EVALUATION OF THE ANTIOXIDANT ACTIVITY OF BANANA STEM MEDIATED COPPER NANOPARTICLES - AN IN VITRO STUDY

ABSTRACT

Background: *Musa sapientum*, commonly known as banana, is an herbaceous plant of the Musaceae family. Copper-based nanoparticles (Cu-NPs) enhance the scavenging capacity of various antioxidants and improve the treatment of ROS-related diseases. The aim of this study is to evaluate the antioxidant activity of banana stem mediated copper nanoparticles.

Materials and Methods: Banana stems weighing 200 grams were cut into small pieces. These fragments were powdered and mixed with 200 cc of sterile distilled water in a blender. CuCl2H2O (0.5 mmol, 5 mL) was applied to ethanol, and the reaction mixture was allowed to react with 1 M NaOH while stirring. Cu-Nps and the prepared banana stem extract were mixed together. The DPPH assay was used to investigate the antioxidant activity of banana stem mediated Cu-Nps. The percent inhibition values were determined using the formula after the absorbance values were measured with a UV spectrophotometer.

Results: At the highest concentration used in the study, the antioxidant activity of banana stem mediated Cu-Nps was 88.10 percent and that of vitamin C was 92.15 percent. This demonstrated that Cu- Nps mediated by banana stems have a concentration-dependent antioxidant effect.

Conclusion: The antioxidant activity of banana stem mediated Cu- Nps tested here was higher than the standard antioxidant drug vitamin c in lower concentration. In higher concentration, vitamin c standard showed higher antioxidant activity.

Keywords: antioxidant; banana stem; copper nanoparticles; Dpph assay; green synthesis

Running title: Antioxidant activity of banana stem mediated copper nanoparticles.

INTRODUCTION

Wounds are physical factors that cause tissue damage and disrupt the normal tissue integrity structure. The wound can be divided into two types based on the extent of tissue damage: open wounds and closed wounds. However, the healing process for such wounds is essentially the same, but the pace of healing is determined by the infection, surgical operation, and medication used(1). The healing process for a tooth extraction includes many steps, including hemostasis (blood clot formation), inflammation (leukocyte infiltration), proliferation (connective tissue formation), granulation and epithelialization stages, and remodelling level(2). As a result, local therapy may be used to reduce systemic effects and avoid unnecessary bleeding, ensuring that the healing process is not hampered(3). Inflammation is often a complicated reaction to the injury's causative agents, such as microbial and cell harm(4). Since it can kill the causative agents of inflammatory lesions and set off a sequence of events aimed at healing or repairing damaged tissue, the inflammatory response is closely linked to the healing process.

Reactive oxygen species (ROS) are increased by phagocytic cells during tooth extraction. (PMN) (5). Oxidants can be involved in different pathological processes in the body as a result of reactive oxygen compounds(6). Oxidants and free radicals are often confused in the medical field because they have identical properties. Antioxidants are medicines that can reduce the activity of free radicals. At their "physiological concentrations," free radicals or ROS may regulate cell growth, differentiation, cell adhesion, cell senescence, and apoptosis. However, if a high concentration of ROS or more than antioxidants is obtained in the body, ROS can be destructive. As a result, ROS can oxidise fat and protein while also damaging DNA through DNA fragmentation(7). Furthermore, chronic inflammation and tissue damage are thought to be caused by repeated exposure to ROS. Endothelial dysfunction and tissue damage can be caused by increased ROS in inflamed tissue. Antioxidant levels in the body decrease as people age, slowing

the healing process. Antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase can neutralise ROS(8).

