https://doi.org/10.33472/AFJBS.6.11.2024.1629-1638

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

ANTIOXIDANT POTENTIAL OF NANOPARTICLES SYNTHESISED WITH CARICA PAPAYA LEAF EXTRACT: AN IN VITRO STUDY

Urvi Echhpal[1], Dr. Khushali K Shah[2*]

[1]Postgraduate student Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India
[2*]Associate Professor Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Corresponding author: [2*]Dr. Khushali K Shah
Associate Professor Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

Email: [1]drurviechhpal@gmail.com, [2*]khushalik.sdc@saveetha.com

Article Info

Volume 6, Issue 11, July 2024

Received: 21 May 2024

Accepted: 27 June 2024

Published: 12 July 2024

doi: 10.33472/AFJBS.6.11.2024.1629-1638

ABSTRACT:

Background: Carica Papaya, is a herbaceous plant popularly known as pawpaw. The remarkable reduction activity of the extract of the leaf of the papaya plant can possibly be used for the synthesis of nanoparticles. Recently, Carica papaya has become popular for the formulation of some NPs.

Materials and Methods Commercially available papaya leaf powder (Bixa Botanical) was employed for the green synthesis of nanoparticles. A formulated copper sulphate solution was made by adding 0.314 g of Copper Sulphate powder (LABOGENS pvt limited) to 60 mL of water. This solution was combined with papaya leaf powder using an orbital shaker to create the master solution. Scanning electron microscopy (SEM) analysis was carried out. (JSM-IT800, White Lab, Saveetha Dental College)

RESULTSThe copper nanoparticles are successfully synthesised by the papaya leaf extract reducing activity. In the assays for DPPH (67% inhibition for 9µL concentration, 75% inhibition for 20µL, 81% at 30µL, 78% at 40µL, and 88.9% at 50µL concentrations) and H2O2 (56% inhibition at 10µL concentration, 68% at 20µL, 69% at 30µL, 77% at 40µL, and 80% at 50µL concentrations), high values were obtained.

ConclusionSince papaya leaf extract has a high concentration of naturally occurring bioactive components and strong antioxidant properties, using it to make copper sulphate nanoparticles is a good substitute for using harmful chemicals. These nanoparticles can be used in impression materials, root canal irrigants, and oral delivery systems.

Keywords: Nanoparticles, Papaya Leaf Benefits, Metallic nanoparticles

1. INTRODUCTION

Papaya has been found to have multiple health benefits. It is not only rich in nutrients; the leaves of Carica papaya contain an enzyme called papain, which may aid in digestion by breaking down proteins.[1] The leaves are commonly known to be used in the treatment of dengue, as a platelet booster. They have also been found to have antioxidant properties. [2]

The prevalence of viral infections, cancer, and diabetes is increasing at an alarming rate around the world, and these diseases are now considered to be the most serious risks to human well-being in the modern period. There is a widespread practice in Asian countries of using papaya leaves [3]

Oxidative stress, which is generated by free radicals and metabolic biological processes, is also linked to most diseases. [4]

A common drawback of conventional methods of nanoparticle synthesis is the use of toxic chemicals like methoxy polyethylene glycol, and phenyl hydrazines, toxic solvents like sodium

benzyl sulphate, and toxic substances produced .[5] More environmentally friendly techniques for producing metal nanoparticles are being developed as science advances.[6] Here, a range of green generated agents (derivatives of microbial compounds) are used to convert metal salts into nanoparticles.[7] These compounds serve as antioxidant and capping agents in vitro. The use of organic components, like phytochemicals, has been proposed as a potential drug for cancer treatment strategy.[8] These chemicals are readily available, affordable, and don't have many negative side effects. [9]

Recent scientific evidence supports the use of certain metallic compounds as effective antioxidant agents. [10]

In this study,our aim was to produce and characterise copper sulphate NPs along with Papaya leaf extract and to evaluate their antioxidant potential.[11] The null hypothesis stated that there is no anti-inflammatory property of nanoparticles synthesised with papaya leaf extract.

