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ANTIOXIDANT AND ANTICARIOGENIC ACTIVITY OF SYNTHESIZED ZINC NANOPARTICLES USING *ZIZIPHUS MAURITIANA* FLOWER AGAINST DENTAL CARIES PATHOGEN

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ABSTRACT

The present study reveals that the biosynthesized ZnO NPs from the flower extract of *Ziziphus mauritiana* with ethanol as a solvent when examined for antioxidant activity using dpph scavenging studies and anticariogenic activity with the help of disc diffusion method. The ethanolic flower extract confirmed the presence of terppenoids, flavonoids, saponins, tannins, alkaloids, steroids, glycosides, phlobatannins, protein, coumarin, emodin, antraquinone, anthocyanin, carbohydrate, leucoanthocyanin, cardiac glycoside, xanthoprotein, phenolics. The characterization of synthesized ZnO NPs was observed using the FTIR analysis was used to confirm the functional group involved in reduction of Zn2+ ions, XRD analysis to know the crystallographic structure of nanoparticles involved and SEM analysis to evaluate the morphology of the synthesized ZnO NPs. The anticariogenic activity of synthesized ZnO NPs from the flower of *Ziziphus mauritiana* exhibited a maximum zone of inhibition for streptococcus sobrinus with the radius of 13.5 mm at a higher concentration of 100 µg/ml. The antioxidant activity of the synthesized ZnO NPs revealed a maximum inhibition of 84.821% when compared to the standard ascorbic acid (92.857%) at a higher concentration of 100 µg/ml. Thus it is proposed that the synthesized ZnO NPs from the ethanoilc flower extract of *Ziziphus mauritiana* acted as a potential source for anticariogenic activity in dental carries application and played an effective role as antioxidant agent.

Keywords: Antioxidant And Anticariogenic Activity, Synthesized Zinc Nanoparticles, *Ziziphus mauritiana*

INTRODUCTION:

Nanotechnology, an emerging revolution in science and technology deals with nanoscale particles of size below 100nm that provides large surface to volume ratio due to which it exhibits chemical stability and conductivity **[1]**. Synthesis methods involve physical, chemical and biological treatments. Biological synthesis method involves enzymes, fungus, microorganisms, plants and their extracts **[2]**. And their approaches provide an environmental friendly, cost effective, low toxic and efficient protocol to synthesize NPs faster compared to chemical methods that include use of toxic compounds, more processing time and high cost laboratory procedures **[3]**. NPs provide wide range of emerging applications in impacting industries such as healthcare, electronics, and energy. They show antimicrobial activity, behave as an anticancer agent, involved in drug delivery and disease treatment **[4]**. Zn NPs are used as fertilisers and in treating plant growth and seed germination. They also find therapeutic applications for the autoimmune related diseases and cancer treatment. Due to their diverse capabilities they are indispensable in pushing the boundaries of science and technology **[5]**. Dental caries, also known as tooth decay or cavities, is a chronic disease characterized by the localized destruction of dental hard tissues caused by bacterial fermentation of dietary carbohydrates. It progresses slowly and arises from an ecological imbalance between tooth minerals and oral biofilms (plaque) **[6]**. Surveys indicate that adolescents have the highest prevalence of dental caries, followed by children and adults. Infants are particularly susceptible to a severe form of caries called "rampant caries" or "nursing bottle caries," affecting their deciduous teeth **[7]**. Classification of dental caries includes acute or chronic forms and can affect enamel, dentin, or cementum **[8]**. Certain Complications are Cavernous sinus thrombosis, Toothache, pulpitis, tooth loss and dental discoloration **[9]**. Treatment options range from arresting non-cavitated lesions through dietary changes to restoring cavitated lesions **[10]**. Natural remedies, such as maintaining personal hygiene, consuming calcium-rich foods, and utilizing fluoride, can complement conventional treatments.To restore the destroyed tooth, removal of the decayed tooth is performed. Regular dental check-ups are essential for optimal oral health **[11]**. *Ziziphus mauritiana* belongs to the family of Rhamnaceae and its commonly known as Indian jujube, Indian plum, Chinese date, chine apple, ber and dunks **[12]**. It's widely distributed in tropical regions of Northern Australia, Africa, Southern eastern parts of Asia. Its origin native is India **[13]**. The chemical constituent of the plant are protein,

amino acid, flavonoids, alkaloids, glycoside, fibers, terpenoids, saponins, tannin and phenolic compounds **[14]**. *Ziziphus Mauritiana* is an effective herbal remedy that aids weight gain, improves muscular strength, increases stamina and strengthen liver function. It also promotes hair growth. Its pharmacological activities include antitumor, antioxidant, antifungal, antidiarrheal, anti-diabetic **[15, 16]**.

MATERIALS AND METHODS:

COLLECTION OF MEDICINAL PLANT:

The flowers of *Ziziphus mauritiana* are collected from local markets.

PREPARATION OF ETHANOLIC EXTRACT FROM FLOWER OF *ZIZIPHUS MAURITIANA*:

The collected flowers of *Ziziphus mauritiana* are totally washed with tap water and dried in shadow place without sunlight. The dried flower is crushed using mortar and pestle. Then the powder is collected and stored in dark and dry place for further studies. The extract is prepared using Soxhlet method, a type of hot peculation method. To 50g of powered sample 200ml ethanol is added and incubated for 2 days in the extractor. The ethanolic extract of plant sample is prepared.

PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT FROM FLOWER OF *ZIZIPHUS MAURITIANA*: (QUALITATIVE ANALYSIS)

1. TEST FOR TERPENOID:

To 2ml of plant extract add few drops of chloroform and few drops of sulphuric acid. The appearance of reddish brown colour indicates the presence of terpenoid.

2. TEST FOR FLAVANOIDS:

To 2ml of plant extract add few drops of concentrated sulphuric acid. The appearance of yellow color indicates the presence of flavonoids.

3. TEST FOR SAPONIN:

To 2ml of plant extract add few drops of distilled water or olive oil and shake vigorously, continuous forth formation indicates the presence of saponins.