Musa sapientum, commonly known as banana, is an herbaceous plant of the Musaceae family. Polyphenols, flavonoids, saponins, anthraquinone, and tannin found in banana stem sap can trap free radicals and prevent cell damage(9). Furthermore, due to the activation of clotting factors and the response of endothelial glycoprotein-Ib, fresh banana stem extract can reduce bleeding and clotting time (GPIB). Glycoprotein aids platelet adhesion to endothelium, allowing activated platelets to release granule contents during the healing process. Copper-based nanoparticles (Cu-NPs) enhance the scavenging capacity of various antioxidants and improve the treatment of ROS-related diseases. Copper (Cu) is one of the best elements for human use, as it is involved in tyrosinase and the Cu–ZnSOD enzyme. Connective tissue biosynthesis, cellular respiration, neurotransmitter processing, iron homeostasis, peptide biogenesis, pigment formation, and antioxidant protection are all examples of bioactivities that trigger Cu enzyme activity(10). Cu-NPs, in particular, have excellent catalytic activity for scavenging H2O2 and O2 but not OH, and can also stimulate electron transfer reactions to inactivate H2O2 or OH. The catalytic activity of Cu compounds in organic reactions prompted us to concentrate our research on the use of Cu-Nps. As a consequence, we believe that Cu-Nps potential serves as both a catalytic and an antioxidant activity at the same time. Furthermore, the stability of Cu-NPs can be greatly improved, which improves the overall ROS scavenging ability (10,11). This research aims to evaluate the antioxidant activity of banana stem mediated copper nanoparticles.

MATERIALS AND METHODS

Preparation of Extract

Banana stems were cut into small pieces weighing 200 grams in the middle. These fragments were powdered and mixed with 200 cc of sterile distilled water in a blender. Five minutes were spent blending those parts until they were fully smooth. Whatman filter paper no. 1 was used to filter it. (Figure 1).

Preparation of Cu-Nps

CuCl2H2O (0.5 mmol, 5 mL) was applied to ethanol, and the reaction mixture was allowed to react with 1 M NaOH while stirring. The blue precipitate was formed after adding 15 mL of water to the reaction mixture. The precipitate was filtered and distilled using appropriate methods. (Figure 1) The prepared banana stem extract and Cu-Nps were mixed together.

Antioxidant assay – DPPH assay

The DPPH (1,1-diphenyl-2-picryl-hydrazil) free radical scavenging activity of prepared banana stem extract was determined according to the method of Rajeshkumar (Figure 2). Different concentrations (2-10 µg/ml) of banana stem extract were mixed with 1 mL of 0.1 mM DPPH in methanol solution and 450 µL of 50 mM TrisHCl buffer (pH 7.4) and incubated for 30 minutes. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. BHT was used as control. The percentage inhibition was calculated from the following equation :

$$\% \text{ INHIBITION} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

where the absorbance of control is 1.

