

PREPARATION OF MOUTHWASH USING BANANA STEM EXTRACT MEDIATED COPPER NANOPARTICLES AND ITS CYTOTOXIC EFFECT - AN IN VITRO STUDY

ABSTRACT:

Background:

Copper nanoparticles have recently attracted researchers due to their low cost, availability and other novel properties. Various parts of *Musa sapientum* which belong to the family Musaceae have been used for various medicinal purposes. The aim of the study is to assess the cytotoxic effect of mouth wash prepared using banana stem mediated copper nanoparticles.

Materials and method: 100 mL of distilled water is mixed with 1g of banana stem extract and distilled. The filtrate is mixed with 0.477g of copper sulphate mixed in 60 mL of distilled water. There were a total of 10 shrimps per dilution. The artificial seawater upto 10 mL per test tube is controlled. The test tube was left uncovered under a lamp. The number of surviving shrimps were counted and recorded after 24 hrs. The percentage of motility was calculated by dividing the total number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death of nauplii is attributed to the compounds present in nanoparticles.

Results: Percentage of lethality for the banana stem extract mediated copper nanoparticles mouthwash at concentrations of 5 μ L, 10 μ L, 20 μ L, 40 μ L, 80 μ L were 60%, 70%, 100%, 100%, 100% respectively.

Conclusion: Thus, with an increase in concentration of the banana stem mediated copper nanoparticles mouthwash, the cytotoxic activity increases. Thus from the study, it is evident that natural mouthwash can be prepared with banana stem mediated copper nanoparticles with less side effects.

Keywords : Banana stem extract, Copper nanoparticle, Cytotoxicity, Mouthwash, Green synthesis

Running title: Banana stem extract mediated copper nanoparticles mouthwash and its cytotoxic effect

INTRODUCTION

Nanobiotechnology deals with nano sized materials in different fields of biological sciences. Biosynthesis of metallic nanoparticles using plant extract is eco friendly (1). Metal nanoparticles have gained more attention in recent years due to its use in various fields. Copper nanoparticles have recently attracted researchers due to their low cost, availability and other novel properties. These properties are different from their bulk metals (2,3). Copper nanoparticles are used in various aspects. They are used in wound dressings. Copper nanoparticles have potential industrial use such as gas sensors, catalytic processes, high temperature superconductors, solar cells and so on. In addition to the anti-inflammatory and antioxidant properties, copper nanoparticles also have the characteristics of cytotoxicity(4).

Musa sapientum which is also known as banana, commonly grown in South India for its fruits, stem, flowers, roots and leaves. Various parts of *Musa sapientum* which belong to the family Musaceae have been used for various medicinal purposes. The area of interest here is banana stem extract since it is cost efficient and is widely distributed in the region where research is carried out. The major uses of banana pseudo-stem fiber are in making specialized and high-quality sanitary products such as baby pampers, textiles, and papers such as banknotes(5,6). The banana pseudo-stem fiber can also be used for ropes such as marine rope since this fiber has good resistance to sea water and has buoyancy properties. Other uses of this fiber are for making coffee and tea bags, filter cloths, as reinforcement fibers for plaster, disposable fabrics, and light-density woven fabrics (5–7). Banana stem is used to treat disorders such as dysentery diarrhea, inflammation, pain and snake bite. The antimicrobial compound present in the banana stem extract is tannic acid which plays a huge role in the study since it helps in the cytotoxic effect of banana stem extract(8). Banana stem is also a source of polyphenols, gentic acid which plays an important role in its antioxidant activity (9,10).

Oral rinses, also called mouthwashes, are most often used to reduce the microbial load in the oral cavity or to control or reduce bad breath, depending on the ingredients in the particular oral rinse(11). Oral rinses also contain chemicals, phenols glycerin as a humectant, sodium benzoate as a buffer, flavors, coloring, and emulsifiers that serve as stabilizers. These mouth wash provides strength to gums thereby preventing gingival recession and other periodontal disease (12) . Mouth wash also acts in healing wounds in the oral cavity to a certain extent. Cytotoxicity is an important characteristic of mouthwash since it eliminates micro organisms which serve the purpose of mouthwash. Similar research has been carried out by fellow researchers(13) (14) (15) (16). The aim of the study is to assess the cytotoxic effect of mouth wash prepared using banana stem mediated copper nanoparticles.

