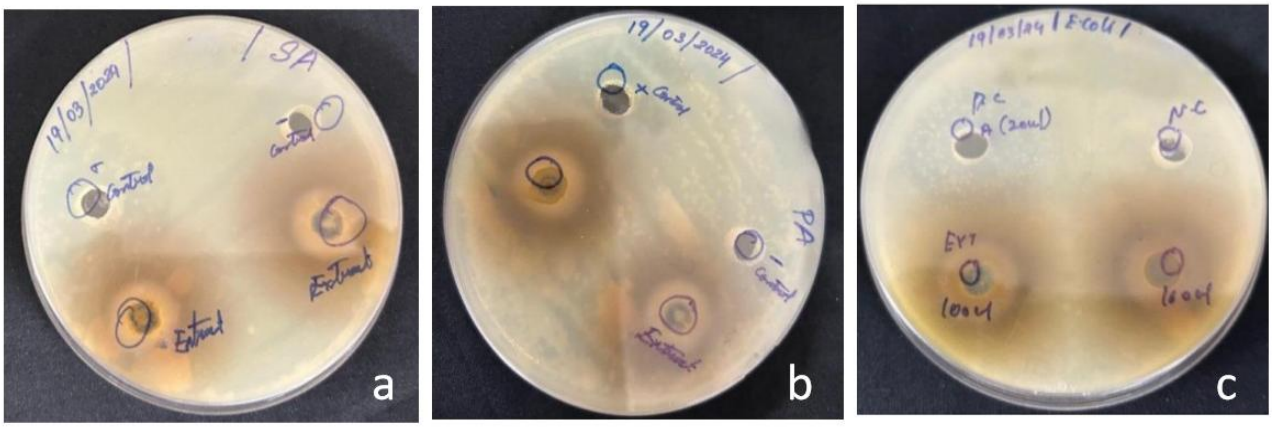

Figure 8. Antimicrobial activity of plant, and synthesized nanoparticles against *E.Coli* (8a), *Staphylococcus* (8b), and *Pseudomonas* (8c).

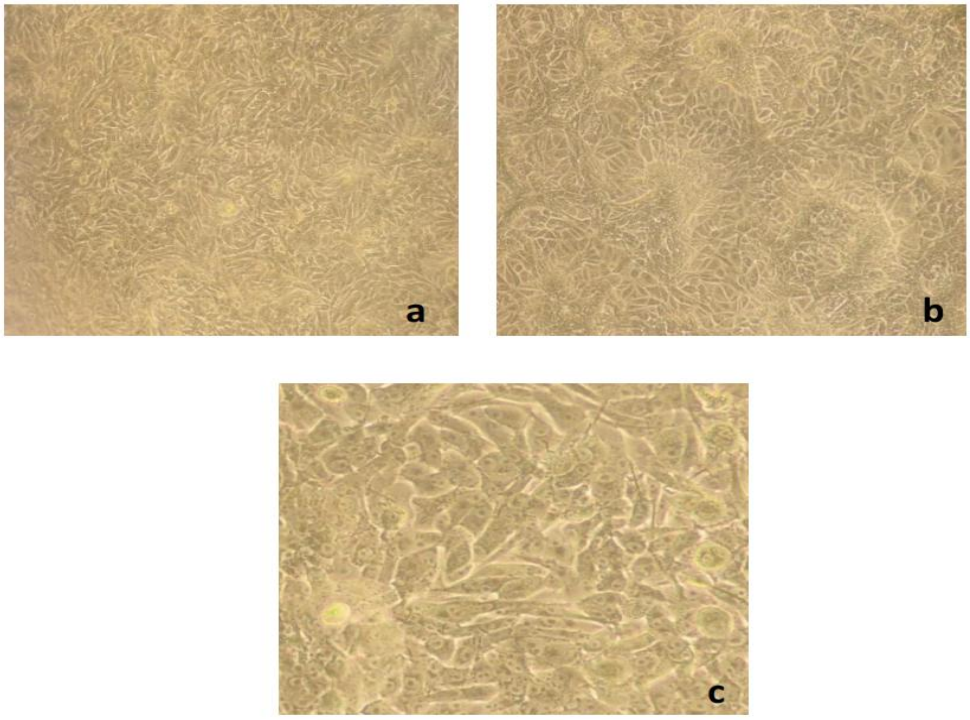

Figure 9. Anti-cancer activity: Control cells of MCF-7 (9a); Cells treated with nanoparticles at 100 µg/ml (9b); Cells treated with nanoparticles at 200 µg/ml (9c).

