Antimicrobial activity and cytotoxic effect of copper nanoparticles synthesized using *Ficus benghalensis* extract against oral pathogens

Pratheebha C[1], S. Rajeshkumar[2*], D. Ezhilarasan[3], T Lakshmi[4]
[1,3,4] Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai - 600077, Tamilnadu, India;
[2]Nanomedicine Lab;Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077,Tamilnadu,India;
[*]**Corresponding Author:Dr.S.Rajeshkumar;**
Email:rajeshkumars.sdc@saveetha.com,

Abstract :
Medicinal plants have always been used as an excellent source for treating disorders or diseases for centuries because they have therapeutic value. *Ficus benghalensis* belongs to the family Moraceae. It is a very large tree height of 20–30 m, with wide-spreading branches bearing aerial roots. To boost the immune system its root extract has been used in medicine since ages. They are very useful in treatment of erysipelas, ulcers, vomiting, biliousness, vaginal complaints, fever, inflammations, and leprosy. To evaluate the antimicrobial activity and cytotoxic effect of copper nanoparticles synthesized using Ficus benghalensis extract against oral pathogens.The antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans*. MHA agar was utilized for this activity to determine the zone of inhibition and Brine shrimp lethality assay was performed to test the cytotoxic effect.Copper nanoparticles exhibited good antimicrobial effect against oral pathogens and also exhibited good cytotoxic effect.From the present study it can be concluded that *Ficus benghalensis* has a good antimicrobial effect and cytotoxic effect and it can be used in the pharmaceutical industries.

Keywords: antimicrobial, cytotoxic, inhibition, oral pathogens, inflammation,copper nanoparticle, green synthesis, innovative technology, eco friendly.

Background :
Medicinal plants are highly effective and they are fully utilized by the primary health care workers. *Ficus benghalensis.* L belongs to the family Moraceae, it has many other medicinal properties like they are effective and can be taken for dysentery, diarrhoea, leucorrhea, menorrhagia, for vaginal and UTI [1]. Herbs and Medicinal plants are used in combination with Ayurveda and they are utilized in folklore medicine as they exhibit good cytotoxic activity [2]. Ficus species are very strong because of its biological and its cytotoxic properties and also it will allow it to diffuse the toxic free radical , because of this property it can be used as a food additive or in pharmaceutical and nutraceutical industries [3].
Medicinal plants are always used as an excellent source for treating disorders or diseases for centuries as an alternative remedy because they contain numerous active constituents of therapeutic value [4]. The development of microbial resistance to antibiotics has led the researchers to investigate the alternative sources for the treatment of resistant strains [5]. The profile of *Ficus benghalensis* roots is explored to have good antimicrobial effect and it is exploited for developing a novel antimicrobial agent of high potential. Plants are now considered as an important source of developing the high potential and useful structures for developing the new chemotherapeutic agents [6]. In the previous studies it has been reported that latex and extracts of different species of Ficus are cytotoxic to some human cancerous cell lines. In the previous study, the cytotoxicity of leaf extract and fruits were analysed as well as the latex of Ficuscarica L. on HeLa cell line were evaluated, and they have got [7].
The chemical and physical properties of these nanoparticles can be modified using different synthetic strategies , chemical reactions and conditions and/or via postsynthetic chemical treatments has been largely responsible for the rapid growth of interest in these nanomaterials and their applications in catalysis [8][9],[10].
Our team has extensive knowledge and research experience that has translated into high quality publications [11–15],[16],[17],[11],[18],[19],[20],[21][13,22,23],[24–28][29] [30] [31] [32] [32,33] [34] [35] [36] [37] [38] [39]

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[40] [41] [42] [43] [44] [45]. In the present study we evaluated the antimicrobial activity and cytotoxic activity of copper nanoparticles synthesized using *Ficus benghalensis* extract against oral pathogens.