2. MATERIALS & METHODS

PREPARATION OF COPPER SULPHATE SOLUTION

An aqueous solution of Copper sulphate (CuSO4) (1.59g) was prepared using double-distilled water. A volume of 60 ml of CuSO4 solution was introduced into a conical flask. (Figure *1*)

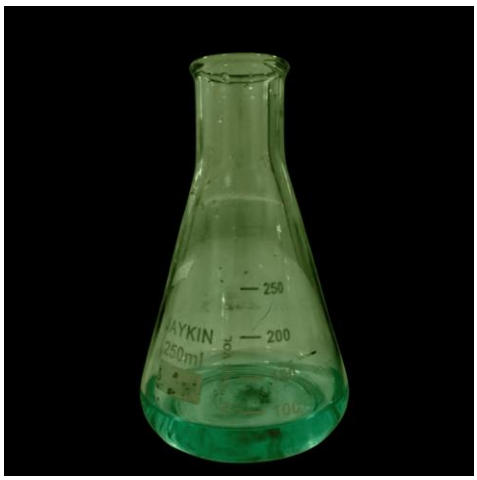

Figure 1: Copper Sulphate Solution

ADDITION OF PAPAYA LEAF POWDER

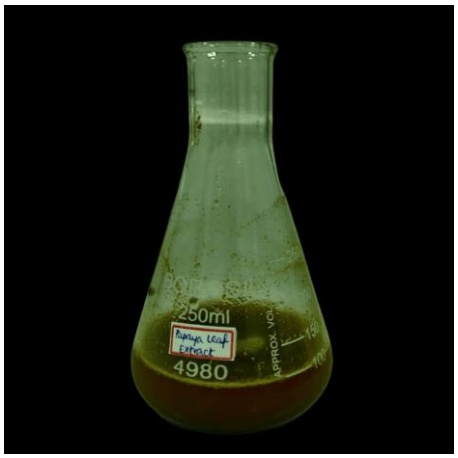

Figure 2: Addition of papaya leaf powder

1 gram of papaya leaf powder was added to the copper sulphate solution and stirred well. It was transferred to a sterile conical flask and kept in an orbital shaker for 1 day. This was done to reduce metal ions and allow nanoparticle formation.(Figure *2*)

Figure 3: ORBITAL SHAKER REMI RS-24

BOILING AND FILTRATION

After the addition of papaya leaf powder, the solution is sealed using aluminum foil, and then is put under orbital shaking in a laboratory orbital shaker (Figure *3*)for 2 hours. After this the solution was boiled down. This process helps in the reduction of the ions of metal and allows for NP formation. (Figure *4*)

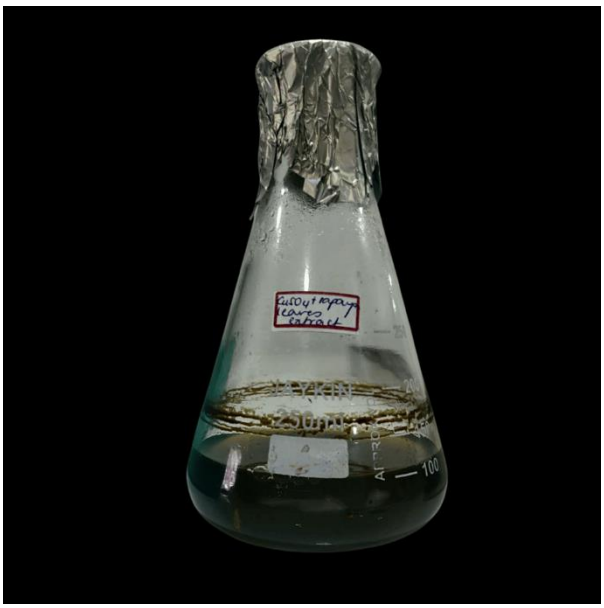

Figure 4: Boiled Down Extract

Filtration of extract was performed using Whatman Filter paper, over the span of 2 hours and repeated twice after first filtration. (Figure *5*)