4. TEST FOR TANNIN:

To 2ml of plant extract add few drops of distilled water and few drops of ferric chloride. The appearance of green precipitate indicates the presence of tannin

5. TEST FOR ALKALOID:

To 2ml of plant extract add few drops of glacial acetic acid and few drops of ammonia solution. The appearance of yellow colour indicates the presence of alkaloid.

6. TEST FOR STEROID:

To 2ml of plant extract add few drops of chloroform and few drops of concentrated sulphuric acid. The appearance of reddish brown ring indicates the presence of steroid **[17]**.

7. TEST FOR GLYCOSIDES:

To 2ml of plant extract add few drops of chloroform and few drops of glacial acetic acid. The appearance green or violet or blue colour indicates the presence of glycosides.

8. TEST FOR PHLOBATANNINS:

To 2ml of plant extract add few drops of hydrochloric acid. The appearance of red precipitate indicates the presence of phylobatannins.

9. TEST FOR PROTEIN:

To 2ml of plant extract add few drops of concentrated sulphuric acid. The appearance of white precipitate indicates the presence of protein.

10. TEST FOR COUMARINS:

To 2ml of plant extract add few drops of sodium hydroxide. The appearance of yellow colour indicates the presence of coumarins.

11. TEST FOR EMODIN:

To 2ml of plant extract add few drops of ammonia solution and few drops of benzene. The appearance of red colour indicates the presence of emodin.

12. TEST FOR ANTRAQUINONE:

To 2ml of plant extract add few drops of benzene and few drops of ammonia solution. The appearance of pink or red or violet colour indicates the presence of antraquinone.

13. TEST FOR ANTHOCYANIN:

To 2ml of plant extract add few drops of hydrochloric acid and few drops of ammonia solution. The appearance of pinkish red into bluish violet indicates the presence of anthocyanin.

14. TEST FOR CARBOHYDRATE:

To 2ml of plant extract add few drops of distilled water, few drops of ethanolic- alpha - napthol and few drops of concentrated sulphuric acid. The appearance of reddish violet colour indicates the presence of carbohydrate.

15. TEST FOR LEUCOANTHOCYANIN:

To 2ml of plant extract add few drops of isoamyl alcohol. The appearance of organic layer into red colour indicates the presence of leucoanthocyanin.

16. TEST FOR CADIAC GLYCOSIDES:

To 2ml of plant extract add few drops of glacial acetic acid, few drops of ferric chloride and few drops of concentrated sulphuric acid. The appearance of violet brown ring formation indicates the presence of cardiac glycosides.

17. TEST FOR XANTHOPROTEIN:

To 2ml of plant extract add few drops of ferric chloride. The appearance of blue black colour indicates the presence of xanthoprotein.

18. TEST FOR PHENOL:

To 2ml of plant extract add few drops of ammonia solution. The appearance of reddish orange precipitate indicates the presence of phenol.

QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUTENTS PRESENT IN *ZIZIPHUS MAIRITIANA*:

1. FLAVONOIDS:

To 0.5g of plant extract add 3ml of methanol in the test tube. Shake the mixture well and filter it. Collect the filtrate and transfer it to watch glass and dry the filtrate solution in room temperature. After drying measure the weight of the dried substance **[18]**.

2. TANNINS:

To 0.5g of plant extract add 3ml of distilled water in the test tube. Shake the mixture well and filter it. Add one drop of ferro cyanide and one drop of potassium ferro cyanide to the collected filtrate and transfer the solution to watch glass and dry the filtrate solution in room temperature **[19]**.

3. SAPONINS:

To 0.5g of plant extract add 3ml of ethanol in the test tube. Shake the mixture well and filter it. To the filtrate add one ml of diethyl ether and shake vigorously. Then discard the upper layer and add one drop of n-butanol of the remaining solution in the test tube. Shake the mixture well and filter it. Collect the filtrate and transfer it to watch glass and dry the filtrate solution in room temperature. After drying measure the weight of the dried substance **[19]**.

4. ALKOLOID:

To 0.5g of plant extract add 3ml of 10% acetic acid in ethanol in the test tube. Shake the mixture well and filter it. To the filtrate add few drops of ammonia solution and transfer it to watch glass and dry the filtrate solution in room temperature. After drying measure the weight of the dried substance **[20]**.

5. PHENOL:

To 0.5g of plant extract add 3ml of distilled water in the test tube. Shake the mixture well and filter it. To the filtrate add few one drop of ammonia solution and one drop of isoamyl alcohol and transfer it to watch glass and dry the filtrate solution in room temperature. After drying measure, the weight of the dried substance **[21]**.

6. TERPENOID:

To 0.5g of plant extract add 3ml of ethanol in the test tube. Shake the mixture well and filter it. To the filtrate add few drops of petroleum ether and transfer it to watch glass and dry the filtrate solution in room temperature. After drying measure the weight of the dried substance **[19]**.

SYNTHESIS OF ZINC NANOPARTICLES:

To 50ml of 1mM zinc acetate add 5ml of plant extract with constant and continuous stirring. The mixture reacts in the environmental condition and Zinc acetate gets reduced to Zn+ ion. After few minutes the colour changes to transparent yellowish white. This shows the formation of Zinc acetate and is confirmed by UV-Spectral analysis.

CHARACTERIZATION TECHNIQUES:

With the help of UV-visible spectra, FT-IR, SEM, XRD and EDAX the synthesized ZnO nanoparticles were characterized.

1. UV-VISIBLE ANALYSIS:

The UV-visible analysis of synthesized ZnO nanoparticles refers to its optical characterization where we can observe the absorption spectra at various concentration and temperature. Addition of zinc acetate to the plant extract leads to formation of whitish yellow nanoparticles that indicates the presence of synthesized ZnO nanoparticles. A single peak of absorption band that lies between the range of 200nm to maximum of 350nm is obtained and any deviations indicates the presence of contaminants **[22]**.

2. FT-IR ANALYSIS:

FT-IR spectroscopy stands for fourier transform infrared spectroscopy. It helps in analysing the chemical properties including organic, inorganic materials and in identifying its functional groups by scanning through infrared light which provides a absorbed band range of 4000 to 400 (cm^-1) **[23]**.

3. SCANNING ELECTRON MICROSCOPE (SEM):

The scanning electron microscope is used to study the surface morphology of the ZnO nanoparticles. The synthesized ZnO nanoparticle is dried to remove the moisture from it and the image is taken through FEI Quanta 250 SEM operating at 10Kv **[24]**.

