

Preparation of *Ficus benghalensis* mediated copper nanoparticles and its based mouthwash and to check its cytotoxic activity

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Abstract:

Ficus benghalensis commonly called the “banyan” is the native and remarkable Indian tree that is useful in a variety of physiological and pathological conditions. It belongs to the family “*Moraceae*”. The tree plays a crucial role in the wound healing process as it produces special bioactive components. It has many beneficial pharmacological activities which are further helpful in various medical fields. Copper nanoparticles are used in this study because of their strong stability in existing both physical and chemical nature. It is also economical and easily available. Cytotoxicity is a process of being toxic to the cells. The cytotoxic activity of the *Ficus benghalensis* is tested to mediate any possible treatments for general disorders. *Ficus benghalensis* plant extract was prepared and then the copper nanoparticles were synthesized and its based mouthwash was prepared. Then the cytotoxic activity at concentration of 10 mg of the prepared mouthwash per μL at volume 5 μL , 10 μL , 20 μL , 40 μL , 80 μL and control in a 6 well cell culture plate was assessed. The cytotoxicity was observed to be 10, 10, 9, 8, 8, 10 live nauplii at the differentiating concentrations of 10 mg per μL of the mouthwash at volume 5 μL , 10 μL , 20 μL , 40 μL , 80 μL , and control wells respectively after 48 hrs. *Ficus benghalensis* mediated copper nanoparticles mouthwash was not toxic to the living nauplii as it did not cause the death of it.

Keywords: nanoparticles, silver, *Ficus benghalensis*, antioxidant activity

Background:

Extensive medicinal research has been conducted on traditional medicinal plants which are rich in natural sources and widely used in preventive, curing, and treatment techniques [1]. Plants and their extract are more significantly used by more than half of the world's population. *Ficus benghalensis* commonly called the “banyan” is the native and remarkable Indian tree that is useful in a variety of physiological and pathological conditions [2]. The tree species vary from around 800 to 2000. The tree has a sacred religious value among the Hindus in India. It is a medicinal plant used in numerous conditions. The secondary constituents or phytoconstituents of *Ficus benghalensis* possess a crucial role in the wound healing process [3]. Plant extract of *Ficus benghalensis* is useful in excision and incision wound healing processes, because it has re-epithelialization capabilities, increases the rate of contraction of the wound, and strengthens skin breathing.

It has many effective pharmaceutical effects, the most important of them is it possesses strong antioxidant activity which might be because of its polyphenolic nature [4]. Respiratory, endocrine, and gastrointestinal disorders can be treated using the fruit of *Ficus benghalensis* [5]. It is used as an Ayurvedic medicine as it treats dysentery, piles, diarrhea, teeth disorders, skin disorders and also boosts the immune system. The fruit of the tree also exhibits anthelmintic and anti-tumor activity. The insulinase activity produced from the liver and kidney is reported by the tree extract. The crown of the *Ficus benghalensis* tree contains an aerial root which is determined to be an ideal plant organ that is useful for study phenomena [6].

Multidisciplinary scientific fields use the application of nanotechnology in various aids [7]. It brings out revolutionary advancements in the developing pharmaceutical markets. Nanomedicine is a newer field that supports the present medical field in providing preventive and treatment measures. It is also used as a diagnostic and screening tool. Metal, carbon bases, ceramic, polymer, and semiconductor nanoparticles are commonly used

nanoparticles [8]. Copper, silver, and selenium are the most commonly used metal nanoparticles that have a strong antibacterial activity [9]. Various authors' works on copper nanoparticles are promising to state that it possesses bacteriostatic and antifungal properties [10]. Copper nanoparticles are the next most commonly used metal nanoparticles to silver [11] because of their great stability in nature both when they are possessed in their physical and chemical nature [12]. They are strong and powerful antimicrobial agents, this property plays a crucial role in the treatment of harmful diseases [13].

Cytotoxicity is a process of being toxic to the cells. Examples of toxic agents include different types of venom present in various plants which are considered to be dangerous [14]. Exposure of the cells to cytotoxic compounds called cytostatics results in different cell outcomes [15]. The cytotoxic agents will alter the chemical and biological nature of the cell leading to its dysfunction [16]. Cytotoxic agents cause cell death, prevent cell growth and proliferation, and inhibit cell function which causes cell failure in varying degrees. The aim of this study is to prepare *Ficus benghalensis* (*F. Benghalensis*) mediated copper nanoparticle (Cu-NP) based mouthwash and its cytotoxic activity is analyzed.