Materials and Method :

Preparation of plant extract :

1 g of *Ficus benghalensis* powder was taken in a beaker and 100 ml of distilled water was measured using a measuring cylinder and transferred into the beaker and boiled using a heating mantle at 60 to 70°C. Then it was filtered using a whatman No. 1 filter paper.

Copper nanoparticle synthesis :

Anhydrous copper sulphate weighed exactly 0.507 g and 70 mL of distilled water was measured using a measuring cylinder and it was added to 30 mL of plant extract that was already prepared before and the resulting solution that was obtained was blue coloured. An uniform dispersion was made with the help of an orbital shaker to initiate the synthesis process and the color change of the solution was observed periodically. The synthesized solution was centrifuged in a Lark refrigerated centrifuge at 8000 rpm for 10 minutes. As a result of centrifuge, the pellet was obtained and washed with distilled water twice. The purified pellet was then dried and heated in a furnace at 100 - 150°C. Finally, the dried powder was collected and stored in an airtight Eppendorf tube for further analysis.

Characterization of Copper Nanoparticles

The copper nanoparticles synthesized were measured optically using double beam UV–vis spectroscopy. It is mostly used in analytical chemistry for quantitative determination of different ions, compounds and biological macromolecules at different wavelengths. The synthesized CuNPs were optically measured at different wavelengths ranging from 250 nm to 350 nm.

Antibacterial Activity

Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans*. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and let stable for solidification. The wells were cut using the well cutter and the test organisms were swabbed.The nanoparticles with different concentrations were loaded into the wells and the plates were incubated for 24 hours at 37 ° C. After the incubation period was over the zone of inhibition was measured and the antimicrobial results were obtained.

Brine shrimp lethality assay :

Salt water preparation :

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well cell culture plates were taken and 10-12 ml of saline water was filled, followed by adding 10 nauplii to each well. The aqueous solution of copper nanoparticle synthesis using *Ficus benghalensis* was slowly added to each well at different concentrations (5µL,10 µL,20 µL, 40 µL,80µL). The plates were incubated for 24 hours. After 24 hours, the cell culture plates were observed and noted for the number of live nauplii present and calculated by using the following formula [Fig 3].

% death = Number of dead nauplii / Number of dead nauplii − number of live nauplii × 100

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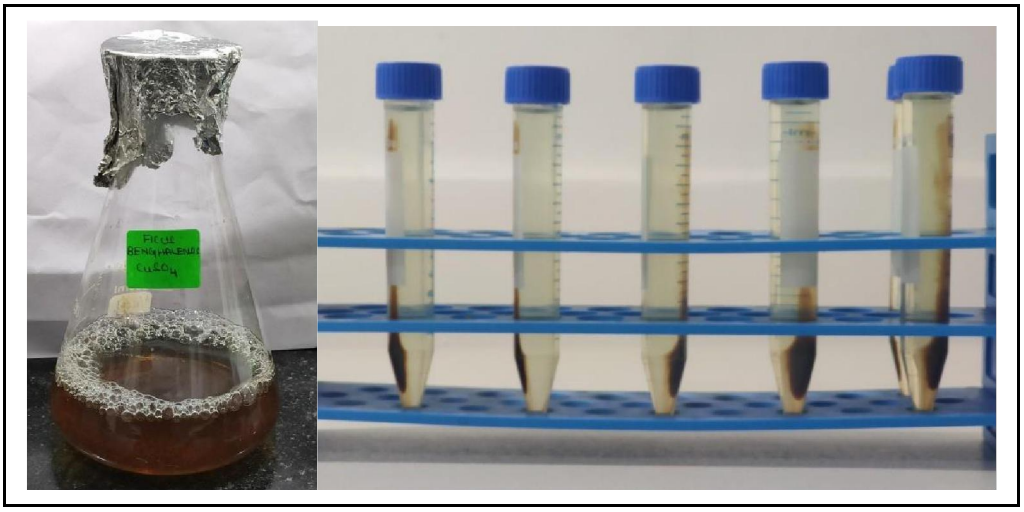

Figure 1 : The plant extract was prepared by dissolving 1 g of *Ficus benghalensis* powder in 100 ml of distilled water.