4. X-RAY DIFFRACTION (XRD):

X-ray diffraction analysis is used for structural study of nanomaterials and also helps in observing the value of the material **[25]**.

5. ENERGY DISPERSIVE X-RAY SPECTROMETER (EDAX):

The energy dispersive X-ray spectrometer is used to analyse and prove the presence of ZnO nanoparticles. It also contains two axis namely horizontal and vertical **[26]**.

ANTIOXIDANT ACTIVITY (DPPH ASSAY):

Antioxidant activity of the synthesized ZnO NPs of the flower extract of *Ziziphus mauritiana* was performed on the basis of scavenging effect along with the stable DPPH free radical activity. 250µl of DPPH belonging to different concentrations (20 to 100µl/ml) of ascorbic acid that is a standard antioxidant drug to which 980µl of ethanol solution is added. By vigorously shaking the process is repeated with different concentrations of synthesized ZnO nanoparticles. Using UV spectrometry absorbance was taken at 540nm. To find the test samples radical scavenging activities, percentage of inhibition was found using the below equation:

Percent (%) inhibition of DPPH activity= [(C-T)/C] x100

Note: T and C denotes the absorbance values of the blank and test samples **[27]**.

COLLECTION OF TEST PATHOGEN:

The anti-cariogenic activity of synthesized ZnO nanoparticles extracted from the flower of *Ziziphus mauritiana* exhibited against *Streptococcus mutants* (MTCC 890), *Streptococcus salivarius* (MTCC 25923), *Streptococcus sobrinus* (MTCC 33479) and *staphylococcus aureus* (MTCC 25923) were prepared as test organisms. From the microbial type culture and collection (MTCC) at Chandigarh, India all bacterial strains can be purchased.

DETERMINATION OF ANTI-CARIOGENIC ACTIVITY BY DISC DIFFUSION METHOD:

ZnO NPs extracted from the flower of *Ziziphus mauritiana* using disc diffusion is used for the study of its anti-cariogenic activity and sometimes the synthesized nanoparticles are allowed to bind with the paper discs. To a sterile petri dish pour 25ml of Muller-Hilton agar medium which was already prepared and inoculated with a test organism at different concentration of isolated compounds of 60, 80 and100 mg/ml. 10µl amoxicillin was prepared and filter paper disc was loaded to this acts as a positive control. Whereas ethanol used as a solvent here was also used in preparation of negative control. The plates were incubated for 24 hours at 37 ºC and the zone of inhibition was recorded in millimetre **[28]**.

DETERMINATION OF MIC AND MBC:

Take multiple test tubes and add 2ml of nutrient broth to each tube. Inoculate each test tube with 50µl of various cultures representing different test organisms. Add different concentrations of isolated nanoparticles (10, 20, 40, 60, 80 and 100 µl/ml) to separate test tubes. Repeat the same process using a standard antibiotic, such as amoxicillin, instead of nanoparticles. Include a control tube with only nutrient broth, which will be seeded with the test organisms. Incubate the test tubes at 37 ºC for 24 hours to allow bacterial growth. After incubation, examine the tubes for turbidity indicating microbial growth. Identify the tubes without visible growth (turbidity) as the ones which are potentially inhibiting microbial growth (MIC determination). To determine the Minimal Bacterial Concentration (MBC), take a loopful of broth from the tubes which did not show any growth in the MIC determination. Streak the broth on the inoculated sterile nutrient agar plates. Use nutrient agar plates without nanoparticles or antibiotics as control plates. Incubate the plates at 37 ºC for 24 hours. After incubation, observe the plates and identify the lowest concentration (of nanoparticles or antibiotics) with no visible bacterial growth. This concentration represents the Minimal Bacterial Concentration (MBC) **[29]**.

RESULT AND DISCUSSION:

QUALITATIVE ANALYSIS OF SYNTHESISED ZINC NANOPARTICLES FROM *ZIZIPHUS MAURITIANA*:

The phytochemical screening of *Ziziphus mauritiana* flower extract using different tests standards are represented in (**Table 1**) showed the presence of terpenoids, flavonoids, saponin, tannin, alkaloids, steroids, glycosides, phlobatannins, emodins, anthraquinone, anthocyanins, carbohydrates, leucoanthocyanins, cardiac glycoside, xanthoproteins, phenols which are known to possess physiological and medicinal activities while, proteins and coumarin were moderately present **(Figure 1)**. Qualitative analysis of *Zizphus mauritiana* flower extract revealed the presence of phytochemical constituents which are primarily responsible for their biological activity.

The previous studies showed that, Mishra and Bathia, 2014, identified that the ethanol extract of *Ziziphus mauritiana* seeds contain alkaloids, terpenes, tannins, flavonoids, saponins, sterols and phytosterols. Thomas A.N.S, 2004 showed that the ethanol extract of *Ziziphus mauritiana* root contains alkaloids, flavonoids, glycosides, saponins and essential oils. The ethanol extract of *Zizphus mauritiana* stem which revealed the presence of alkaloids, anthocyanins, anthraquinone, glycosides, carbohydrates, cardiac glycosides, flavonoids, steroids, terpenoids, tanins, saponins was proven by Thomas A.N.S, 2004. Suzie *et al.,* preformed phytochemical screening of ethanol extract of *Ziziphus mauritiana* fruits and found that it contains phenolic compounds **[30]**.

QUANTITATIVE ANALYSIS OF SYNTHESIZED ZnO NPs FROM *ZIZIPHUS MAURITIANA*:

The qualitative analysis of *Ziziphus mauritiana* flower extract reported the presence of different amount of phytoconstituents. Tannin is the phytoconstituent with higher quanity followed by alkaloids, flavonoids, saponins, phenols and terpenoids. The compositions of phytoconstiuents are tanins (0.019mg/g), alkaloids (0.015mg/g), flavanoids (0.013 mg/g), saponins (0.011mg/g), phenol (0.011mg/g) and terpenoids (0.008mg/g) **(Figure 2)**.