Materials and methods:

Preparation of the plant extract:

Commercially available dry powder of *Ficus benghalensis* was used for this experiment. The experiment was carried out by dissolving 1g of *Ficus benghalensis* in 100ml of distilled water. The mixture was then boiled in a heating mantle at 70 degrees celsius for up to 10 minutes. The boiled mixture was then filtered using a Whatman number 1 filter paper to obtain the plant extract. Then 40 ml of plant extract was measured using a measuring cylinder and the mixture was a

Preparation of the mouthwash:

To a centrifuge tube, 10 ml of distilled water was taken then 0.3 g of sucrose was measured using an electronic weighing scale and was added to the tube containing the water, the mixture was mixed well and then 0.01 g of sodium lauryl sulfate and 0.001 g of sodium benzoate were added and mixed well. Then 12 drops of the *Ficus benghalensis* plant pellets were added to the above mixture followed by adding 2 drops of peppermint oil and it was mixed well.

Cytotoxic activity:

Brine shrimp lethality assay:

Saltwater preparation:

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water. Then 6 well of the cell culture plates were taken and 10-12 ml of saline water was filled respectively. 10 mg per μL of concentration was then added according to the volume level specified 5 μL , 10 μL , 20 μL , 40 μL , 80 μL and control to the prepped 6 ELISA wells respectively. Then the 10 live nauplii were slowly added to each well in the ELISA microtiter plate.

The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for a number of live nauplii present and calculated by using the following formula:

Cytotoxic activity: $\text{number of dead nauplii} / \text{number of dead nauplii} + \text{number of live nauplii} \times 100$

Results:

The *Ficus benghalensis* plant extract mediated copper nanoparticles is shown in **Figure 1**, the brownish yellow discoloration of the extract concluded the synthesized copper nanoparticles. The mouthwash then prepared is shown in **Figure 2**, whose cytotoxic activity was then tested. The cytotoxic activity was performed and after 48 hrs the cell culture plates shown in **Figure 3**, the following were observed and concluded that the wells shown in **Figure 4** contained the nauplii with different prepared mouthwash concentration with 10 mg of mouthwash per μL were 10 number of nauplii in the 5 μL and 10 μL and the rest of the wells with gradient increase in the mouthwash concentration contained 9 number of nauplii in 20 μL and there were 8 nauplii alive in 40 μL , 80 μL , wells and control well contained 10 nauplii. **Table 1** shows the results tabulated clearly of the cytotoxicity tested using the prepared *Ficus benghalensis* copper nanoparticles and its based mouthwash.

Table 1: Cytotoxic activity of the mouthwash prepared using *Ficus benghalensis* plant extract with its mediated copper nanoparticles.

The concentration of copper nanoparticles based mouthwash (10 mg per μL)	Number of living organisms	
	First day	Second day
5 μL	10	10
10 μL	10	10
20 μL	10	9
40 μL	10	8
80 μL	10	8
control	10	10

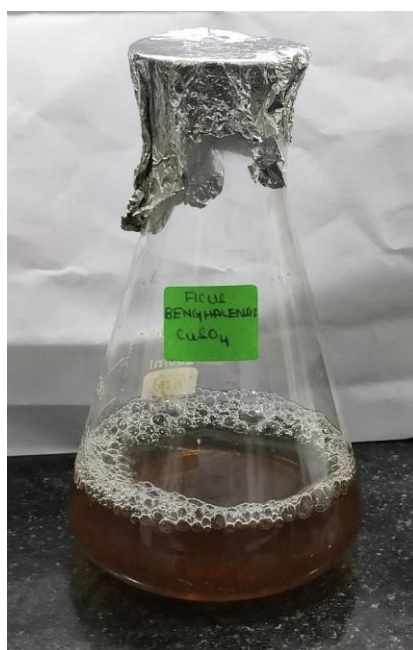


Figure 1: *Ficus benghalensis* plant extract copper nanoparticles synthesized indicative due to the formation of brownish-yellow discoloration.



Figure 2: Mouthwash prepared using the already synthesized *Ficus benghalensis* copper nanoparticles extract.