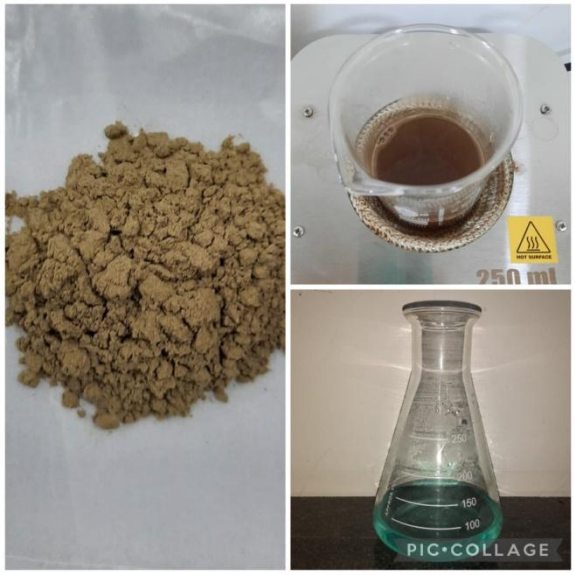

Figure 1: Preparation of Banana stem extract and Cu- Nps

Figure 2: Dpph assay

RESULTS

The results of the antioxidant activity were tabulated and studied as shown in table 1 and figure 3 The results suggest that the % inhibition values are higher than the standard vitamin C at every concentration and so was the antioxidant activity as shown in Figure 3. Descriptive statistics was followed. The antioxidant activity of the banana stem mediated Cu- Nps at 10 µL was 86.10% whereas that of the vitamin c was 25.03 %. The banana stem mediated Cu- Nps showed a higher antioxidant activity by 61.07%. The antioxidant activity of banana stem mediated Cu- Nps at 20 µL was 86.10% whereas that of the vitamin c was 51.13 %. The antioxidant activity of banana stem mediated Cu- Nps at 30 µL was 87.60% whereas that of the vitamin c was 65.54 %. The antioxidant activity of banana stem mediated Cu- Nps at 40 µl was 87.90% whereas that of the vitamin c was 78.68%.

The banana stem mediated Cu- Nps showed a higher antioxidant activity compared to control, Unlike other concentrations of banana stem mediated Cu- Nps, at the highest concentration (50 µL) vitamin C showed a higher antioxidant activity when compared to the banana stem mediated Cu- Nps. The antioxidant activity of banana stem mediated Cu- Nps was 88.10% and that of vitamin C was 92.15%. Hence, it could be concluded that the antioxidant activity of banana stem mediated Cu- Nps tested here was higher than the standard antioxidant drug vitamin c in lower concentration. In higher concentration, vitamin c showed higher antioxidant activity (Table 1, Figure 3).

Table 1: Table compares the percentage inhibition produced by banana stem mediated Cu-Nps with standard Vitamin C

CONCENTRATION (µL)	% INHIBITION OF VITAMIN C	% INHIBITION OF TEST
10	25.03	86.10
20	51.13	86.10
30	65.54	87.60
40	78.68	87.90
50	92.15	88.10