Figure 5: Filtration of master solution

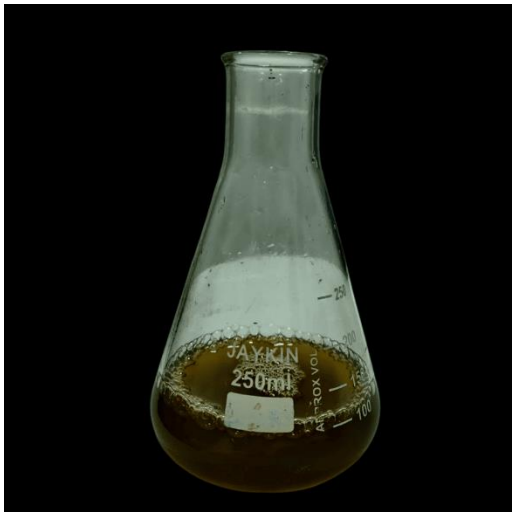

Figure 6: Filtered Master solution

INCUBATION

The filtered solution (Figure *6*) was then placed in Orbit kTM(Figure *7*) for 2 hours , at a temperature of 36 degrees celsius.

CENTRIFUGATION

The solution was centrifuged at 9000 revolutions per minute for 12 minutes to allow for separation of the NPs from the bulk of the solution.

CHARACTERIZATION

SEM ANALYSIS

After synthesis, utilising SEM, the size and shape of the nanoparticles were investigated. A high-resolution microscope was used and the synthesised nanoparticles were examined after being placed on a sample holder after being concealed in a shallow layer of a conductive material.

EDX ANALYSIS

The surface of the NPs that were separated from the Carica papaya extract was subjected to an electron beam scan, which allowed the electrons to strike and activate the material. Each element released X-rays with distinct energies and unique wavelengths almost instantly as it reverted to its initial energy state. The X-ray wavelength and intensity were plotted on the X

and Y axes, respectively, and the associated elements were labeled. The elemental composition of the sample was determined by comparing the peak values on the X-axis with the known wavelengths of each element. (Bruker Corporation, Billerica, Massachusetts; D8 Advance Diffractometer)

TESTING

DPPH ASSAY

Antioxidant activity was assessed using the DPPH (2,2 - diphenyl -1- picrylhydrazyl hydrate) test. The freshly created nanoparticles were diluted using Four hundred and fifty µL of 50 mM Tris-HCl buffer (pH 7.4) and 1 mL of 0.1 mM DPPH in methanol. The mixture was then incubated for 30 minutes at varying concentrations (ten µL, twenty µL, thirty µL, forty µL, and fifty µL). The amount of DPPH free radicals that were decreased was calculated using the absorbance at 517 nm. Ascorbic acid was the standard that was applied. The following formula was used to determine the percentage of inhibition: % inhibition = (control absorbance minus test sample absorbance) times 100/control absorbance. Every concentration's percentage inhibition readings were recorded and added up.

H2O2 ASSAY

0.5 mL of 1 mm ferrous ammonium sulphate, 0.13 mL of 5 mM H2O2, and 3 mL of the generated nanoparticles at various concentrations of 10 µL, 20 µL, 30 µL, 40 µL, and 50 µL were placed into five test tubes. Every test tube was let to stand at room temperature in the dark for five minutes. Next,three millilitres of 1 mM 1,10-phenanthroline was put in each combination, and the samples were shaken to ensure even mixing. The solution was kept at room temperature for ten minutes. After that, the reaction mixture was incubated for ten minutes at room temperature. At five hundred and ten nanometer, the reaction mixture's absorbance was measured. Gallic acid was the typical medication used as control. The amount of hydrogen peroxide scavenged was obtained from the following equation:- % inhibition = (Absorbance of control-Absorbance of test sample) × 100/Absorbance of control The values of % inhibition at each concentration were recorded and tabulated.

3. RESULTS

SEM ANALYSIS

SEM analysis was done at White Lab, Saveetha Dental College (SkyScan2214TM) SEM analysis was used to check for size and shape of the synthesized nanoparticles. The conical shaped nanoparticles with a uniform size distribution were visible in the SEM images. By measuring several nanoparticles, the average particle size was ascertained to be 65 nm in size. The SEM study demonstrated the surface properties of the nanoparticles and offered visual confirmation of their structure. (Figure *7*)