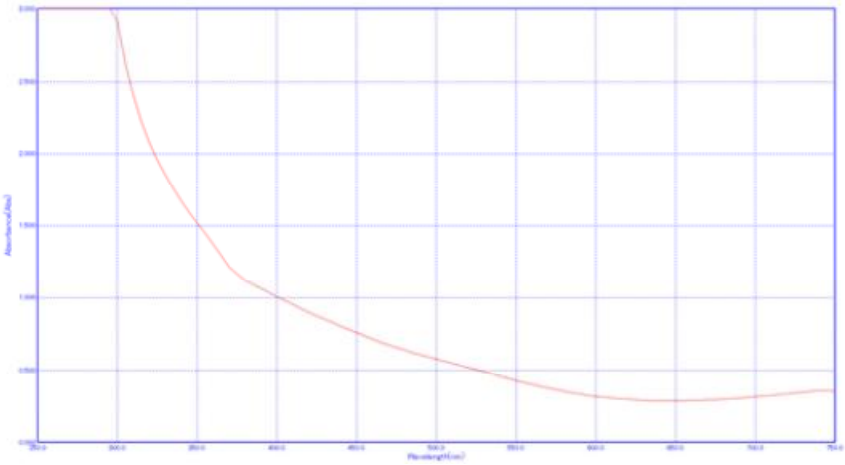

Figure 2 : Graph showing the absorbance of copper nanoparticles synthesized using *Ficus benghalensis.*

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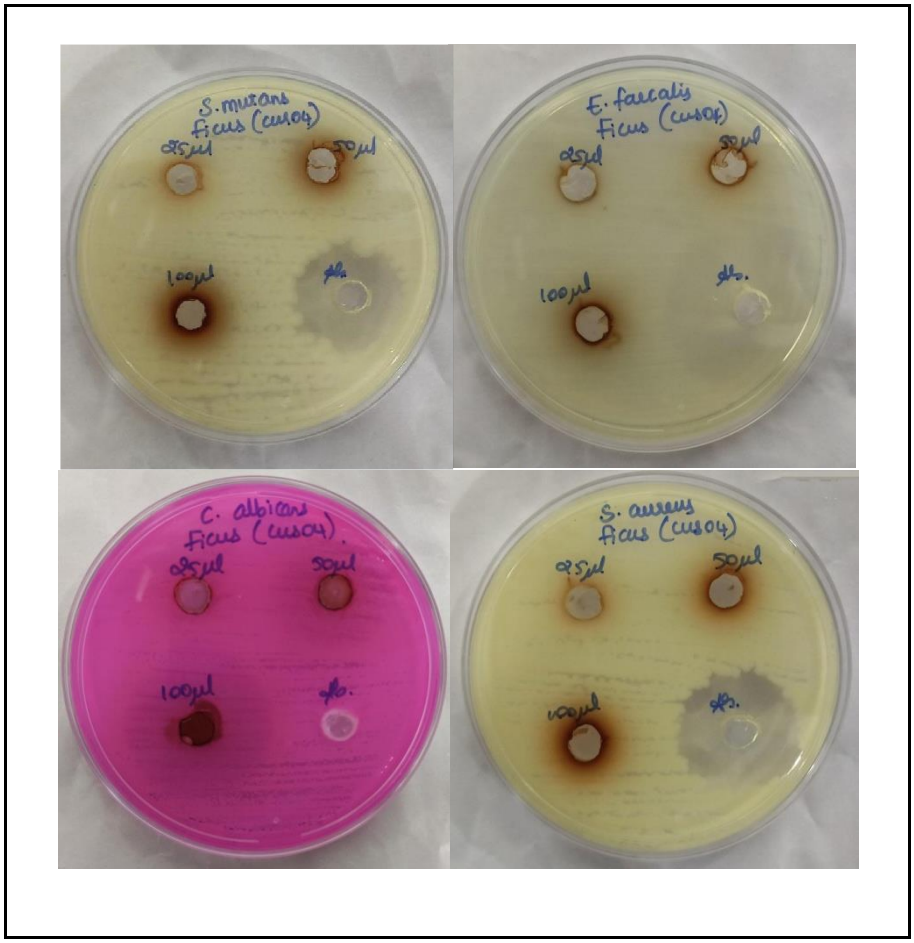

