Journal of Research in Medical and Dental Science
2021, Volume 9, Issue 11, Page No: 242-246

Available Online at: www.jrmds.in
eISSN No.2347-2367: pISSN No.2347-2545

Preparation and Free Radical Scavenging Activity of Centella Asiatica Mediated Silver Nanoparticles

Obuli Ganesh Kishore S[1], Abirami Arthanari[1*], Rajeshkumar S[2]

[1]*Department of Forensic Odontology, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, India*

[2]*Department of Pharmacology, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, India*

ABSTRACT

Introduction: Centella asiatica commonly known as vallarai is a herbal plant. It is a plant with skin disease healing properties. Numerous preparations of this plant in various pharmaceutical forms recommended for several indications including neurological disorders are available all over the world. Free radical scavenging activity is the ability to delay ageing which can be simply termed as antioxidant property.

Aim: The objective of this study is to assess the free radical scavenging activity of Centella asiatica mediated silver nanoparticles.

Materials and methods: The plant extract was first prepared and the silver nanoparticles were added and mixed well. Silver nitrate was mixed with 50ml of water. 50ml vallarai extract was mixed with 50ml of the silver nanoparticle. This extract with properly synthesized nanoparticles was used to assess the antioxidant activity of Centella asiatica mediated silver nanoparticles. DPPH assay was performed to assess the free radical scavenging activity.

Results: Centella asiatica along with silver nanoparticles has a positive outcome in the present study. It has also shown that it has a potent effect on antioxidant activity. The bioactive compounds may have significant health implications. These compounds may serve as antioxidants, enzyme inhibitors and inducers, receptor inhibitors and inducers, and gene expression inducers and inhibitors.

Conclusion: Centella asiatica mediated silver nano particles was found to be a potent antioxidant.

Key words: *Centella asiatica*, Vallarai, Free radicals, DPPH, Silver nitrate, Percentage of inhibition, antioxidant, innovative technique

HOW TO CITE THIS ARTICLE: Obuli Ganesh Kishore S, Abirami Arthanari, Rajeshkumar S, Preparation and Free Radical Scavenging Activity of Centella Asiatica Mediated Silver Nanoparticles , J Res Med Dent Sci, 2021, 9(11): 242-246

Corresponding author: Abirami Arthanari
e-mail ✉ : abiramia.sdc@saveetha.com
Received: 13/09/2021
Accepted: 10/11/2021

INTRODUCTION

Nanotechnology in medicine is currently being developed to employ nanoparticles to deliver drugs, heat, light or other substances to specific types of cells, such as cancer cells. This technique reduces damage to healthy cells in the body and allows for earlier detection of disease. The use of engineered nanoparticles offer the ability to transport therapeutics to specific sites of a disease, thus reducing the off-target toxicity of many drugs. The safety issues with nanoparticles are not very well known but their potential for danger is evident due to the high surface area to volume ratio, which can make the particles very reactive or catalytic [1,2]. In addition, these are able to pass through cell membranes in organisms and may interact with biological systems. Nanotechnology could eliminate diseases, disabilities, and illnesses such as diabetes, malaria, HIV, cardiovascular disease, damage from injuries and accidents, heal wounds, reduce child mortality, regenerate limbs and organs, eliminate inflammatory/infectious diseases, and so on and so forth [1,2].

Centella asiatica, commonly known as vallarai, Indian pennywort and Asiatic pennywort, is a herbaceous, perennial plant in the flowering plant family Apiaceae. It is native to the wetlands in Asia. It is used as a culinary vegetable and as a medicinal herb. *Centella asiatica* aka vallarai has a variety of benefits for the body. It is used to repair nervous tissue due to spinal injury, neuromuscular disorders, and to increase general brain function and memory [3]. Vallarai is also used in skin treatments for a wide spectrum of skin conditions [4]. Numerous preparations of this plant in various pharmaceutical forms recommended for several indications including neurological disorders are available all over the world. Taking this fact into consideration, many researchers have focused on the neuroprotective effect of *C. asiatica* in order

to confirm its traditional use on a scientific basis [5]. *Centella asiatica* is a medicinal plant that has been in use since prehistoric times. Its active constituents include pentacyclic triterpene derivatives. Studies have been conducted in particular to investigate the madecassoside and asiaticosides [6].

Aging is a natural process which is related to several morphological and biochemical changes that happen from maturity to senescence, making the organism vulnerable to diseases and toxicity, and eventually leading to cellular death [7].

According to the hypothesis of oxidative stress on aging, the loss of functional capacity associated with senescence comes from accumulation of molecular oxidative damages brought about by (toxic) free radicals produced during normal breathing. Free radicals were previously reported as being capable of damaging a lot of cellular components such as proteins, lipids and DNA [8].