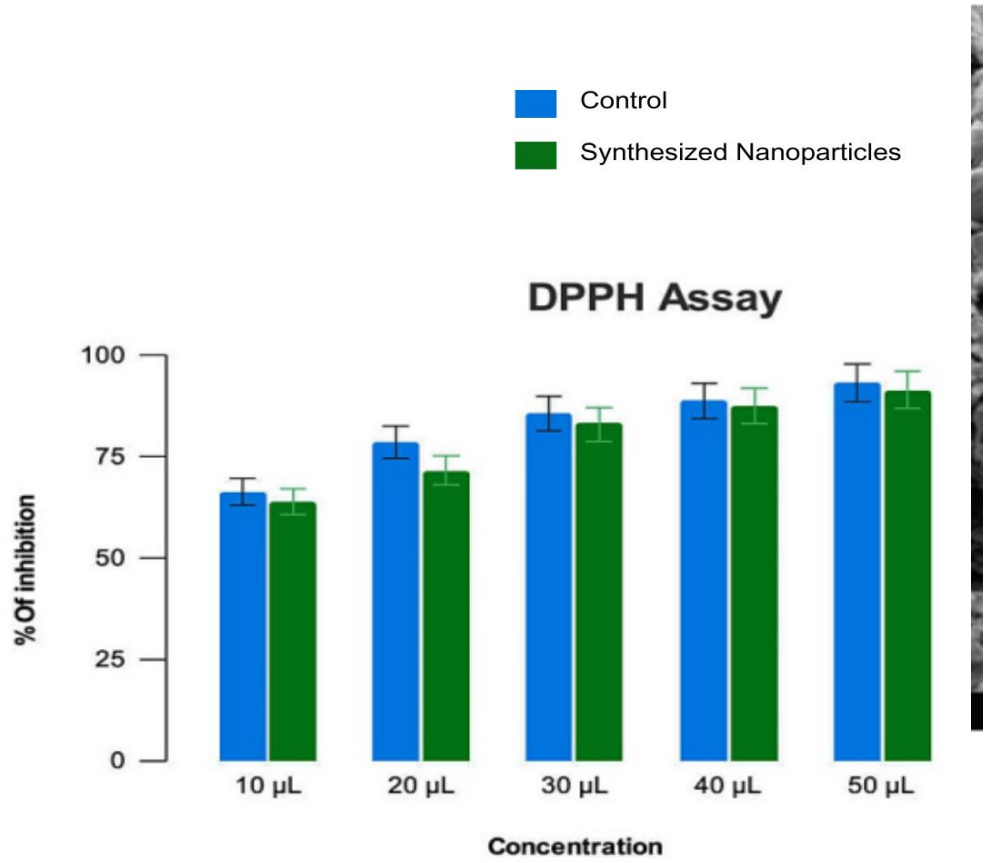

Figure 7: SEM ANALYSIS

EDX ANALYSIS

To check the ionic presence of the newly NPs, an EDAX analysis was done . The existence of Copper (Cu) elements was confirmed and therefore demonstrated the successful production of Copper Sulphate nanoparticles . (figure 8)

