ANTI MICROBIAL ACTIVITY OF MUSA SAPIENTUM MEDIATED COPPER NANOPARTICLE AGAINST ORAL PATHOGENS - AN INVITRO STUDY

ABSTRACT

BACKGROUND: Musa sapientum is a useful medicinal plant from the Musaceae family. Copper nanoparticles with a high surface to volume ratio can also be used as antifungal and antibacterial agents. The aim of the study is to access the antimicrobial activity of musa sapientum mediated copper nanoparticle against oral pathogens

MATERIALS AND METHOD: Plant extract was prepared by 1g of musa, was weighed aseptically and then dissolved in 100ml of distilled water. Then the solution is boiled for about 5 minutes at a temperature of about 60-80 degree Celsius and then allowed to cool down followed by filtration of extract. The copper solution was prepared by dissolving 20 millimolar of copper sulphate in 80ml of distilled water followed by 20ml of plant extract and then the mixture is placed in the shaker for the synthesis and then allowed to mix for about 1 hour then the first reading was taken using UV spectrum and noted down. Antimicrobial activity was done against the strain of S. aureus, S. mutans, E. faecalis. Muller Hinton agar was utilised for this activity to determine the zone of inhibition different concentrations were loaded in the plates and incubated for 24 hrs 37 degree celsius after the incubation time the zone of inhibition was measured. Antifungal activity of zinc oxide nanoparticles was done against the strain Candida Albicans. **RESULT:** For the study, descriptive statistics was used. For 100µL concentration 11mm zone of inhibition was seen when compared to the standard drug Amoxyrite which had a zone of inhibition of 36mm, which means that the antimicrobial activity was low in case of E. Faecalis. In C.Albicans, at 100µL concentration 14mm zone of inhibition was seen, but the standard fluconazole had only a zone of inhibition of 10mm, which means antimicrobial activity was good in case of C. albicans which showed more zone of inhibition. For S.aureus, at 100µL 13mm zone of inhibition was seen, the standard value showed 28mm zone of inhibition which means there is less zone of inhibition when compared to standard drug. For S. mutans, at 100µL concentration

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18mm zone of inhibition was seen and when compared to the standard value which was 23mm which showed poor antimicrobial activity.

CONCLUSION: The research concludes that musa sapientum mediated copper nanoparticles showed a moderate antimicrobial activity against the pathogens Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans.

KEYWORDS: musa sapientum, oral pathogens, copper nanoparticles, green synthesis, antimicrobial activity

INTRODUCTION

Musa sapientum var. sylvesteris is a useful medicinal plant from the Musaceae family. Bangladesh has a large population of this plant. Bananas are commonly eaten as a dessert, cooked as a vegetable or converted into a variety of confections(1). The leaves are used to bandage cuts, blisters, and ulcers by the tribals of India's Western Ghats. The Musa genus contains a wide range of taxa, each with its own set of pharmacological investigations. In rats, the banana plantain has been proven to have ulcer-healing properties(2). They concluded that wound healing was aided by the antioxidant action of plantain bananas as well as many wound healing biochemical parameters. In normal and non-insulin-dependent diabetic mellitus rats, methanolic extract of M. sapientum var. paradisiaca displayed antiulcer and mucosal defence properties(3). The extract's ulcer-protective activity could be owing to its major effect on mucosal glycoprotein, cell proliferation, free radicals, and antioxidant systems, according to the researchers. Through its primary effect on numerous mucosal defensive variables, studies with the plantain banana (M. sapientum var. paradisiaca) have suggested its ulcer protecting and healing activities, and they concluded that its antioxidant activity may be implicated in its ulcer protective action. It is a remedy for a variety of ailments, including diarrhea, wounds, stomach ulcers, diabetes, heartburn, inflammation, and more. Banana is one of the most nutritious fruits that provides valuable health benefits to the body. Bananas are more nutrient-dense than apples in comparison. It is high in potassium, has five times the amount of vitamin A and iron, two times the amount of carbs, and three times the amount of calcium(4).

