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Preparation of Mouthwash Using *Centella asiatica* Nanoparticles - An *In-Vitro* Study

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Abstract:

The use of natural products in the field of medicine has a great influence as they have low risk of side effects. Traditional approaches to the treatment for various diseases have yielded a better result in the terms of treatment. It belongs to the family of Apiaceae. This species is generally known for their phytochemicals, as they contain special alkaloids such as the terpenes and diterpenes. Metal silver plays a vital role in the field of medicine as it acts as a potent antimicrobial, catalytic and biological metabolism. Use of the Silver nanoparticles other than other metallic green synthesis is preferred as they show less antibiotic resistance than other metallic compounds. Mouth washes have an effective role in the prevention of chemically formed plaque than mechanical removal such as flossing and brushing. The aim of this study is to determine the anti-microbial and the cytotoxicity activity of the Centella asiatica mediated silver nanoparticles'. Anti-microbial activity and cytotoxicity of the formulated mouthwash was assessed by using deep well agar method on four different microorganisms. These results show that the mouthwash showed a good anti-microbial activity and hence it can be further formulated in form of mouthwash. The results are indication of the potent antimicrobial activity against bacterial strains proving that the Silver nanoparticles synthesized by Centella asiatica and its based chitosan can be used as a very good mouthwash.

Keywords: *Centella asiatica, Antimicrobial, Cytotoxicity, Silver Nanoparticles.*

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Introduction

The use of natural products in the field of medicine has a great influence as they have low risk of side effects. Traditional approaches to the treatment for various diseases have yielded a better result in the terms of treatment. *Centella asiatica*, commonly known as the vallarai keerai in Tamil is cultivated all over the states of India, It is widely used in food is also known for its medicinal value in terms of relieving muscular pain and inflammation in ancient Ayurvedic medicine. It belongs to the family of Apiaceae. This species is generally known for their phytochemicals, as they contain special alkaloids such as the terpenes and diterpenes. **(Vasanth *et al*., 2021; Zweig et al., 2021).** The extract is known for its effective treatment for many diseases, including cancer, inflammation, diabetes, obesity, and hepatotoxicity. Each part of the plant has its specific and iniquity uses such as the leaves of *Centella asiatica* is known for providing nourishment to neurons thus improving the memory capacity**.** Methanolic extract of this plant shows a significant anti-inflammatory property and also it is used as in the treatment for rheumatism, insomnia, palsy and epilepsy**. (Laosuwan, 2019; Maaz, 2018)**.

Metal silver plays a vital role in the field of medicine as it acts as a potent antimicrobial, catalytic and biological metabolism. **(Fard *et al*., 2018)** The use of silver nanoparticles other than other metallic green synthesis is preferred as they show less antibiotic resistance than other metallic compounds also as silver has a tendency to release silver ions in a large amount , it disrupts the bacterial cell wall causing lysis of the bacteria. This can be achieved with the help of nanotechnology**. (Netala *et al*., 2016).**

Nanotechnology deals with materials whose diameter is within 1 -100 nm. The advancement of science and technology lead to the synthesis of metal nanoparticles and nanocomposites is an emerging area of research and exploration in the field of material science for their unique size and shape and features are different from the regular bulk structure **(Demirer *et al*., 2021).** Since the development of nanoparticles, metal nanoparticles are extensively used in wide range of applications including sensing, energy, catalyzing, drug and gene delivery.

Mouth washes have an effective role in the prevention of chemically formed plaque than mechanical removal such as brushing and flossing **(Demirer *et al*., 2021)**. Chemically prepared chlorhexidine mouthwashes is considered as the gold standard chemical treatment for dental plaque control, but chlorhexidine mouthwashes can lead to the staining of teeth and tongue, disturbance in taste sensation and adverse effects on long term use on the oral mucosa causes ulceration. Herbal preparation contains a mixture of phytochemicals such as catechins, tannins, sterols has the same remedial effect but does not harm the mucosa to that extent even on prolonged usage **(Luck, 1967; Demirer *et al*., 2021).** Also in comparison with synthetic mouthwashes they also contain anti-oxidant and anti-inflammatory properties which further enhances the oral health and hygiene. The aim of the study is to prepare a silver nanoparticle based mouthwash of the *Centella asiatica* and assess its cytotoxic effect and anti-microbial activity **(Koch, 1967).**

Methodology

Preparation of the Plant Extract

The *Centella asiatica* fruit is purchased and it is cleaned, dried and powdered into fine granules. 0.5 g of *Centella asiatica* powder are weighed separately and taken. Now 50 ml of Distilled Water is added to dissolve the weighted extract in conical flasks mixed well. This mixture is boiled at 60 degree Celsius for 7 minutes with the help of heat mantle. Then the boiled extract is filtered with the help of filter paper.