Table 6. Anti-cancer activity

Conc. µg/mL	% of cell death
100	11.63
120	32.82
140	46.12

160	54.11
180	68.03
200	77.82

Human breast cancer cells were used to test the anticancer activity of silver nanoparticles synthesised from *Chryzopogon zizanioides* root extracts. The cytotoxic reaction was found to be concentration dependent. According to the results, there was also a substantial rise in ROS, and lipid peroxidation as well as a decline in MMP, and glutathione levels. Biosynthesised silver nanoparticles caused cell death in the cells, implying that silver nanoparticles have anticancer capacity, Table 6, and Fig 9.

4. CONCLUSION

Chryzopogon zizanioides biosynthesis produces silver nanoparticles with strong antioxidant, anti-inflammatory, antibacterial, and anticancer properties. It is clear that silver nanoparticles are effective anti cancer activity against breast cancer. Given its strong performance in antioxidant, anti-inflammatory, antibacterial, and anticancer properties, more research on silver nanoparticles, and their potential therapeutic uses in clinical settings may be conducted in future. The study's limitation was that it was conducted in vitro, so it cannot be assumed that the results of antioxidant activity could be translated into clinical effectiveness.

List of Abbreviations

ATP : Adenosine tri phosphate
DPPH : 2.2-diphenylpicrylhydrazyl
mgQE/g : Quercetin equivalents per gram
GAE/g : Gallic acid equivalents per gram
HDI : Human Development Index
WHO : World Health Organization
Ag NPs : Silver nanoparticles
HRBC : Human Red Blood Cell
MMP : Matrix Met alloproteinase
MCF-7 : Michigan Cancer Foundation-7

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Author Contributions:

Study conception, and design: D.S.Pushparani
Data collection: Keerthana.M
Data analysis, and interpretation of results: Keerthana.M, D.S.Pushparani
Draft manuscript: D.S.Pushparani.
All authors reviewed the results, and approved the final version of the manuscript.

Ethical Approval

The study was approved by the Institutional Ethics Committee of DG Vaishnav College, Arumbakkam. As per the plant regulations followed in the Department of Biochemistry, Dwaraka Doss Goverdhan Doss Vaishnav College, Arumbakkam, Chennai-600106, Tamil Nadu, India.; the authors observed the anti-microbial, anti-inflammatory, and anti-cancer activities of *Chryzopogon zizanioides* root. The ethical guidelines for plants & plant materials are followed in the study for observation, identification & experimentation. Also, MCF-7

cells were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. The ethical guidelines for cell-lines are followed in the study for experimentation.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

Data that support the findings of this study are embedded within the manuscript.