The previous studies conducted by, Fluck, 1973 showed that 1.4% flavonoids were present in dried bark sample of *Ziziphus mauritiana* Lam. Chung *et al.,* 1998 performed quantitative tests and found that 87.05% saponins were present in bark sample and 33µg/ml tannins were identified in bark sample of *Ziziphus mauritiana* **[31]**.

CHARACTERIZATION TECHNIQUES:

1. UV SPECTROSCOPY:

The absorbance spectra at various concentration and temperature of the synthesized ZnO NP was analyzed through

UV visible spectroscopy. A single peak of absorbance band at a height of 1.522 AU and wavelength of 263.75 nm was obtained with no deviations which indicated the absence of contaminants **(Figure 3)**.

Diallo. A *et al.,* 2015, analysed that the MWL and MWS of synthesised ZnO NPs from leaf and seed extracts of *Malva neglecta wallr* annealed at 400°C showed the absorption band at 372 and 376 nm also confirmed the absence of contaminants **[32]**. Gupta A *et al.,* 2014, has revealed through his studies that the ZnO NPs synthesized using *Mimosa pudica*, leaf extract showed maximum peaks at 235nm and 300nm in UV spectrum **[33]**.

2. FT-IR SPECTROSCOPY:

By scanning through the infrared light the functional group and chemical properties of the synthesized zinc NPs was analyzed. FT-IR spectrum of ZnO NPs synthesized from *Ziziphus mauritiana* was recorded at the range of 4000 to 400 cm^-1, observation showed that the bands of ZnO NPs occurred at 3436.02cm^-1 attributed to OH and NH2 groups, alkynes stretch was observed at 2078.94 cm^-1, the stretching vibrations of C=O and C=C bonds on the aromatic rings of phenolic compounds were observed at 1634.05 cm^-1 and 1416.85 cm^-1, the peak at 1016.20cm^-1 represented the stretching vibration of the hydroxyl C-O bond of flavonoids and phenolic compounds and C-H bend was observed at 688.90cm^-1 **(Figure 4)**.

Becheri *et al*., 2008 reported that the FTIR spectrum of ZnO NPs lie between the range of 4000-500cm^-1, and it also showed that the bands of ZnO NPs synthesized from *Mimosa pudica*, leaf extract occurred between 3903.4 cm−1 and 682.6 cm−1. The FTIR result of synthesized ZnO NPs from leaf and seed extracts of *Malva neglecta wallr* showed that the ZnO functional group was found at low wavenumber. N. Matinise *et al.,* 2017 showed that the FTIR spectrum of MWL and MWS has a broad peak around 3400 cm^-1 with the stretching vibrations of OH and –NH2 functional groups of the polyols and the stretching vibrations of C=C and C=O bonds on the aromatic rings of phenolic compounds around 1400 and 1600 cm^1 **[32]**.

3. SCANNING ELECTRON MICROSCOPE (SEM):

The morphology of the surface of synthesized ZnO NPs was evaluated using the SEM image. The synthesized ZnO NPs from flower extract of *Ziziphus mauritiana* was within the range of 200nm. From **(Figure 5)** it was revealed that the obtained SEM image of synthesized ZnO NPs was spherical in shape.

The previous analysis by Anatol Degefa *et al.*,2021 has exhibited through the SEM analysis of the synthesized ZnO NPs extracted from the carrot, tomato, cabbage,

onion. The result images depicted the morphology incase of tomato and onion the spherical surface was obtained while in case of cabbage and carrot it was nanotube and nanorod **[34]**. Ajay Kumar Tiwari *et al.,* 2022 helped in obtaining the data to confirm the rod shape morphology thorugh SEM with the thickness of 25-65 nm and length 250-600 nm for the spherical and cubical shapes of biologically synthesized ZnO NPS **[35]**.

4. X-RAY DIFFRACTION (XRD):

From the X-ray crystallography the crystalline nature of zinc oxide nanoparticles was confirmed from **(Table 3)**. The synthesized ZnO NPs pattern obtained by XRD was represented in **(Figure 6)**. The diffraction peaks obtained by ZnO NPs was observed at 13.64, 21.83, 27.94, 30.83, 32.76, 59.45, 68.75 in the 2Ɵ range. The X-ray diffraction peaks corresponded to the lattice plane of (100), (002), (101), (102), (110), (112) and (201) suggested the face-centered cubic crystal structure of ZnO NPs. Joint Committee on Powder Diffraction standards (JCPDS) was used as a reference to assign the lattice planes according to the peaks obtained.

The previous studies by Ashmalina Rahman*et al.*, 2021 has resulted the XRD patterns of the synthesized ZnO NPs from the aqueous leaf extract of *Ziziphus mauritiana lam*. Which showed the diffraction peaks at 2θ values 31.74°, 34.41°, 36.26°, 47.54°, 56.58°, 62.84°, 66.32°, 67.97°, 69.10° 72.56° and 76.94°, which can be indexed to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes of the hexagonal wurtzite structure **[36]**. Ajay Kumar Tiwari *et al.,* 2022 revealed the XRD patterns of ZnO NPs to have a hexagonal crystalline structure with the space group P63mc (186). The particle sizes were found to be 47 nm and 55 nm for the chemically and biologically synthesized ZnO NPs, respectively, along with the high intensity orientation (002) **[35]**.

5. ENERGY DISPERSIVE X-RAY SPECTROMETER (EDAX):

To confirm the elemental signal of ZnO NPs energy dispersive X-ray (EDX) spectrometer analysis was performed. The presence of supplementary peaks of oxygen (O) and carbon (C) in the EDAX spectra of synthesized ZnO nanoparticles (NPs) from the flower extract of Ziziphus mauritiana indicates the attachment of biomolecules onto the surfaces of the ZnO NPs is shown in **(Figure 7)**. The weight of zinc in ZnO NPs was reduced by the flower extract of *Ziziphus mauritiana* was found to be 54.38%.

The previous studies by Vidhya S, 2021 showed that the weight of the silver in AgNPs was reduced by the seed extract of *Linum usitatissimum* to be 56.42% and the weight of silver in the silver nanoparticles

was reduced by *Terminalia chebula* bark was found to be 65.81% **[37]**. Abdol-Majid Cheperli *et al.,* 2022 revealed the weight percentage of zinc and oxygen elements were received in 72.08, 20.50%, for MWL ZnO-NPs, and 62.64, 25.64% for MWS-ZnO-NPs, respectively. Also some weight loss and weak signal can be realted to the compounds of the plant used for synthesizing ZnO NPs from seed and leave extracts of *Malva neglecta wallr* **[32]**.