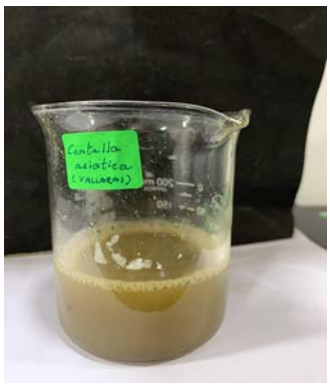


Figure No. 1: The Crude Extract

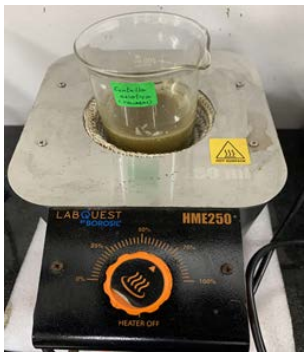


Figure No. 2: Boiling of Extract

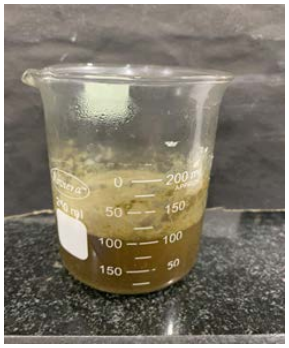


Figure No. 3: Filtered Extract

Synthesis of Silver Nanoparticles

This *Centella asiatica* extract is treated with 0.016 g of the Silver Nitrate and 90 ml of Distilled Water and it is placed in a semi-automatic shaker at 900 rpm. With the help of a double beam UV spectrophotometer, synthesis of the nanoparticles for every one hour is noted. Then this formulation is placed in a centrifuge for 10 minutes. Now the synthesized nanoparticles which are settled at the bottom are collected.

Figure No. 4: The Synthesized Nanoparticles

Cytotoxic Effect

Now a twelve well micro plate is taken and cleaned. Then the various readings are marked and then salt water is added to this well. Now salt water brine shrimps are collected and placed into these wells. Ten brine shrimps are now present in each of these wells. Now estimated volume of the *Centella asiatica* coated silver nanoparticles extract is added on the brine shrimps with the help of micro dripper. This setup is kept for 48 hours. Now the number of brine shrimps that are alive on day 1 and day 2 are counted and tabulated.

Results and Discussion

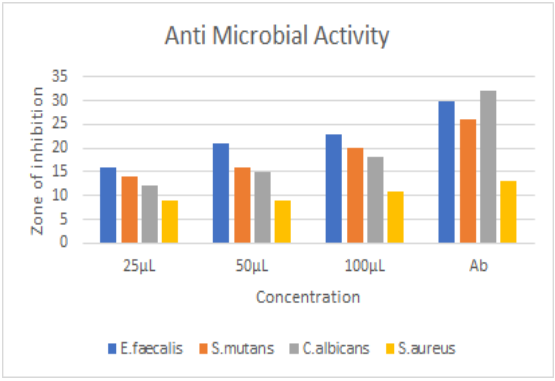
UV Spectrophotometric Analysis

The above material had taken in a conical flask and kept in a bright and airy environment. The piper longum extract is observed under naked eye. On visual observation the *Centella asiatica* extract was pale green in color Fig. After addition of the Silver Nitrate it is placed in semi-automatic shakers for 12 hours. After a period of 15 hours it is observed that the solution turned into brown in colour Fig. Then the solution is sent for synthesis of silver nanoparticles. After complete synthesis of silver nanoparticles the formulation was found to be in dark brown color Fig. The UV- spectrometer readings were recorded and the peak at 485nm confirms the nanoparticle synthesis. Then the formulation is added to the brine shrimps and the mortality of the brine shrimps is calculated.

Antimicrobial Activity

Table No. 1: The Antimicrobial Assay

Organism	25	50	100	Ab
S.mutans	16	21	23	30
S.aureus	14	16	20	26
E.faecalis	12	15	18	32
C.albicans	9	9	11	13



Graph No. 1: The Antimicrobial Assay

Table No. 1 shows the MIC of bacterial and fungal growth at varying concentrations of biosynthesized silver nanoparticles. The anti-bacterial activity of the

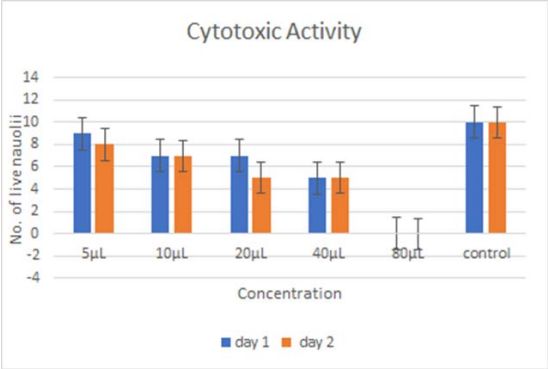
Silver Nanoparticles was evaluated based on their zone of inhibitions and the results were compared with the standard antibacterial agent the anti-bacterial activity of the silver nanoparticles against S. aureus showed a zone of inhibition of 14 mm at the zone of inhibition at the concentration of 25µl. The antibacterial activity of silver nanoparticles against S. aureus showed a zone of inhibition of 16mm at the concentration of 50µl activity of silver nanoparticles against S. aureus showed the zone of the inhibition of 20mm at 100µl concentration which is comparatively less than the used silver nanoparticles against S. mutans showed a zone of inhibition of 16mm at 25µl and 21 mm at 50µl.