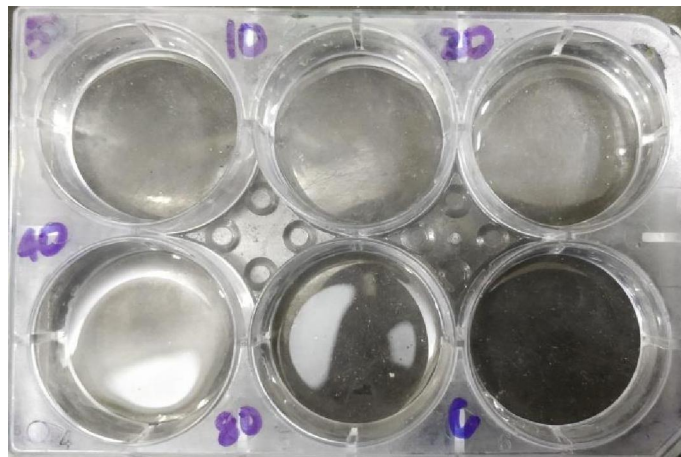


Figure 3: The microtiter ELISA plate which shows the various concentrations (10 mg prepared mouthwash per μL) from 5 μL , 10 μL , 20 μL , 40 μL , 80 μL , and control wells loaded with the prepared *Ficus benghalensis* copper nanoparticles cytotoxic activity observed after 2 days.

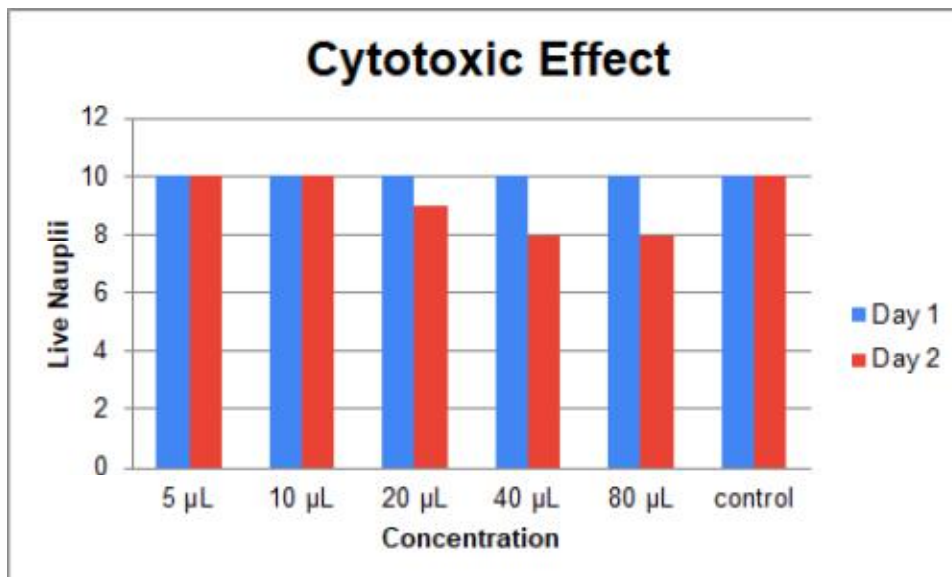


Figure 4: This bar graph represents the cytotoxic activity of the prepared copper nanoparticles of the *Ficus benghalensis* plant extract, the cytotoxicity was observed to be 10, 10, 9, 8, 8, 10 at the differentiating concentrations (10 mg prepared mouthwash per µL) of 5 µL, 10 µL, 20 µL, 40 µL, 80 µL, and control wells after 48 hrs.

Discussion:

Copper nanoparticles are the cheap version of silver nanoparticles [17]. Copper nanoparticles are chosen for this specific present study because they are economical and have strong stable chemical and physical properties. Specific properties of the copper nanoparticles are extremely useful in nanotechnology which is influenced in nanomedicine and biomedicine. Next to silver nanoparticles, the copper nanoparticles have special optical properties in which surface plasma resonance is one crucial part. The intensity of the synthesized copper nanoparticles depends upon the surface plasmon resonance. They are commonly used as catalysts for organic reactions.

Nanoparticles are extremely minute and they are measured in nanometers. Especially copper nanoparticles possess high antibacterial activities which may be useful in further exploring fields. But while exploring on the not-so-bright side, the consumption of any metal nanoparticles for a longer duration causes toxic effects on the human body [18]. They were reported to cause damage to the kidney, liver, and spleen which was noted to be pathological. The synthesization of the copper nanoparticles is shown in **Figure 1** due to the formation of brownish-yellow discoloration leading to the determination of the production of nanoparticles using the *Ficus benghalensis* plant extract.