ANTIOXIDANT ACIVITY OF FLOWER OF *ZIZIPHUS MAURITIANA*– DPPH METHOD

The result showed that the flower of *Ziziphus mauritiana* has a better percentage of antioxidant activity at high concentrations when compared with ascorbic acid. The compound showed 84.821% activity at higher concentration of 100 µg/ml while ascorbic acid gave 92.857% at the same concentration **(Table 5)**.

In the previous study Biapa P.C. *et al.,* 2007 performed DPPH free radicals scavenging activity of methanolic extract of *Ziziphus mauritiana* leaves and found that it had a significant level of antioxidant activity. And this result was compared with the results of ascorbic acid which is an standard antioxidant agent. Perumal *et al.,* 2012 claimed that the antioxidant activity of methanolic extract of *Z.mauritiana* Lam leaves might be due to the presence of antioxidant compounds such as flavonoids and ascorbic acid **[38]**.

ANTICARIOGENIC ACTIVITY:

The streptococcus species demineralizes the teeth and leads to dental caries. The streptococcus species used for the test are *Streptococcus mutans, Staphylococcus aureus, Streptococcus sobrinus* and *Streptococcus salivarius*. The anticariogenic activity of synthesized ZnONPs from the flower of *Ziziphus mauritiana* against these bacteria was investigated and results were tabulated in the **(Table 6)**. The results suggested that the synthesized ZnO NPs showed maximum inhibition against *streptococcus sobrinus* at the concentration of 100µg/ml.

The previous study by Bhuvaneshwari, 2021 suggested that the maximum inhibition against the streptococcus salivarius at concentration of 100µg/ml by the synthesized silver nanoparticles from seeds of *Linum usitatissimum.* Hamid Reza Ghorbani, 2017 proved that the maximum inhibition by biosynthesized AgNPs from *salmonella typhirium* was against *lactobacillus casei* **[37]**.

MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC):

The minimum inhibitory concentration (MIC) for the biosynthsised ZnO NPs using ethanolic extract of flower of *Ziziphus*

mauritiana was found and minimum bactericidal concentration was evaluated which is presented in **(Table 7)**.

From the **Table 5**, it has been noted the flower extract is very effective against the strain Streptococcus mutans at lower concentration followed by *Streptococcus salivarius*, *Staphylococcus aureus, Streptococcus sobrinus*. So it is understood that against the four bacterial strains that has been tested, the flower extract of *Ziziphus mauritiana* showed different anticariogenic activities.

A recent study carried out by Garibo D *et al.,* 2020 proved that the synthesised Ag NPs from *Lysiloma acapulcensis* showed good antimicrobial activity against *staphylococcus aureus* **[32]**. Ali Al Ghasham *et al.,* 2017 revealed that the methanolic extract of *Ziziphus mauritiana* Lam, leaves exhibited a significant level of antimicrobial activities against *Staphylococcus aureus* and *Streptococcus pneumoniae*. Abdullah *et al*., 2016 reported that, the methanolic extract of *Ziziphus mauritiana* leaves has a significant level of antimicrobial activities against Bacillus cereus and Proteus vulgaris **[38]**.

Table 1: List of phytochemical constituents present in ethanolic extract of *Ziziphus mauritiana* flower

S. No.	Metabolites	Observation	Concentration
1.	Terpenoids	Reddish brown	+++
2.	Flavonoids	Yellow colour	+++
3.	Saponins	Blue colour	+++
4.	Tannins	Brownish green	+++
5.	Alkaloids	Yellow colour	+++
6.	Steroids	Reddish brown	+++
7.	Glycosides	Violet, blue	+++
8.	Phlobatannins	Red	+++
9.	Proteins	White	++
10.	Coumarins	Yellow	++
11.	Emodin's	Red	+++
12.	Anthroquinones	Pink, violet	+++
13.	Anthocyanins	Pinkish red, Bluish violet	+++
14.	Carbohydrates	Reddish pink	+++
15.	Leuco anthocyanin	Red	+++
16.	Cardiac glycosides	Brown ring, violet	+++
17.	Xanthoproteins	Blue / black	+++
18.	Phenols	Reddish orange	+++