Free radicals have been hypothesized to play an important role in ageing process. There exists an imbalance between free radical production and antioxidant defence mechanism, which may lead to cell death during ageing [9].

Silver nanoparticles have a potential in the treatment of cancer or degenerative Alzheimer's disease because of their antioxidant properties [10].

Silver nanoparticles synthesized through bio-green method has been reported to have biomedical applications to control pathogenic microbes as it is cost effective compared to commonly used physical and chemical methods [11].

AgNPs are known to have antioxidant and antimicrobial properties. AgNPs are used in coating or embedding for medical purposes. In addition to their medical uses, AgNPs are also used in clothing, food industry, paints, electronics and other fields [11,12].

Our team has extensive knowledge and research experience that has translate into high quality publications [13-32]. The objective of this study is to assess the free radical scavenging activity of *Centella asiatica* mediated silver nanoparticles.

MATERIALS AND METHODS

Preparation of Plant Extract

Dried, crushed and powdered samples of *Centella asiatica* were purchased from the market. 1g of this powdered plant extract was added to 100 ml of distilled water and was mixed well. It was boiled well for 5 to 10 minutes at 50 to 60 degrees Celsius (Figure 1).

To the prepared extract the selenium nanoparticles were added and were mixed well. The prepared extract was centrifuged and then it was filtered using a Whatman No 1 filter paper.

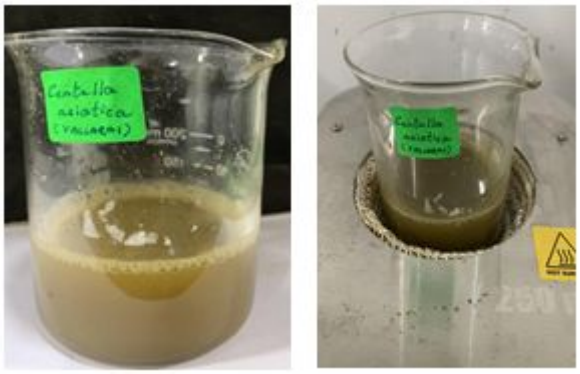

Figure 1: Image representing preparation and synthesis of *Centella asiatica* mediated silver nanoparticles.

Synthesis of silver nanoparticle

About 1 mill molar of silver nitrate was dissolved in 90 ml of double distilled water. The plant extracts of *Centella asiatica* were added with the metal solution, and this solution was made into a 100 ml solution. The colour change was observed visually and photographs were taken for the record (Figure 2). The solution was then kept in a magnetic stirrer/orbital shaker for nanoparticle synthesis. The synthesis of nanoparticles is primarily characterized using UV-vis spectroscopy. 3 ml of the solution is taken in a cuvette and scanned in UV-vis spectrometer under 350 nm to 550 nm wavelength. The results were recorded for graphical analysis. This extract with properly synthesized nanoparticles was used to assess the antioxidant activity of *Centella asiatica* mediated silver nanoparticles.

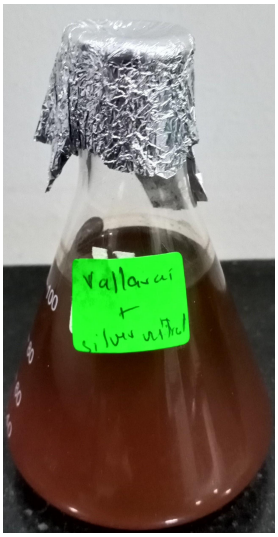

Figure 2: Image representing colour change observed after centrifuging and filtering the synthesised *Centella asiatica* mediated silver nanoparticles extract.

Estimation of antioxidant activity

DPPH radical scavenging assay was performed to estimate the antioxidant activity of *Centella asiatica* mediated silver nanoparticles. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical which reacts with

compounds that can donate a hydrogen atom. DPPH acts as free radical which induces oxidation. The anti-oxidant can donate an electron to DPPH radical and change in absorbance at 517 nm will follow. There was a colour change to pale yellow gradually. 2ml of plant extract was added to five test tubes. 50% of the methanol solution (buffer), 0.1mm of DPPH solution was added to five test tubes. Plant extracts with silver nanoparticles were added to five test tubes in different concentrations ranging from 10-50µL. The mixture was then incubated for 30 minutes in a dark place at room temperature. The absorbance value was spectrophotometrically analysed at 517 nm. The blank used was methanol solution.

Methanol solution mixed with 0.1mM of DPPH solution was used as a control. Ascorbic acid was used as a standard.

The minimum inhibitory concentration value was calculated using, DPPH radical scavenging (%)=Control optical density-Sample optical density X 100/ control

RESULTS

The *Centella asiatica* mediated silver nanoparticles showed different percentage of DPPH inhibition at different concentrations as mentioned in Table 1 and Figure 3.