REFERENCES

1. Aarthi N, Murugan K. Effect of *Vetiveria zizanioides* L. root extracts on the malarial vector *Anopheles stephensi*. Asian Pac J Trop Dis. 2012;2:154–158.
2. Ai Y, Wang Y, Zhou J. Effects of biochar on the physiology and heavy met al enrichment of *Vetiveria zizanioides* in contaminated soil in mining areas. J Hazard Mater. 2023.
3. Ali SJ, Arthanari A, Rajeshkumar S. Antioxidant activity of silver nanoparticles synthesized using *Vetiveria zizanioides*: an in vitro study. J Res Med Dent Sci. 2021;9(10):199–203.
4. Ali SJ, Preetha S, Jeevitha M, Prathap L, Rajeshkumar S. Antifungal activity of selenium nanoparticles extracted from *Capparis decidua* fruit against *Candida albicans*. J Evol Med Dent Sci. 2020;9:2452–2456.
5. Champagnat P, Heitz A, Carnat A, Fraisse D, Carnat AP, Lamaison JL. Flavonoids from *Vetiveria zizanioides* and *Vetiveria nigritana* (Poaceae). Biochem Syst Ecol. 2008;36:68–70.
6. Chandra R, Rustgi R. Biodegradable polymers. Prog Polym Sci. 1998;23(7):1273–1335.
7. Chen D, Qiao X, Qiu X, Chen J. Synthesis and electrical properties of uniform silver nanoparticles for electronic applications. J Mater Sci. 2009;44:1076–1081.
8. Cheng X, Su J, Zhou X. Advances in enzyme-assisted extraction of natural products. In: Proc 3rd Int Conf Mater Mech Manuf Eng (IC3ME); 2015.
9. Chomchalow N. Other uses and utilization of vetiver. In: Proc 3rd Int Conf Vetiver; 2003; Guangzhou, China.
10. Chou ST, Shih Y, Lin CC. Vetiver grass (*Vetiveria zizanioides*) oils. In: Preedy VR, editor. Essential oils in food preservation, flavor and safety. San Diego: Academic Press; 2016. p. 843–848.
11. Dankovich TA, Gray DG. Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. Environ Sci Technol. 2011;45:1992–1998.
12. David A, Fărcaș A, Socaci SA. An overview of the chemical composition and bioactivities of *Vetiveria zizanioides* essential oil. Trends Food Sci Technol. 2023;140:104153.
13. David A, Wang F, Sun X, Li X, Chen H. Chemical composition, antioxidant and antimicrobial activities of *Vetiveria zizanioides* essential oil extracted by carbon dioxide expanded ethanol. Molecules. 2019;24:1897.
14. Ganapathy D. Nanobiotechnology in combating COVID-19. Bioinformation. 2020;16:828–830.
15. Gligor O, Mocan A, Moldovan C, Locatelli M, Crișan G, Ferreira ICFR. Enzyme-assisted extractions of polyphenols: a comprehensive review. Trends Food Sci Technol. 2019;88.
16. Gnansounou E, Alves CM, Raman JK. Multiple applications of vetiver grass: a review. Int J Environ Sci. 2017;2:125–141.
17. Gupta R, Xie H. Nanoparticles in daily life: applications, toxicity and regulations. J Environ Pathol Toxicol Oncol. 2018;37:209–230.
18. Jackson K. Cytotoxic potentials of silibinin-assisted silver nanoparticles on human colorectal HT-29 cancer cells. Bioinformation. 2020;16:817–827.
19. Jiao J, Zhang X, Li B. Enzyme-assisted microwave hydrodistillation of essential oil from *Fructus forsythia*: chemical constituents and antimicrobial and antioxidant activities. Food Chem. 2012;134:235–243.
20. Kannappan A, Gowrishankar S, Srinivasan R, Pandian SK, Ravi AV. Antibiofilm activity of *Vetiveria zizanioides* root