+ = Trace ++ = Moderate +++ = Strong A = Absence

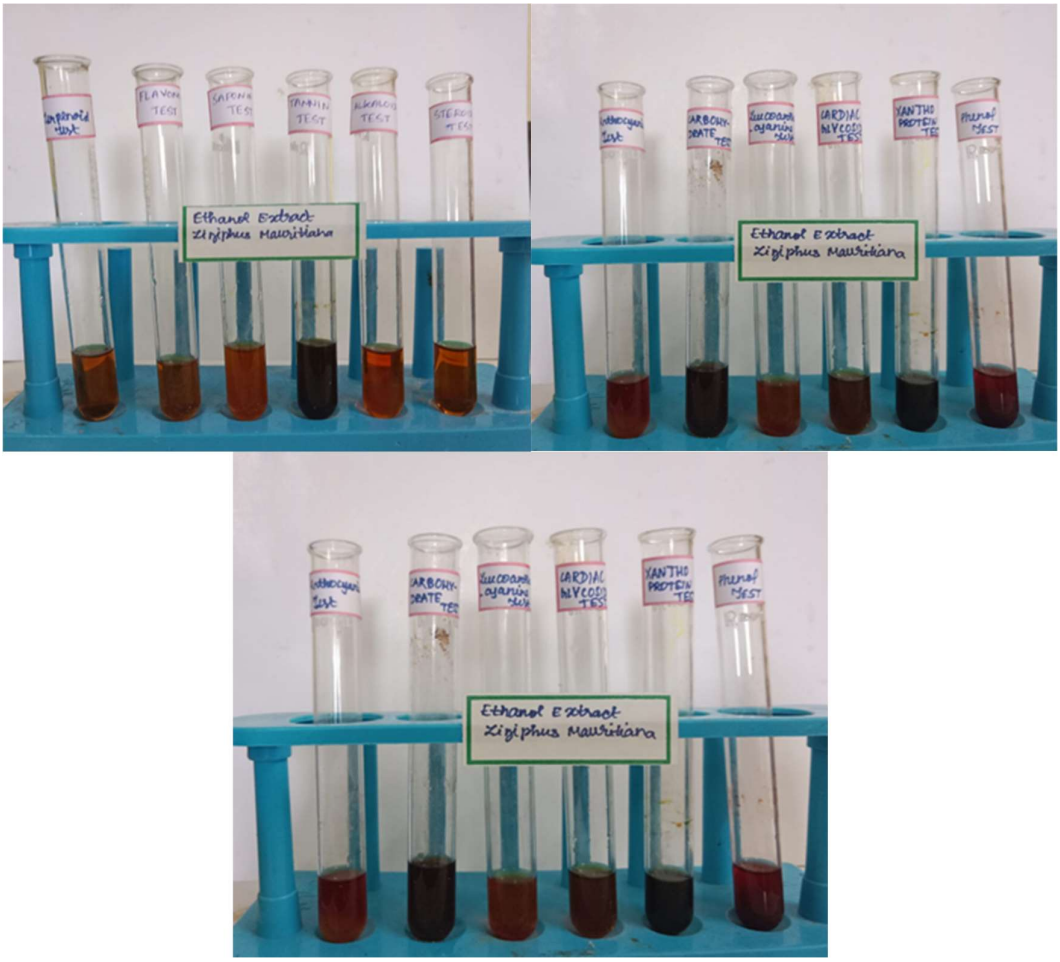


Figure 1: Qualitative analysis of *Ziziphus mauritiana* flower extract

Table 2: Quantitative analysis of Ziziphus mauritiana flower

S. No.	Phytochemical constituents	*Zizipjus mauritiana* (mg/g)
1	Saponin	0.011
2	Alkaloids	0.015
3	Flavonoids	0.013
4	Phenol	0.011
5	Terpenoids	0.008
6	Tannin	0.019

Figure 2: Quantitative analysis of flower of *Ziziphus mauritiana*

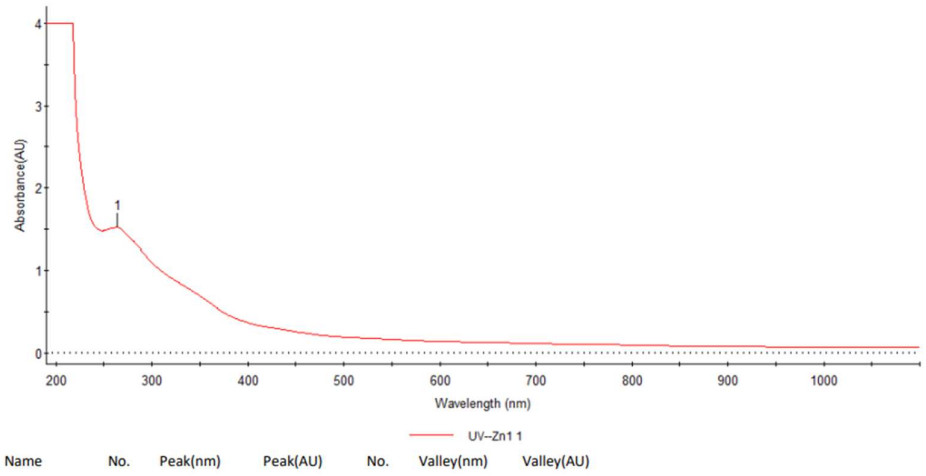


Figure 3: UV-Visible spectroscopy of ZnO NPs

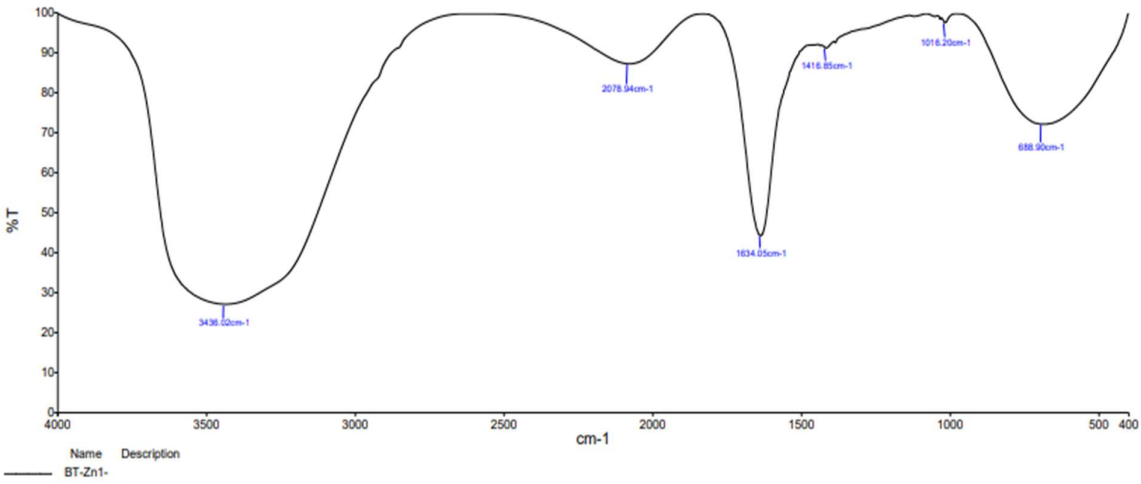


Figure 4: FT-IR spectroscopy of ZnO NPs

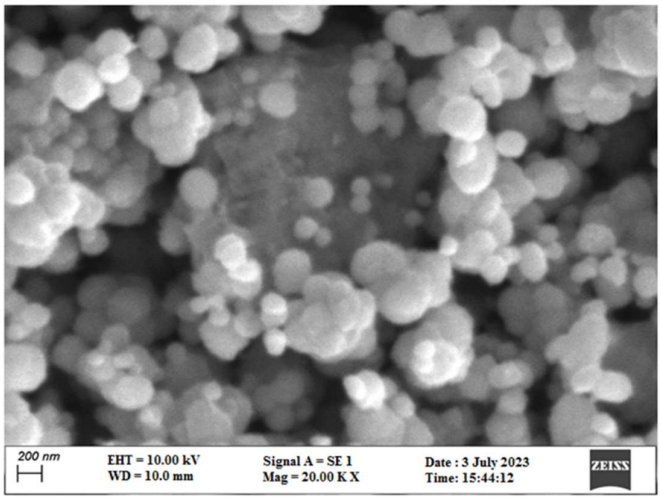


Figure 5: SEM image of ZnO NPs

Table 3: XRD patterns for synthesized ZnO NPs using flower extract of *Ziziphus mauritiana*