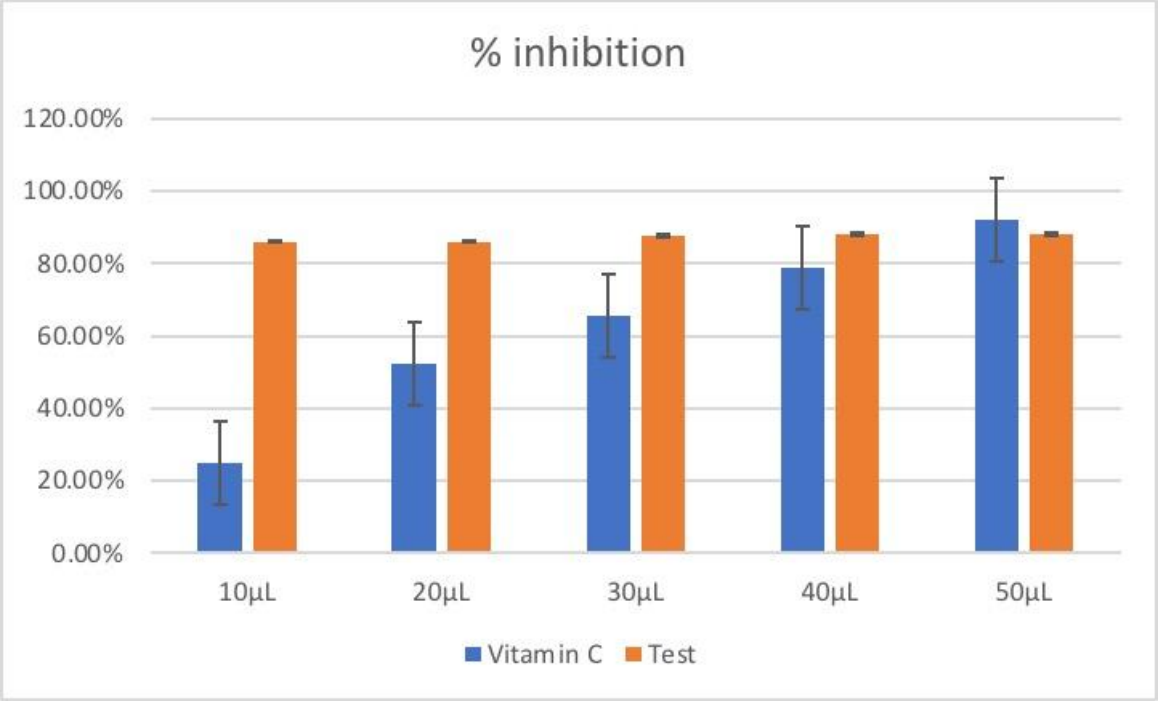

Figure 3: Graph comparing the antioxidant activity of banana stem mediated Cu- Nps with standard vitamin C, while X -axis shows the different concentrations of banana stem mediated Cu- Nps and Vitamin C while Y axis shows % inhibition values obtained. Blue color indicates % inhibition of vitamin C in various concentrations while orange color indicates % inhibition of test sample in various concentrations. The antioxidant activity of banana stem mediated Cu- Nps tested here was higher than the standard antioxidant drug vitamin c in lower concentration. In higher concentration, vitamin c (standard) showed higher antioxidant activity. A concentration dependent antioxidant activity was observed.

DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications (12–24),(25–29),(30),(31). In the present study, activity of banana stem mediating Cu-Nps to scavenge the harmful free radicals or unstable molecules produced during normal metabolism, that possess the ability to damage the cells of our body, was studied by analyzing the ability to scavenge DPPH. Free radical damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, and cancer. Hence, calculating antioxidant activity enables us to nullify these ill effects caused by antioxidants. As

part of the wound healing process, wounds caused by medical attention can trigger an inflammatory response. Vascular permeability can be increased by the release of inflammatory mediators such as bradykinin, histamine, and free radicals from leukocytes. A large number of free radicals can cause cell damage, reduce the cell's ability to adapt to the environment, and inhibit the cell's ability to heal quickly(32).

Ascorbic acid, also known as vitamin C, is a six-lactone carbon atom produced by the liver from glucose. The atoms of vitamin C donate H + or H that is oxidised by ROS, resulting in neutral tricarbonyl ascorbate free radicals. In addition, as compared to other compounds, vitamin C is a compound with a high antioxidant activity. Vitamin C has antioxidant activity of 92.15% in this study, indicating that it can function as an antioxidant according to the norm IC50 (32,33). According to the findings, the antioxidant activity of the banana stem mediated Cu- Nps was 87.70% at 30µL, 87.90% at 40µL, and 88.10 percent at 50µL, indicating that those scores are in line with the appropriate IC50 norm. As a result, the antioxidant activity of the banana stem extract concentrations can be inferred (34). Furthermore, flavonoids found in green banana stem sap can induce antioxidant activity. Since they contain hydroxyl groups, these compounds serve as free radical trappers. Flavonoids can thus serve as hydrogen donors against free radicals as reducing agents (35),(36).

Because of the crude extract, a solution of vitamin C as a positive control (+) has lower antioxidant activity than the banana stem extract used (37). The crude extract does, however, contain several compounds that can be collected, such as flavonoids, saponins, and polyphenols, as well as antioxidant tannins. Vitamin C, on the other hand, is a single molecule. Some of the compounds in the crude extract of banana stem sap have a different ROS damping function, reducing a wide variety of ROS ((38). The copper nanoparticles utilized in this study were not tested in patients which could be a possible limitation of this study. In conclusion, antioxidant activities found in all of the banana stem concentrations indicate that the banana stem has the potential to be developed as a biomaterial for wound healing in the future(39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) ((49,50) (51) (52).

CONCLUSION

The antioxidant activity of banana stem mediated Cu- Nps tested here was higher than the standard antioxidant drug vitamin c in lower concentration. In higher concentration, vitamin c standard showed higher antioxidant activity. A concentration dependent antioxidant activity was observed.

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