MATERIALS AND METHOD

Preparation of plant extract:

The preparation of plant extract involves the selection of banana stem extract in powder form (Figure 1). Each glassware was thoroughly washed with distilled water and dried in a hot air oven prior to use. The plant powder that weighs approximately 1g was taken and mixed in a conical flask with 100 mL of distilled water. The solution is then boiled and filtered through filter paper for 5 to 10 minutes. The extract thus obtained will be purified and then used for further experiments (Figure 2).

Preparation of banana stem mediated Copper Nanoparticles:

0.477 g of copper sulphate ($0.179 * 3$) is weighed and taken for the synthesis of Copper nanoparticles. The CuSO_4 weight is combined with 50 mL distilled water. Now, it is blended with the 50 mL solution for plant extract. Finally, 100 mL of a mixture of the solution CuNP and banana stem extract is collected.

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Green Synthesis of banana stem mediated Copper nanoparticles:

The mixture was put for two days in a laboratory shaker until the changes in color were detected. The reading was taken every hour in the UV-vis spectrophotometer (Beckman DU-20 spectrophotometer) and color variations were also noted. In addition, the reaction mixture was subjected to 10 minutes of centrifugation and the resulting pellet was collected in one tube.

Cytotoxic activity of banana stem mediated Copper Nanoparticles:

There were a total of 10 shrimps per dilution. The artificial sea water up to 10mL per test tube is controlled. The test tube was left uncovered under a lamp. The concentrations taken were ~~5 μ L~~ 5 μ L, 10 μ L, 20 μ L, 40 μ L, 80 μ L and a control group. The number of surviving shrimps were counted and recorded after 24 hrs. The percentage of motility was calculated by dividing the total number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death of nauplii is attributed to the compounds present in nanoparticles.

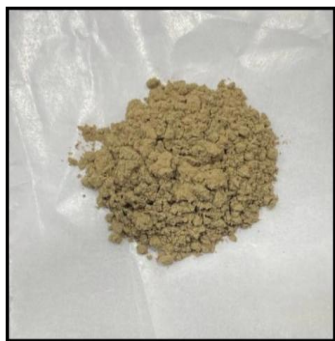


Figure 2 represents the preparation of plant extract.

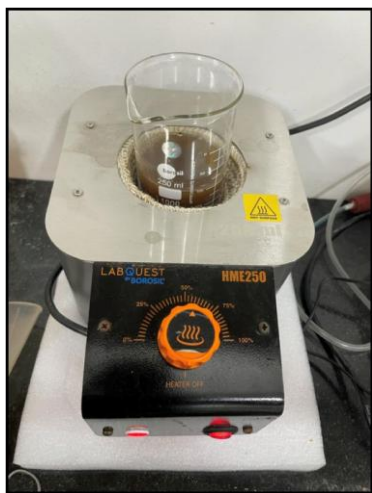


Figure 2 depicts the heating of the solution of banana stem extract.

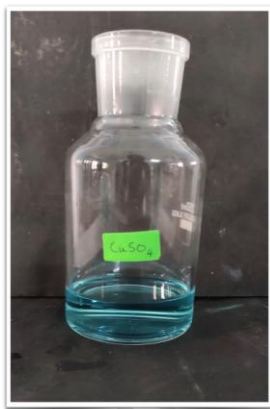


Figure 3 represents Copper sulphate solution.



Figure 4 represents the filtrate prepared.

RESULTS

The figures 1, 2, 3 and 4 represent the methodology in preparation of mouthwash through green synthesis using banana stem extract mediated copper nanoparticles. Descriptive statistics was used. The concentration of the lethality dosage (LD50) was measured at 95% confidence intervals using Probit analysis. LD50 below 100 ppm was considered (active) to be effective. As Meyer and others have stated, the LD50 value of less than 1000 $\mu\text{g} / \text{mL}$ is toxic while the LD50 value of more than 1000 $\mu\text{g} / \text{mL}$ is non-toxic. The mortality percentage (%M) was also calculated by dividing the number of dead nauplii by the total number and multiplying by 100%. This is to ensure nauplii mortality is due to the substances in the nanoparticles. The cytotoxic effect of banana stem mediated CuNP shows that as the concentration increases there is a significant increase in the percentage of lethality. The result shows that a significant cytotoxic effect is achieved at 5 μH reaching 100% of lethality at 20 μH , 40 μH , 80 μH . (Figure 5) With reference to the below table it can be seen that the % of lethality for the concentrations 5 μH , 10 μH , 20 μH , 40 μH , 80 μH were 60%, 70%, 100%, 100%, 100% respectively. (Table 1).