A simple toxicity test was performed using the brine shrimp, in which the present study was done to test the activity of *Ficus benghalensis* as an effective source as a cytotoxic substance. But the results of the present study suggest that copper nanoparticles of the prepared plant extract are not toxic based on the results obtained. Because when the concentration of plant extract nanoparticles gradient increased, the number of nauplii in the respective wells were alive from 8 to a total of 10 in number. As shown in **Figure 2**, there was the preparation of a mouthwash based on the copper nanoparticle plant extract already obtained from the previous step.

The mouthwash prepared was then tested for its cytotoxic activity which revealed that the prepared mouthwash extract was not cytotoxic enough to kill the brine shrimp after 48 hrs. The number of nauplii was found to be 10, 10, 9, 8, 8, and 10 at the concentrations (10 mg of prepared mouthwash per µL) of 5 µL, 10 µL, 20 µL, 40 µL, 80 µL, and control wells are respectively shown in **Figure 3**. The correlating graph representing the cytotoxic activity of the prepared mouthwash is shown in **Figure 4**. The cytotoxic activity is a toxic property that determines the poisonous capacity of a susceptible plant. Thus the data collected and analyzed in this study concluded that the mouthwash prepared using *Ficus benghalensis* mediated copper nanoparticles was less to the nauplii thus they cannot be used as a cytotoxic agent in treating harmful conditions like oral cancer caused in human beings.

A study conducted by [19] was based on the assessment of cytotoxic activity on various human cancer cell lines. Cytotoxicity is the basic test that acts as a preliminary screening assay in determining the toxicity of a substance

[20]. The number of shrimps was the basis of the cytotoxicity test and the present study was determined by the alive number of nauplii present after adding the prepared *Ficus benghalensis* plant extract copper nanoparticle mouthwash.

The test clearly acts as a primary preliminary tool to determine whether some species of plants have cytotoxic nature against pathological diseases [21]. Opposing findings reveal the antibacterial and cytotoxic nature of the plant *Hibiscus sabdariffa* was found to be promising against human cancer cell lines [22] especially orofacial cancers. Other oral cavity diseases can be treated with various drugs, one of them includes the effectiveness of mouthwash. A mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque. It may contain alcohol, glycerine, synthetic sweeteners, surface active agents, flavouring agents, colouring agents and more. Mouthwash formulation can be herbal in nature which is effectively used against oral pathogens [23]. The cytotoxic activity of the prepared mouthwash was found to be less in the present study. On the other hand, work conducted by [24] concluded that using *Agave cannula* the cytotoxic activity was exhibited to be less than usual. This cytotoxic activity assay is also useful to assess the presence of toxins, heavy metals, fungal agents, and pesticides in aberrant areas. Brine shrimp bioassay is a useful tool in determining the isolates being bioactive compounds of different plant components. Our team has extensive knowledge and research experience that has translate into high quality publications [25–29],[30],[31],[25],[32],[33],[34],[35][27,36,37],[38–42] [43] [44] [45] [46] [46,47] [48] [49] [50] [51] [52] [53] [54] [55] [56] [57] [58] [59].

The limitations of the present study were using less sample size, and the study was an *in vitro* study. *Ficus benghalensis* is the plant used in the present study, where there could have been the involvement of various other plants. There was the assessment of only the cytotoxic activity of the plant selected [60].

According to the present study conducted, the cytotoxic activity of the prepared *Ficus benghalensis* plant extract copper nanoparticles and its based mouthwash prepared was found to have less effect on the alive brine shrimp. This clearly concludes by stating that the mouthwash prepared using *Ficus benghalensis* mediated copper nanoparticles extract has least outcome on the brine shrimp nauplii, thus they can be used as a treatment option against various human pathological conditions under different trial setups. The future scope of this study is the properties of synthesizing nanoparticles which are further implied in nanotechnology which may be useful for nanomedicine, biomedicine and other sourcing fields. Furthermore research works are needed to potentiate the various aspects of the use of nanoparticles in different fields.

Conclusion:

The study conducted at the present concluded that the increased concentration of *Ficus benghalensis* mediated copper nanoparticles mouthwash was not toxic to the living nauplii as it did not cause the death of it. Previous studies state that the extensive use of copper nanoparticles has shown high levels of cytotoxicity. But the plant extract-based copper nanoparticles have less to no cytotoxic activity. Further studies, with different nanoparticles associated with various plant extracts, are needed for improving a better understanding and creating a possible treatment for pathological conditions.

Conflict of interest:

The authors declare there was no conflict of interest in the present study.

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