The antibacterial concentration of 100µl showed 23mm of zone inhibition which is almost near to that of standard antibiotic which showed the zone of inhibition of 30mm. The anti-bacterial activity of the Silver Nanoparticles against S. mutans. The anti-bacterial activity of the Silver Nanoparticles against E. faecalis showed a zone of inhibition of 12mm, 15mm & 18mm at the concentrations of 25, 50 & 100 µl respectively. The anti-fungal activity of the silver nanoparticles against C.albicans showed the zone of inhibition of 9mm at the concentration of 25µl , 9mm at 50µl and the maximum zone of inhibition against Candida albicans was seen at 11µl which if comparatively more than that of the standard antibiotic which showed a zone of inhibition of 13 mm only.

Cytotoxicity

Table No. 2: The Lethality Assay of the Brine Shrimps

Concentration	No of alive nauplii -day1	No of alive nauplii-day 2
5	9	8
10	7	7
20	7	5
40	5	5
80	0	0
control	10	10



Graph No. 2: Brine Shrimps Lethality Assay

After visual observation, the solution is sent for nano particle synthesis and for cytotoxicity assay. Brine shrimp lethality assay was performed using the formulated solution. The results obtained from the assay are tabulated and converted into a bar graph for easy representation. The X axis represents the concentration of the formulated solution used. The Y axis represents the number of live nauplii. The formulated solution is given to the nauplii for two days at different concentrations. 5 µl concentration of solution is given and observed for two days consequently. It is seen that 9 of the nauplii are alive on day 1 and 8 are alive on day 2. Similarly 10 µl of solution is given and observed for two days. It is seen that on day 1 and 2, 7 of the nauplii are alive. Then the solution is given at 20 µl concentration.7 of the nauplii are alive on day 1 whereas only 5 of them are alive on day 2. At 40 µl concentration 5 of the nauplii are alive on day 1 and 5 on day 2. At 80 µl concentration all the nauplii none of the nauplii are alive.
In the test control no extract is added and though all the nauplii are alive for both the days.

In the present study, we adopted a simple procedure for synthesizing silver nanoparticles from *Centella asiatica* extract **(Vasanth *et al.*, 2021)**. This could be achieved due to the presence of various flavonoids and phenolic compounds that are responsible for its antioxidant activity and reducing property. Silver nanoparticles are cheaper to obtain and have a wide range of applications **(Maaz, 2018).** Silver nanoparticles particularly contain special properties such as antimicrobial properties as they continuously release a low level of silver ions that provides a continuous protection against bacterial cell wall. Silver nanoparticles also have unique properties such anti larvicidal, anti carcinogenic, catalytic, wound healing etc. **(Fard *et al.*, 2018)**.

Nanoparticles can be synthesized from other metal ions such as zinc, gold, copper, selenium and cerium but due to the above mentioned properties silver nanoparticles are adopted in this study **(Laosuwan, 2019).** Our results clearly demonstrate the ability of *Centella asiatica* based AgNPS mouthwash to scavenge free radicals which could also be **(Vasanth *et al*., 2021)** due to the presence of functional groups present on the surface of nanoparticles, this property can be used to kill the microorganisms that are present in the oral cavity which can be used in the form of mouthwash. But we need to find whether this formulation is cytotoxic or non- cytotoxic. As our study showed that our mouthwash **(Vasanth *et al*., 2021; Zweig et al., 2021)** has a less percentage of cytotoxicity and as the concentration and exposure of the mouthwash increases this formulation is found to be harmful to the nauplii and may be harmful to the gingival tissues and hence it can be further tested on cell line and may be formulated in the form of mouth washes cleanses, toothpaste and even as medications in endodontic treatments at low concentrations **(Netala *et al*., 2016; Fard *et al*., 2018).** The same cytotoxicity property was also demonstrated with the AgNPs synthesis using belladona which showed more cytotoxicity than the *Centella asiatica*.

Conclusion

From the above study, it is evident that silver nanoparticles synthesized by *Centella asiatica* shows a good antimicrobial activity. It can be used as a potent antimicrobial agent against all microorganism infections in the form of gels for external use and in the form of mouthwashes to control oral thrush. Also in the field of forensic science it can be used as a preliminary step for obtaining dermatoglyphics and rugae patterns in dead bodies to obtain a germ free evidence. Also further studies can be done to assess its antioxidant, anti-inflammatory and cytotoxic activity which could be used in the treatment of numerous diseases.

Acknowledgement

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Conflicts of interest: Nil

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