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
13.6423	38.29	0.8348	6.48234	13.64
21.8356	40.37	0.9417	4.04850	16.19
27.9452	82.66	0.5621	3.28336	36.24
30.8376	61.29	1.1147	2.89417	27.18
32.7650	230.53	0.2463	2.73528	100.00
59.4547	85.27	0.6394	1.55423	35.43
68.7583	27.06	1.5843	1.36354	17.40

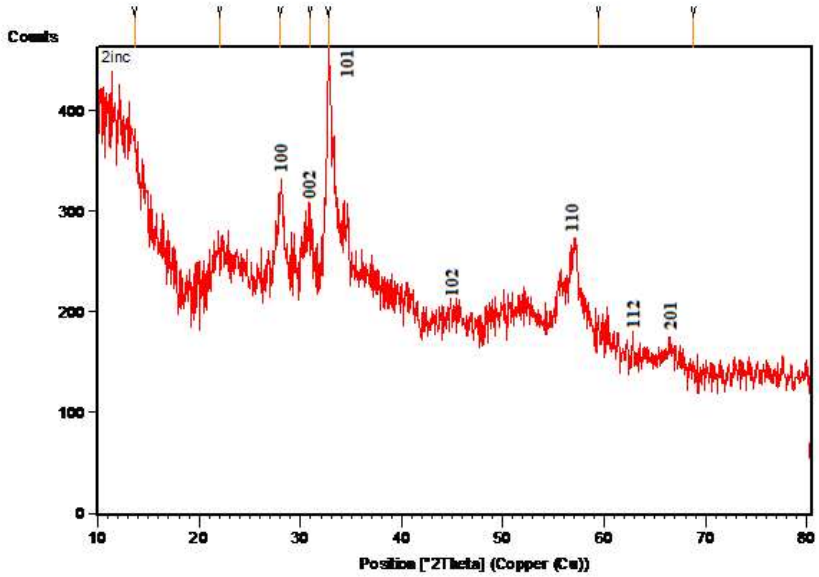


Figure 6: XRD analysis of synthesized ZnO

Table 4: Quantitative XRD Results for: Base (951)

Element line	Weight %	Weight % error	Atom %
C K	32.38	± 0.99	39.18
O K	13.20	± 0.89	44.58
Zn K	54.38	± 1.91	16.24
Zn L	---	---	---
Total	100.00		100.00

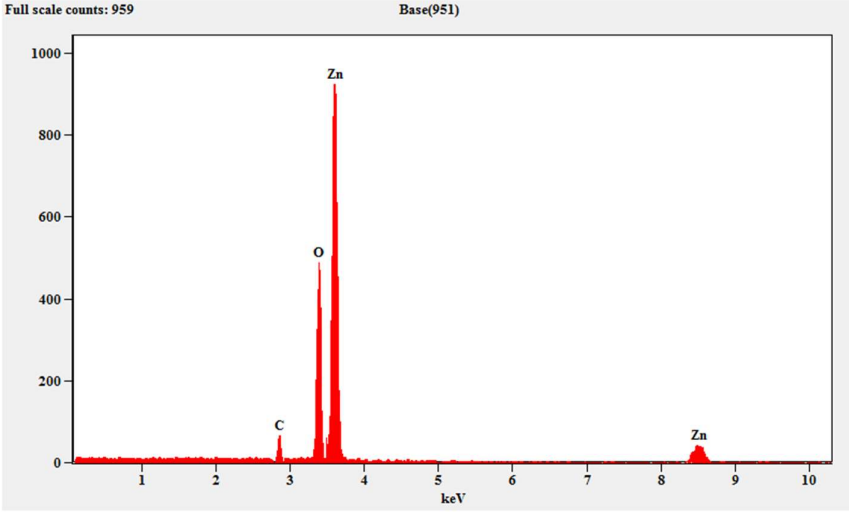
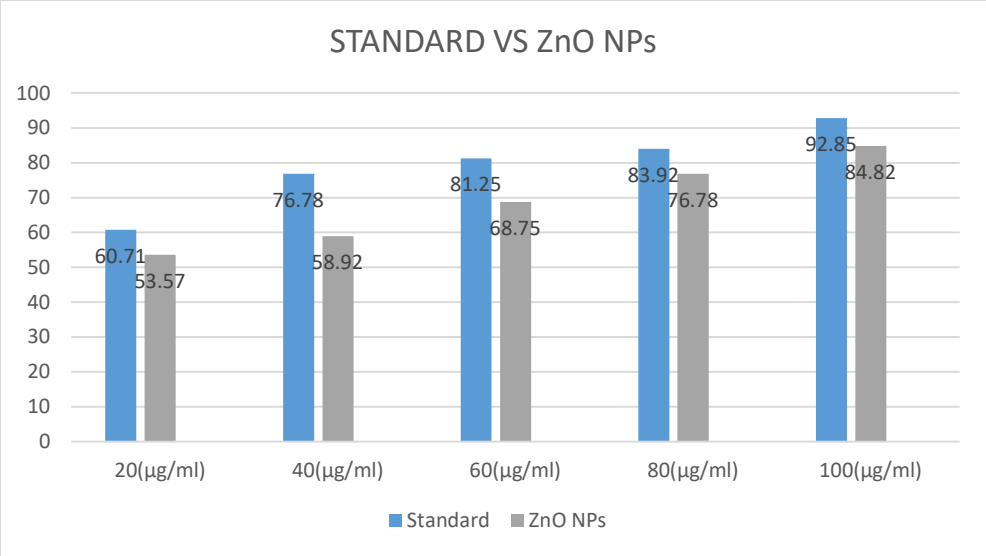