Figure 3 : Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans.* MHA agar was utilized for this activity to determine the zone of inhibition.

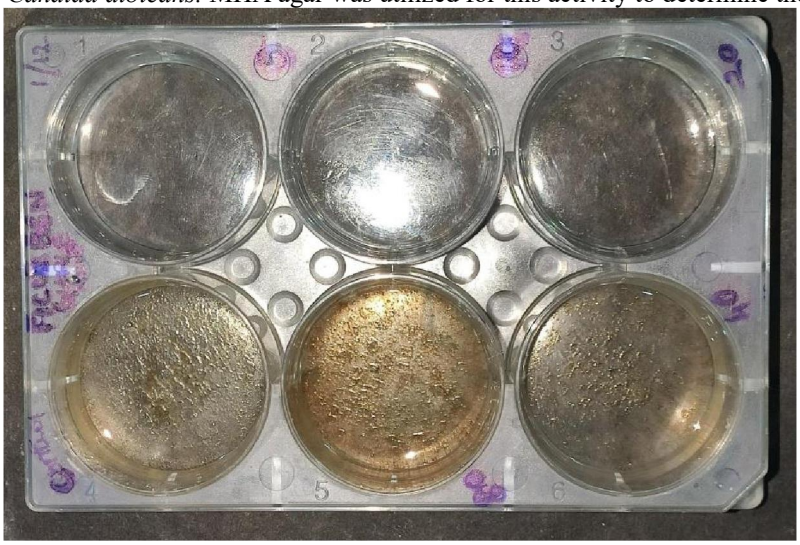

Figure 4 : Brine shrimp lethality assay was conducted by 2g of iodine free salt weighed and dissolved in 200ml of distilled water.

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Results and Discussion :

Figure 1 shows the plant extract preparation by dissolving 1 g of *Ficus benghalensis* powder in 100 ml of distilled water and the purified pellet that was collected after centrifugation. **Figure 2** shows strong peaks at 520 nm confirm the CuNPs synthesis using *Ficus benghalensis*. **Figure 3** shows the Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans.* MHA agar was utilized for this activity to determine the zone of inhibition. The cytotoxic effect of copper nanoparticles is confirmed using brine shrimp lethality assay shown in **Figure 4**.