Nanoparticles (NPs) have a lot of catalytic activity, which is useful for chemical processes in both industry and research purposes(5). Energy conversion and storage, chemical manufacture, biological applications, and environmental technologies are just a few of the sectors where NPs are used.(6) The enormous interest in catalysis utilising nanomaterials has driven the synthesis and analysis of varied highly functionalized NPs, including graphene-based catalysts, nanocarbon catalysts, core/shell nanocatalysts, magnetite-supported nanocatalysts, integrated nanocatalysts, and also various metal nanostructures(7). Rapid advances in synthetic techniques have permitted the synthesis of NPs with customizable compositions, forms, sizes, and structures, either on their own or supported on other materials, facilitating the study of these systems.(8)

Cu NPs are particularly appealing due to copper's abundant natural supply and low cost, as well as the numerous practical and simple approaches to make Cu-based nanomaterials. Despite the extensive history of bulk Cu uses in a variety of sectors, the usage of Cu NPs is limited due to Cu's inherent instability under air settings, which makes it susceptible to oxidation(9). Many efforts to develop methods and supporting materials that boost the stability of Cu NPs by modifying their sensitivity to oxygen, water, and other chemical entities have prompted the development of more complex Cu-based NPs, such as core/shell Cu NPs or copper oxide systems. Copper nanoparticles have properties that are not seen in commercial copper, such as catalytic and antifungal/antibacterial activity. To begin with, copper nanoparticles have a very high catalytic activity, which is related to their huge catalytic surface area(10). When used as reagents in organic and organometallic synthesis, nanoparticles have a better reaction yield and a quicker reaction time due to their tiny size and high porosity. Copper nanoparticles with a high surface to volume ratio can also be used as antifungal and antibacterial agents(11). Their antibacterial activity is triggered by their intimate contact with microbial membranes and the metal ions they release into solutions. Cupric ions are released from nanoparticles when they slowly oxidise in liquids, and when the lipid membrane is adjacent, they can form harmful hydroxyl free radicals. The free radicals then use oxidation to break down lipids in cell membranes, causing the membranes to degenerate. As a result, intracellular chemicals leak out of cells due to degraded membranes; cells are no longer able to function. Finally the alterations caused by the free radicals cause cell death(12). The main aim of the study was to evaluate the antimicrobial activity of musa sapientum mediated copper nanoparticles against oral pathogens.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT

1g of musa was weighed aseptically and then dissolved in 100ml of distilled water. Then the solution is boiled for about 5 minutes at a temperature of about 60-80 degree Celsius and then allowed to cool down followed by filtration of extract.

PREPARATION OF NANOPARTICLE

The copper solution was prepared by dissolving 20 millimolar of copper sulphate in 80ml of distilled water followed by 20ml of plant extract. Then the mixture is placed in the shaker for the synthesis and then allowed to mix for about 1 hour then the first reading was taken using UV spectrum and noted down and then again the extract was placed in the shaker for 1 hr and the second set of readings were taken.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity was done against the strain of S. aureus, S. mutans, E. faecalis. Muller Hinton agar was utilised for this activity to determine the zone of inhibition different concentrations were loaded in the plates and incubated for 24 hrs 37 degree celsius after the incubation time the zone of inhibition was measured. Antifungal activity of zinc oxide nanoparticles was done against the strain Candida Albicans. Sabouraud's Dextrose Agar was used to prepare the medium; the plates were incubated at 28°C for 48 to 72 hours and after the incubation time the zone of inhibition was measured.

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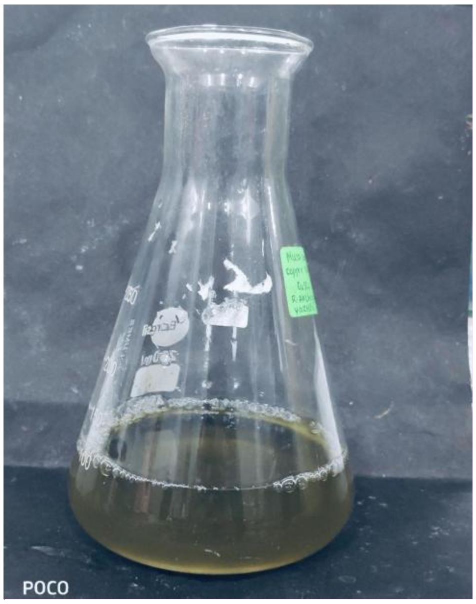

Figure 1 - It shows musa sapientum mediated copper nanoparticles

Figure 2 - It shows the zone of inhibition of Candida Albicans which was loaded with different concentrations of musa sapientum mediated copper nanoparticles to check the antimicrobial activity.