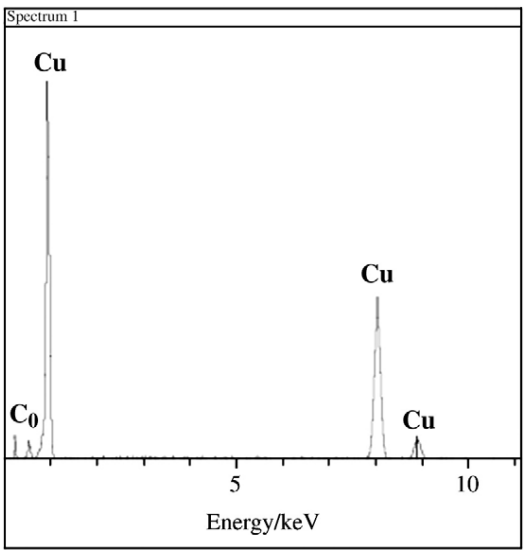

FIGURE:8 EDX

FIGURE 9: Antioxidant activity analysis by DPPH assay

FIGURE 9 shows the Antioxidant activity analysis by DPPH assay

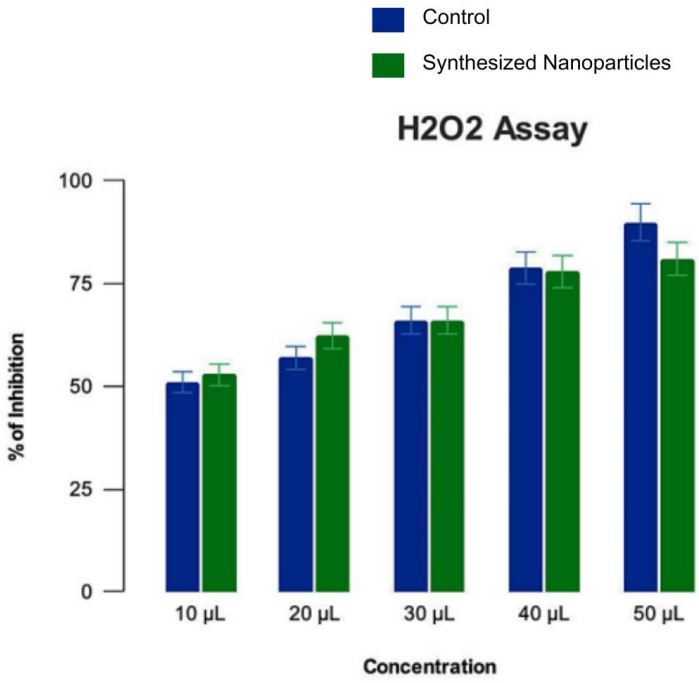

FIGURE 10: H2O2 ASSAY

The synthesised extract exhibited greater antioxidant activity at lower doses. Twenty one ml of the extract had the most high reducing potential of 85% (p = 0.012) compared to the control, with a total of 10 microliters having an antioxidant potential of 80% (p = 0.000).
(Figure 10)

4. DISCUSSION

Carica Papaya is a nutritionally a strong source of multiple vitamins like A, B and C and also a fair source of calcium and iron. [12] The use of papaya leaf in nanoparticle synthesis is a novel process .It not only provides an environment friendly and economic option to commonly used agents, but also has been found to be significantly useful. [13]The mechanism of action of biologically active is for them to act as reducing agents,therefore allowing the change from metal ions to NPs. Effective antioxidant activity was observed in this study.
The largest number of peaks related to elemental copper is displayed by the Energy Dispersive X-ray (EDX) analysis. This indicates with certainty that the test sample contains copper-influenced nanoparticles. As a result, we can now affirm that the papaya leaf extract was used to create the copper sulfate nanoparticles.
The 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay is a commonly used , fast, executable , and affordable technique for figuring out how much of an antioxidant a substance is. In the assay, free radicals are used to assess a substance's ability to donate hydrogen or participate in free-radical scavenging (FRS). The process has to do with eliminating the stable free radical DPPH, which forms a noticeable purple peak at 517 nm in the absorbance spectrum when it combines with an odd electron.

Free radical elimination is a phenomenon that is used to evaluate a compound's radical scavenging ability as a proxy for its antioxidant capacity.9. The amount of free radical eliminated, or the number of H2 atoms released by the test substance, is indicated by the% inhibition result that was achieved. The supplemental H2O2 assay and the DPPH assay for the produced nanoparticles both indicated that the percentage of inhibition was highest at 50µL concentration. The outcomes were consistent with what had been previously noted.[14]
There is a clear indication that antioxidant activity follows a dosage-dependent trend, rising in proportion to dosage concentration. The concentration of the nanoparticles was higher than that of the standard. The results of this study show that the DPPH scavenging activity is recognized by a visual colour change from blue to yellow at 518 nm, which shows that the absorbance values of the nanoparticles have increased in a concentration-dependent manner. Unlike a related study, DPPH exhibits an absorption band at 515 nm. which diminishes upon reduction by an antiradical compound [13,15].

5. CONCLUSIONS

Because of its quick, economical, and environmentally friendly protocol that offers a simplified method of producing novel NPs, the use of biomaterials for this purpose has attracted a lot of interest from researchers

Limitation

The study is limited to in vitro environments, which may not adequately replicate the complex interactions that occur in the human body. To confirm the observed effects in a more complete biological setting, in-vivo studies are necessary. Furthermore, harvesting methods, regional climate, and geographic location can generate variabilities in the content of plant extracts, which could jeopardise the findings' reproducibility.