Table 1: Table representing the percentage of DPPH inhibition of the plant extract. At 10 µg/ml concentration the DPPH inhibition value was found to be 35, at 20 µg/ml concentration the DPPH inhibition value was found to be 50, at 30 µg/ml concentration the DPPH inhibition value was found to be 55, at 40 µg/ml concentration the DPPH inhibition value was found to be 60 and at 50 µg/ml concentration the DPPH inhibition value was found to be 60.

S.No	Concentration of Plant Extract (µg/ml)	DPPH inhibition
1	10	35
2	20	50
3	30	55
4	40	60
5	50	65

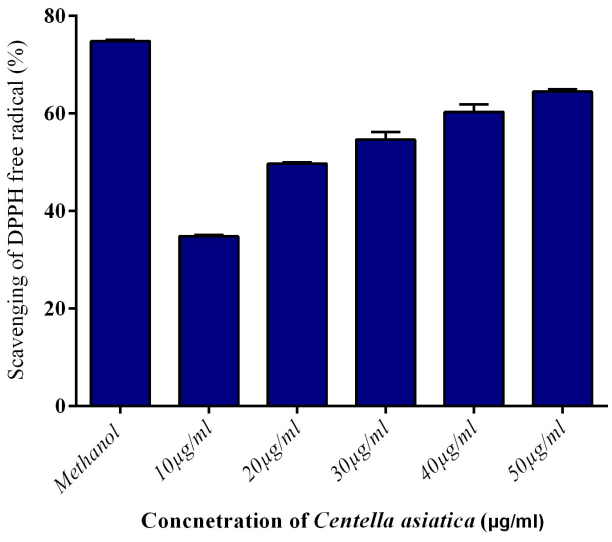

Figure 3: Bar graph representing the percentage of inhibition of DPPH free radical scavenging by the plant extract at different concentrations and the standard solution at 50µL concentration. X axis represents the concentration of the solutions in µg/dl and Y axis represents the percentage of inhibition of DPPH free radical.

DISCUSSION

Centella asiatica along with silver nanoparticles has a positive outcome in the present study. It is also evident that the prepared extract has proved itself to be a potent antioxidant. *Centella asiatica* has shown to have a broad range of biological functions and possible health advantages, including anti-oxidative, anti-diabetic, antimicrobial, anticancer, and anti-inflammatory properties.

A study demonstrated cognitive-enhancing and antioxidant properties of *Centella asiatica* in normal rats. The effect of an aqueous *Centella asiatica* extract (100, 200 and 300 mg/kg for 21 days) was evaluated in intracerebroventricular streptozotocin-induced cognitive impairment and oxidative stress in rats. The rats treated with *Centella asiatica* showed a dose-dependent increase in cognitive behaviour in passive avoidance and elevated plus-maze paradigms [33]. From a previous study conducted the IC50 value of the DPPH and hydroxyl radical scavenging activity of methanol extract showed 0.07 mg/ml and 500 µg/ml respectively. Reducing power assay results also followed in the same way [34].

From the present investigation using *Centella asiatica*, the DPPH scavenging activity was recorded at 10, 20, 30, 40 and 50µL concentrations. The standard used was methanol solution. The percentage of inhibition of standard was observed to be 75%. The percentage of inhibition of the prepared extract was 35%, 50%, 55%, 60% and 65% at 10, 20, 30, 40 and 50µL concentrations respectively. The maximum value was recorded at 50µL concentration. Even though it wasn't more than the standard value, it can be considered as a potent antioxidant as we can perform various tests with various concentrations to improve its potential. From a previous

study *Centella asiatica* as well as Bacopa monnieri, exhibited an antioxidant activity in a dose-dependent manner. The methanol extract of Bacopa monnieri leaf at different doses exhibited significantly ($P<0.05$) higher antioxidant activity as compared to *Centella asiatica* leaf [35].

The limitations of this study were, the measurement of solution in a micropipette showed small errors which had to be rectified. Further studies should be done to identify *Centella asiatica* as a therapeutic agent, and also to provide detailed information about compounds present in the preliminary compounds. There are very limited studies done about the biological and physiological effects. Therefore furthermore studies can also be done based on these topics. Further investigations could make the production and marketing of nanoparticle based natural medicines possible which will be a turning point in the pharmaceutical industry. Also other activities of the same plant and nanoparticles can be assessed.

CONCLUSION

From the study done, we may conclude that *Centella asiatica* mediated silver nanoparticles is a potent antioxidant.

ACKNOWLEDGEMENT

Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University.

SOURCE OF FUNDING

The present study is funded by the following agencies.

Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Saveetha University.

Sarkav Health Services.

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