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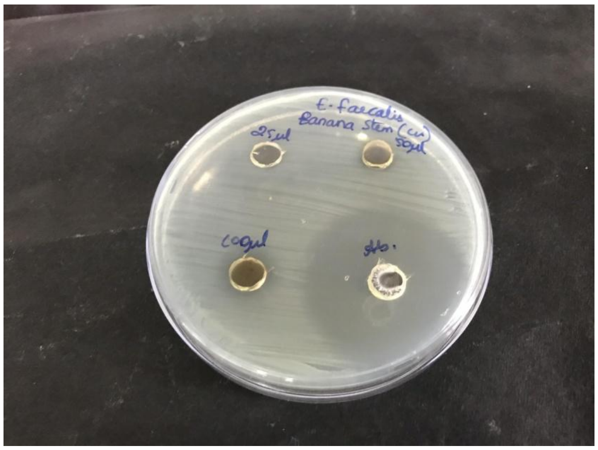

Figure 3 - It shows the zone of inhibition of E faecalis, which was loaded with different concentrations of musa sapientum mediated copper nanoparticles to check the antimicrobial activity.

Figure 4 - It shows the zone of inhibition of S.mutans, which was loaded with different concentrations of musa sapientum mediated copper nanoparticles to check the antimicrobial activity.

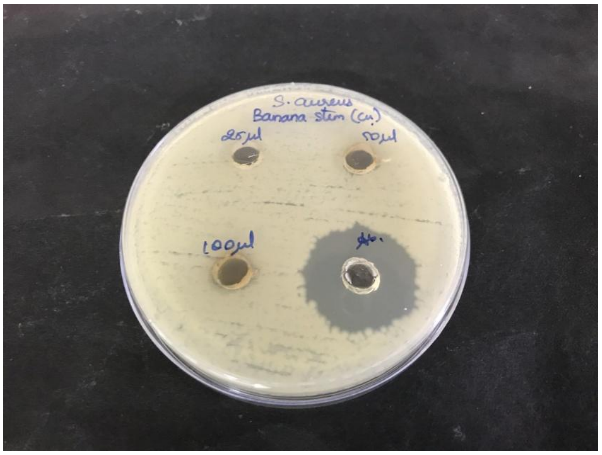

Figure 5 - It shows the zone of inhibition of S.aureus, which was loaded with different concentrations of musa sapientum mediated copper nanoparticles to check the antimicrobial activity.

RESULTS

For the study, descriptive statistics was used. For 25µL concentration 10mm zone of inhibition was seen in E.faecalis group, at 50µL concentration also 10mm zone of inhibition was seen, in 100µL concentration 11mm zone of inhibition was seen when compared to the standard drug Amoxyrite which had a zone of inhibition of 36mm, which means that the antimicrobial activity was low in case of E. Faecalis. In C.Albicans, for 25µL concentration 10mm zone of inhibition was seen, at 50µL concentration 10mm, at 100µL concentration 14mm zone of inhibition was seen, but the standard fluconazole had only a zone of inhibition of 10mm, which means antimicrobial activity was good in case of C. albicans which showed more zone of inhibition. For S.aureus, at 25µL concentration 10 mm zone of inhibition was seen, at 50µL concentration 13mm zone of inhibition was seen , at 100µL 13mm zone of inhibition was seen, the standard value showed 28mm zone of inhibition which means there is less zone of inhibition when compared standard drug. For S. mutans, for 25µL concentration 10mm zone of inhibition was seen, at 50µL concentration 10mm zone of inhibition was seen, for 100µL concentration 18mm zone of inhibition was seen and when compared to the standard value which was 23mm which showed poor antimicrobial activity. (Table 1 and figure 6)

Table 1- Antimicrobial activity of musa sapientum mediated copper nanoparticles against different microorganisms at different concentrations.