DISCLOSURES

Subjects from humans: Every author has attested that neither human subjects nor human tissue were used in this investigation.

6. REFERENCES

1. S K, Panneer Selvam S, Shanmugam R, Ramadoss R, Sundar S, Ramani P. Exploring the Antimicrobial Potential and Cytotoxic Effects of Different Brassica oleracea Varieties. Cureus. 2024 May;16(5):e59613.
2. Singh SP, Kumar S, Mathan SV, Tomar MS, Singh RK, Verma PK, et al. Therapeutic application of Carica papaya leaf extract in the management of human diseases. Daru. 2020 Dec;28(2):735–44.
3. Sharma A, Sharma R, Sharma M, Kumar M, Barbhai MD, Lorenzo JM, et al. L. Leaves: Deciphering Its Antioxidant Bioactives, Biological Activities, Innovative Products, and Safety Aspects. Oxid Med Cell Longev. 2022 Jun 9;2022:2451733.
4. Prabhu Venkatesh D, S G, Ramani P, S R, Ramalingam K. In Vitro Evaluation of Antioxidant and Anti-inflammatory Potentials of Herbal Formulation Containing Marigold Flower (Calendula officinalis L.) Tea. Cureus. 2023 Aug;15(8):e43308.
5. Nasim I, Rajesh Kumar S, Vishnupriya V, Jabin Z. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. Bioinformation. 2020 Nov 30;16(11):831–6.
6. Thiraviarajan A, Shanmugam R, Munusamy T. Preparation of Andrographis Paniculata and Rosa formulation and its antibacterial activity against urinary tract infection causing pathogens. J Pharm Bioallied Sci. 2024 Apr;16(Suppl 2):S1360–4.
7. Sneka, Santhakumar P. Antibacterial Activity of Selenium Nanoparticles extracted from

Capparis decidua against Escherichia coli and Lactobacillus Species. J Adv Pharm Technol Res. 2021 Aug 6;4452–4.

8. Nasim I, Professor and Head, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and technical Sciences, Saveetha University, Chennai, India. Green synthesis of reduced graphene oxide nanoparticles, its characterization and antimicrobial properties against common oral pathogens. Int J Dent Oral Sci. 2021 Feb 22;1670–5.
9. Rajagopal S, Sugumaran S. The Antibacterial Effectiveness of Citrullus lanatus-Mediated Stannous Nanoparticles on Streptococcus mutans. Cureus. 2023 Sep;15(9):e45504.
10. Chatterjee S, R J, S R. Green Synthesis of Zinc Oxide Nanoparticles Using Chamomile and Green Tea Extracts and Evaluation of Their Anti-inflammatory and Antioxidant Activity: An In Vitro Study. Cureus. 2023 Sep;15(9):e46088.
11. Rajasekar R, Shanmugam R, Anandan J. Biosynthesis of Cissus rotundifolia Stem-Mediated Titanium Dioxide Nanoparticles and Their Anticariogenic Activity against Streptococcus mutans and Lactobacillus sp. J Pharm Bioallied Sci. 2024 Apr;16(Suppl 2):S1350–3.
12. Sandhya Rani S, Vedavijaya T, Sree P K, Shanmugasundaram J, Deepalatha C, Raju MG, et al. A Comprehensive Analysis of Phytochemical Composition, Acute Toxicity Assessment, and Antioxidant Potential of Ethanolic Extract of Carica Papaya Seeds. Cureus. 2023 Nov;15(11):e49686.
13. Yi-Fang Chu †., Jie Sun †., Xianzhong Wu †. And, Liu*†‡ RH. Antioxidant and Antiproliferative Activities of Common Vegetables. 2002 Oct 10 [cited 2024 Jan 24]; Available from: https://pubs.acs.org/doi/abs/10.1021/jf020665f
14. Shalini V, Shanmugam R, Manigandan P. Cytoplasmic leakage and protein leakage analysis of Ocimum gratissimum stem extract-mediated silver nanoparticles against wound pathogens. J Pharm Bioallied Sci. 2024 Apr;16(Suppl 2):S1354–9.
15. [No title] [Internet]. [cited 2024 Jan 24]. Available from: https://doi.org/10.1093/mutage/5.4.327