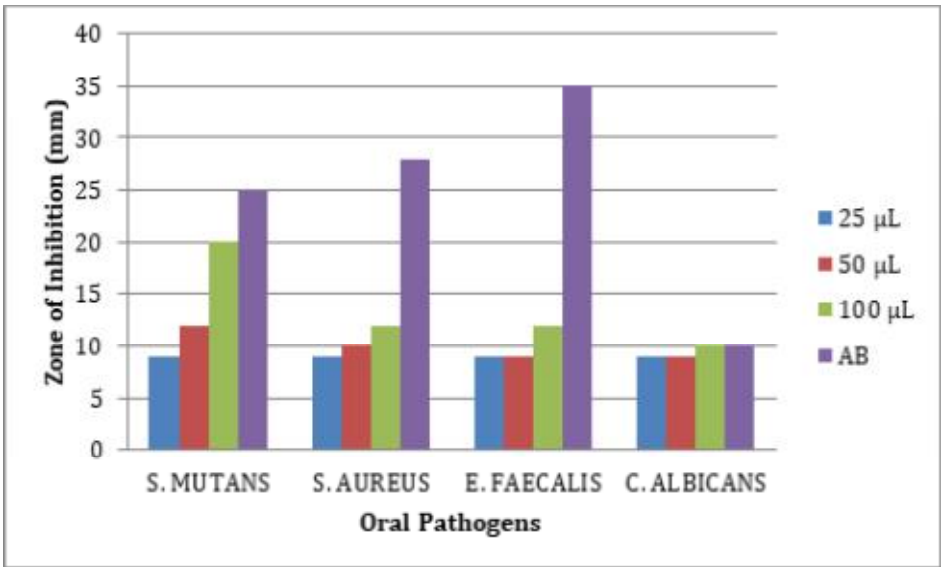

Figure 5 : The above graph represents the zone of inhibition by *Staphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans* representing its antibacterial activity of respective nanoparticles against the strain. The X-axis indicates the concentration of nanoparticles and Y-axis indicates the zone of inhibition.

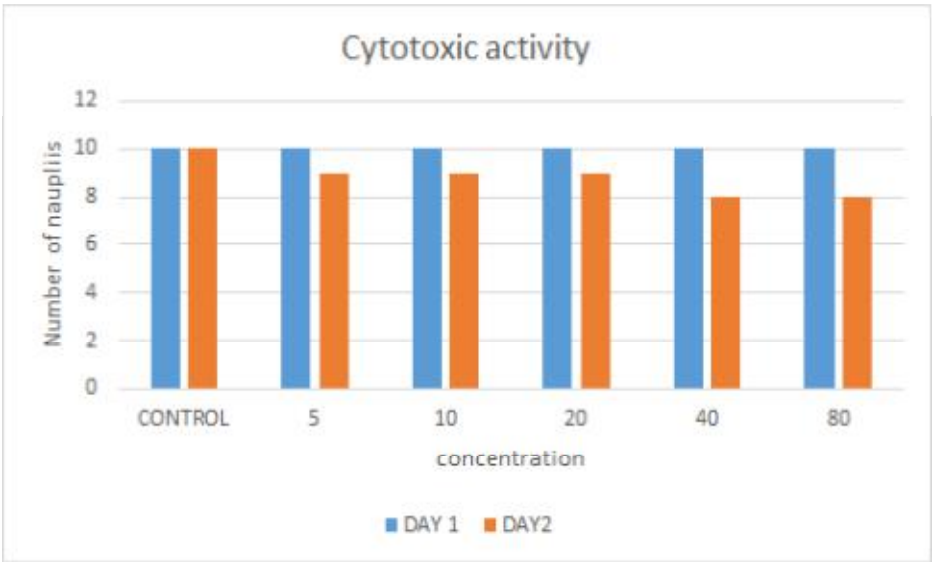

Figure 6 : The above graph represents the cytotoxic activity of *Ficus benghalensis* in different concentrations. The X-axis indicates the concentration of nanoparticles and the Y-axis indicates the number of live nauplii.

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Antimicrobial activity :

In the present study, a test for antimicrobial activity was carried out using the agar well diffusion activity was carried out using the agar well identifying the inhibitory effect over S*taphylococcus aureus*, *Streptococcus mutan*s, *E.Faecalis*, *Candida albicans* [Fig3]. Each plate had 4 wells each with different nanoparticle concentrations being 25µL, 50µL, 100µL, while the fourth one is a standard that is used as a control. Against S*taphylococcus aureus*, the diameter of inhibition of the nanoparticles at 25µL, 50µL, 100µL, Ab is observed to be 9 mm, 10mm, 12mm and 28 mm respectively. With *Streptococcus mutan*s, the at 25µL, 50µL, 100µL, while the fourth one is a control is observed to be 9 mm, 12mm, 20mm, 25mm. Against E.faecalis the diameter of inhibition of the nanoparticles at 25µL, 50µL,100µL,Ab is observed to be 9 mm, 9mm, 12 mm, 35 mm.Against *Candida albicans,* the diameter of inhibition of the nanoparticles at 25µL, 50µL, 100µL, AB is observed to be 9mm, 9mm, 10mm, 10mm. The results of *Ficus benghalensis* mediated synthesis of copper nanoparticles show effective antimicrobial activity against oral pathogens. [Fig 5].