Table 1: represents the data of concentration of the mouthwash and its respective cytotoxic effect.

Concentration in μL	% of lethality
Control	0%
5 μL	60%
10 μL	70%
20 μL	100%
40 μL	100%
80 μL	100%

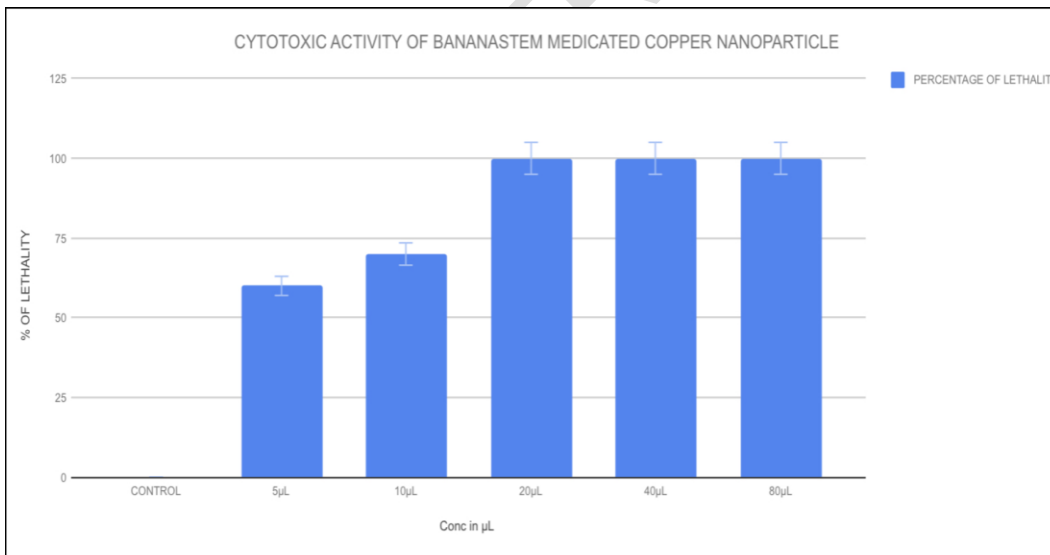


Figure 5: Graph showing the cytotoxic activity of banana stem mediated copper nanoparticles. X

X-axis represents concentration in μL and Y axis represents % of lethality. The graph shows that with increase in concentration, the lethality of the mouthwash increases.

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications (17–29),(30–34),(35),(36). From the results, it is seen that the cytotoxic effects increase with concentration reaching 100% lethality at 80 μL . This shows that banana stem mediated copper nanoparticles has a significant cytotoxic effect and plays an important role in the characteristics of the mouth wash. This attribute helps in leading to reduced microbial count in the oral cavity at a reduced time.

In a study, Avinash et al has described the cytotoxic effect of copper nanoparticles also explaining about the bioactivity mechanism of action (37). Reza et al, has also examined the cytotoxicity of copper nanoparticles, in which the aim of their experiment was the green synthesis of copper nanoparticles using *Allium saralicum* and assessment of their cytotoxicity, antioxidant, antibacterial, antifungal, and cutaneous wound healing effects under *in vitro* and *in vivo conditions*. These nanoparticles were characterized by Fourier transformed infrared spectroscopy (FT-IR), UV–visible spectroscopy, field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) (38).

Oral cavity is a mirror of an individual's healthy state. Maintaining the oral cavity is essential for everyone to prevent themselves from many gingival and periodontal diseases that include aggressive and chronic periodontitis which further leads to tooth loss. Thus, each individual has to be aware of their dental status and maintain their oral hygiene by using the mouthwashes as mentioned to minimise the effect of microbial growth within the oral cavity. Since this research is underway, further modifications have to be done like adding some flavouring agents and a better mode of preparing the mouth wash to reduce the loss of the flavonoids present in the mouthwash. Clinical trials are to be done. This study will be helpful in preparation of eco friendly mouthwash which is completely devoid of chemical substances and has low adverse effects in the future. The study also helps in providing a better mouthwash which can provide good results in a shorter time.

CONCLUSION

Thus with an increase in concentration of the banana stem mediated copper nanoparticles mouthwash, the cytotoxic activity increases (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) ((49,50) (51) (52). Thus from the study, it is evident that natural mouthwash can be prepared with banana stem mediated copper nanoparticles with less side effects.

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