extract against methicillin-resistant *Staphylococcus aureus*. Microb Pathog. 2017;110:313–324.
21. Ketaubon P, Ritthikasem N, Tanheng P, Prapagdee B. Enhancing heavy metal phytoremediation in landfill soil by *Chrysopogon zizanioides* through bacterial-biochar pellets. Environ Technol Innov. 2024;35:103738.
22. Kim HJ, Chen F, Wang X, Rajapakse NC. Evaluation of antioxidant activity of vetiver oil and identification of its antioxidant constituents. J Agric Food Chem. 2005;53:7691–7695.
23. Kumar P, Singh VP, Tagade A, Sawarkar AN. Thermochemical characterization of post-phytoremediated vetiver for bioenergy potential. Ind Crops Prod. 2023;191:115964.
24. Lavania U. Vetiver oil. In: Proc 3rd Int Vetiver Conf; 2003; Guangzhou, China.
25. Lee SH, Jun BH. Silver nanoparticles: synthesis and application for nanomedicine. Int J Mol Sci. 2019;20:865.
26. Luqman S, Kumar R, Kaushik S, Srivastava S. Antioxidant potential of the root of *Vetiveria zizanioides*. Indian J Biochem Biophys. 2009;46:122–125.
27. Martinez J, Rosa PT, Menut C, Leydet A, Brat P, Pallet D, Meireles MAA. Valorization of Brazilian vetiver oil. J Agric Food Chem. 2004;52(21).
28. Mohapatra S, Leelavathi L, Rajeshkumar S, Lakshmi T. Assessment of cytotoxicity, anti-inflammatory and antioxidant activity of zinc oxide nanoparticles. J Evol Med Dent Sci. 2020;9:1859.
29. Moon UR, Durge AA, Kumar M. Value addition of vetiver root extracts by elicitor and heat treatment. Ind Crops Prod. 2020;144:112037.
30. Morsy NF. Essential oil as green preservative obtained by ecofriendly extraction techniques. In: Essential oils: advances in extraction and biological applications. 2022. p. 47.
31. Morsy NF, Hammad KS. Extraction of essential oil from *Ocimum canum* using enzyme pretreatment. J Food Sci Technol. 2021;58.
32. Mosmann T. Rapid colorimetric assay for cellular growth and survival. J Immunol Methods. 1983;65:55–63.
33. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine. 2007;2:129–141.
34. Nasim I, Kumar SR, Vishnupriya V. Cytotoxicity and antimicrobial analysis of silver and graphene oxide nanoparticles. Bioinformation. 2020;16:831–836.
35. Paillat L, Périchet C, Pierrat JP, Lavoine S, Filippi JJ, Meierhenrich U, Fernandez X. Purification of vetiver alcohols and esters. J Chromatogr A. 2012;1241:103–111.
36. Peng HY, Lai CC, Lin CC, Chen YS. Effect of vetiver oil on melanogenesis in melanoma cells. Sci World J. 2014;213013.
37. Raj RK. β-sitosterol-assisted silver nanoparticles activate Nrf2 and induce apoptosis. J Biomed Mater Res. 2020;108:1899–1908.
38. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Gold nanoparticles preparation using fucoidan. Mater Today Commun. 2021;26:101975.
39. Rao RR, Suseela MR. *Vetiveria zizanioides*: a multipurpose eco-friendly grass of India. Proc 2nd Int Vetiver Conf; 2000; Thailand.
40. Saikia D, Parveen S, Gupta VK, Luqman S. Anti-tuberculosis activity of *Vetiveria zizanioides*. Complement Ther Med. 2012;20:434–436.
41. Seshadri VD, Vijayaraghavan P, Kim YO, Kim HJ, Al-Ghamdi AA. Antioxidant and cytotoxic activities of polyherbal extracts. Saudi J Biol Sci. 2020;27:1475–1481.
42. Shathviha PC, Ezhilarasan D, Rajeshkumar S. β-sitosterol-mediated silver nanoparticles induce cytotoxicity. Avicenna J Med Biotechnol. 2021;13:42–46.
43. Shifa J, Gayathri R, Priya VV. Preliminary phytochemical analysis of *Sambucus nigra*. Drug Invent Today. 2020;13.
44. Shunmugam R, Renukadevi Balusamy S, Kumar V, Prabu HG, Rajeshkumar S. Biosynthesis of gold nanoparticles using *Vibrio alginolyticus*. J King Saud Univ Sci. 2021;33:101260.
45. Sreenivasagan S, Subramanian AK, Rajeshkumar S. Antimicrobial activity and cytotoxic effect of silver nanoparticles. Ann Phytomed. 2020;9:207–212.
46. Syafiuddin A, Salmiati, Salim MR. A review of silver nanoparticles. J Chin Chem Soc. 2017;64:732–756.
47. Tiwari S, Sarangi BK. Comparative antioxidant response of vetiver. Ecol Eng. 2017;100:211–218.
48. Tyagi U, Anand N, Jain AK. Pyrolysis of vetiver in a fixed-bed reactor. Bioresour Technol Rep. 2022;18:101046.
49. Varadharajan S, Kuppusamy A, Muthuswamy U. In vitro antioxidant activity of vetiver root extract. Tanzan J Health Res. 2010;12:265–271.
50. Wang EC, Wang AZ. Nanoparticles and their applications in cell and molecular biology. Integr Biol. 2014;6:9–26.
51. Xu W, Li W, He J, Singh B, Xiong Z. Effects of Zn and Cd on vetiver growth and uptake. J Environ Sci. 2009;21:186–192.
52. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, properties and applications. Int J Mol Sci. 2016;17:1534.