Cytotoxic activity :

The test for cytotoxic properties was assessed using brine shrimps. Ten nauplii were placed in each of 6 wells with one standard and the remaining with nanoparticles concentrations 5 µL, 10µL, 20µL, 40µL, 80µL. LD_{50} concentration was obtained to be 80µL, with half the population of nauplii in the respective well surviving , post - incubation. When the CuONPs was synthesised using Ficus benghalensis were injected into the culture containing shrimp larvae, it was observed that it can inhibit the growth of the cells, which means that they also have the potential to inhibit the growth of normal human cell since its growth pattern resembles that of shrimp larvae. Hence, this can be also applied to carcinogenic cells as a carcinogenic cell is also a normal cell with increased metabolic and rapid division. [Fig 6].

In another study done by Shubharani et al. [46] in his research he demonstrated the antimicrobial and antioxidant potential of SeNPs biosynthesised using ethanol extract of bee propolis. In the previous studies, many other researches were performed regarding Copper nanoparticles for various other activities with various plant extracts like *Persea americana* seeds, Cissus arnottiana plant extract [47], [48],[49], [50]. Recent studies on the synthesis of SeNPs by the aqueous extract of Bougainvillea spectabilis flower, dried Vitis vinifera fruits and Leucas lavandulifolia leaf, and highlighted the likely role of phytochemicals such as amines, alcohols, phenols, terpenoids, sugar, and carboxylic acids in reduction of selenium ions and stabilization of SeNPs. The other study demonstrated by Boromund et al. [51] , selenium nanoparticles were synthesised using chemical reduction methods, which resulted in the spherical shaped Se-NPs without using capping agents.

The results of the study clearly indicated that the growth of S. aureus and S. epidermidis were inhibited by polyvinyl alcohol (PVA) stabilized Se-NPs at concentration of 125 ppm. It showed that PVA coated Se nanoparticles could have significant potential such as it can be used in S. aureus infection-resistant coatings on food supplements, medical devices, and other biomedicine products. In other previous studies, Copper nanoparticles were synthesized to check its anti fungal properties[52],[53]).Other studies involved in the synthesis of copper nanoparticles using *Cissus vitiginea* and its antioxidant and antibacterial activity against urinary tract infection pathogens were reported to exhibit a very good antibacterial and antioxidant activity [54].

Simple zoologic organism (an arthropod) is the brine shrimp. The brine shrimp test (BST) is used as a tool to measure the bioactivity in plant extracts and established as a simple, bench-top, rapid, in-house, and low cost prescreen for cytotoxicity and pesticidal activities [55]. For the last 20 years the research of the studies conducted by Tawaha et al. and Ali et al. The brine shrimp bioassay led to the discovery of the cytotoxic effects of a wide range of plants [56,57]. Sampling error might be there in our study of what we performed. It is now performed as an in vitro study but it can be expanded as an in vivo study for further experimentation. Greener medicines are wanted in our country which is very much required which has limited side effects when compared to synthetic.

Conclusion :

Greener medicine is nowadays gaining more people's attention and the responsibility lies to a medical practitioner to use it as an alternative safer medicine for the people. From the present study, it was concluded that the copper nanoparticle extract prepared by using *Ficus benghalensis* serves as a potent antioxidant, antimicrobial and cytotoxic agent and has a great efficacy as an antibacterial agent especially against *Streptococcus mutans*, thereby indicating

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its potential to be incorporated as a newer avenue of green medicine in the field of dentistry. This research brings in a new medicine and to be used in daily practice.
innovation for the progression of greener.

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Conflict of interest :
The authors declare that there was no conflict of interest

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