Organism	**25µL**	**50µL**	**100µL**	**Ab**
E. Faecalis	**10mm**	**10mm**	**11mm**	**36mm**
C. Albicans	**10mm**	**10mm**	**14mm**	**10mm**
S. aureus	**10mm**	**13mm**	**13mm**	**28mm**
S. mutans	**10mm**	**10mm**	**18mm**	**23mm**

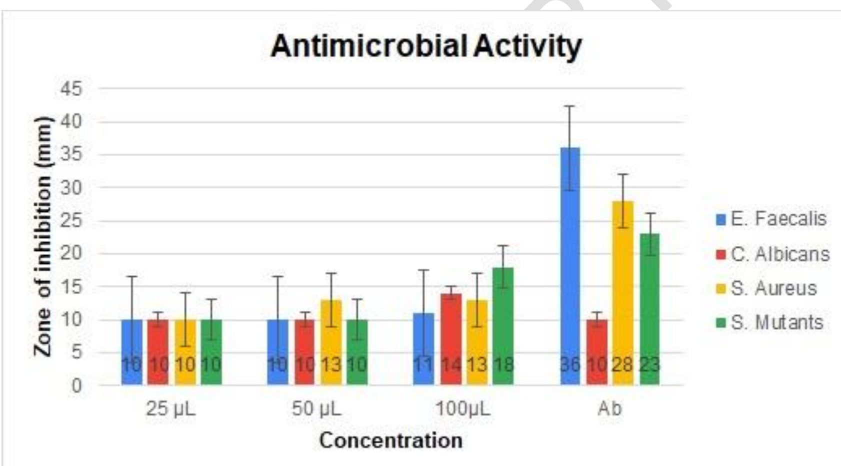

Figure 6 - Graph shows the Antimicrobial activity of musa sapientum mediated copper nanoparticles against different microorganisms at different concentrations. The standard used here is Fluconazole for C Albicans and Amoxyrite is the standard drug used for the rest of the bacteria. The X axis depicts the various concentrations of musa sapientum mediated copper nanoparticles while the Y axis depicts the zone of inhibition. Blue colour depicts E. Faecalis, orange colour depicts C. Albicans, Yellow colour depicts S.Aureus and green colour depicts

S.mutans. Values are compared with the standard values and the zone of inhibition is measured. Thus as the concentration increases the zone of inhibition also increases.

DISCUSSION

A study by Ahmad et al proved that the methanolic extract of the musa sapientum showed a good antimicrobial activity against both gram positive and negative bacteria as it showed a increase in zone of inhibition when compared to its standard drug which was kanamycin.(16) Several studies have been conducted for checking the antimicrobial activity of copper nanoparticles; one such study of different microbial cultures that were performed in the presence of nanoparticles to observe their effect on the growth profile(17). The study showed that silver and copper nanoparticles had great promise as antimicrobial agents against E. coli, B. subtilis and S. aureus. MIC, MBC and disk diffusion tests suggested that for all cultures of E. coli and S. aureus, the antimicrobial action of the sil- ver nanoparticles were superior. Although an oxide layer was formed on the copper nanoparticles, these nanoparticles demonstrated better antimicrobial activity towards B. subtilis.

Our team has extensive knowledge and research experience that has translated into high quality publications(18–30),(31–35),(36),(37). From the study, it can be seen that musa sapientum mediated copper nanoparticles showed a good antimicrobial activity. The availability of copper has made it a better choice to work with, because it shares properties similar to those of other expensive noble metals, including silver and gold. The choice of copper in the present research is attributed to the above-mentioned factors; in addition, copper nanoparticles are reported to have antimicrobial activity against a number of species of bacteria and fungi. Previous studies have indicated that copper nanoparticles have antimicrobial activity against E. coli and Staphylococcus Species, and similar antifungal properties were also reported. Furthermore, musa sapientum mediated copper nanoparticles which showed a good antimicrobial activity against Candida albicans with the highest zone of inhibition when compared with the standard Amoxyrite, can be used as a very good antimicrobial agent(38) (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) ((48,49) (50) (51). We did not evaluate the antimicrobial effect in patients, which can be done in future.

CONCLUSION

The research concludes that musa sapientum mediated copper nanoparticles showed a moderate antimicrobial activity against the pathogens Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans.

- **COMPETING INTERESTS DISCLAIMER:**
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- Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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