Figure 7: EDAX analysis of ZnO NPs

Table 5: Antioxidant activity of flower of *Ziziphus mauritiana* DPPH activity

S. No.	Concentrations	Scavenging Effect (%)		Statistics for antioxidant activity			
	C=1.12	Flower of *Ziziphus mauritiana*	Ascorbic acid	Flower of *Ziziphus mauritiana*	Standard mean deviation	Ascorbic acid	Standard mean deviation
1.	20(µg/ml)	53.57	60.71	53.57±2.88052		60.71±3.66974	
2.	40(µg/ml)	58.92	76.78	58.92±4.8824		76.78±7.30299	
3.	60(µg/ml)	68.75	81.25	68.75±5.32632		81.25±5.51374	
4.	80(µg/ml)	76.78	83.92	76.78±7.51002		83.92±2.54329	
5.	100(µg/ml)	84.82	92.85	84.82±5.18205		92.85±8.147	



Graph: Comparison between the standard Ascorbic acid and the extract from the flower of *Ziziphus mauritiana*

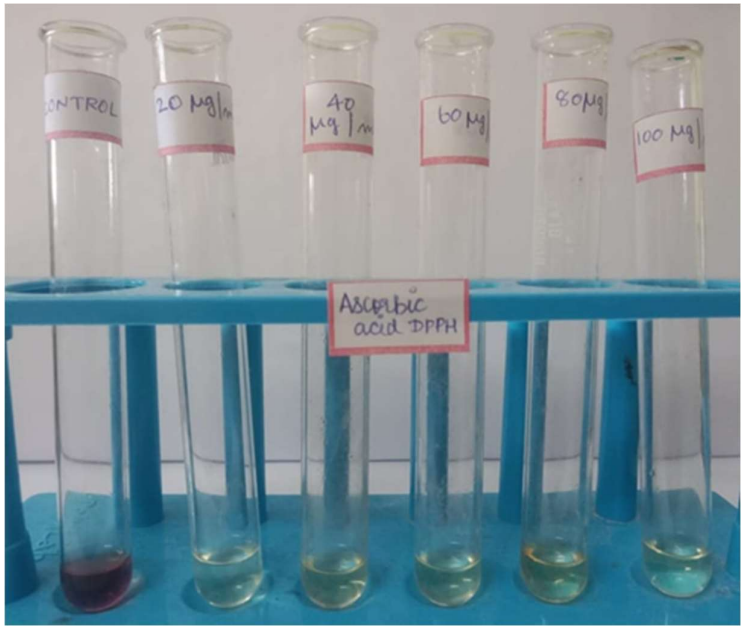


Figure 8: Antioxidant activity of standard Ascorbic acid by DPPH activity

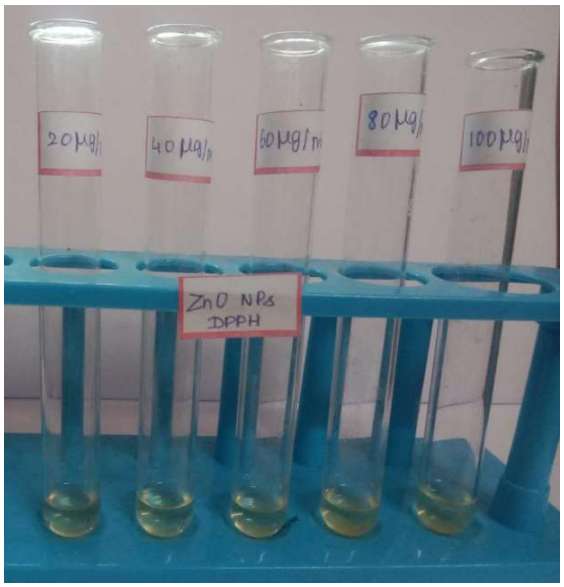


Figure 9: Antioxidant activity of flower of *Ziziphus mauritiana* DPPH activity

Table 6: Anticariogenic activity of flower of *Ziziphus mauritiana*

Zone of inhibition (mm)				
ZnO-NPs concentration(µg/ml)	**Streptococcus mutans-BB1**	**Staphylococcus aureus-BB2**	**Streptococcus sobrinus-BB3**	**Streptococcus salivarius-BB4**
Standard (amoxicillin)	**12mm**	**10mm**	**15mm**	**12.5mm**
60	**6mm**	**8mm**	**12mm**	**11mm**
80	**8mm**	**8.5mm**	**13mm**	**11.5mm**
100	**11mm**	**9.5mm**	**13.5mm**	**12mm**

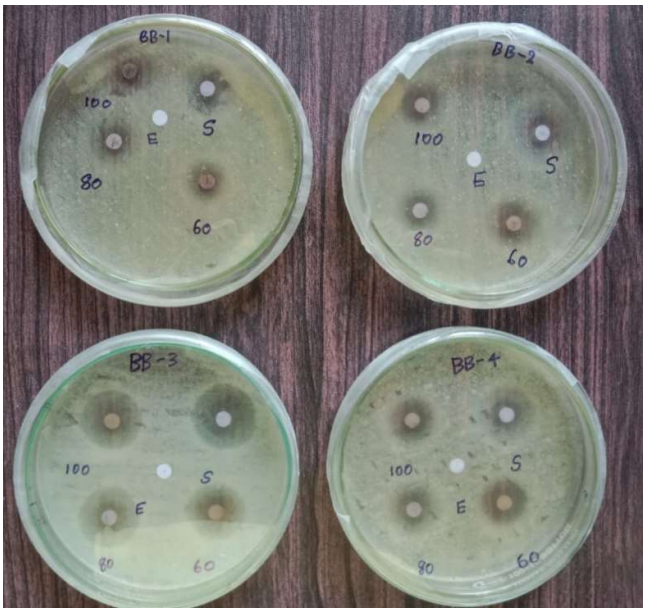


Figure 10: Anticariogenic activity of flower of *Ziziphus mauritiana*

Table 7: Results of MIC and MBC of ethanolic extract of *Ziziphus mauritiana* flower

BACTERIA	MIC (µg/ml)	MBC (µg/ml)
Streptococcus mutans	**20**	**40**
Staphylococcus aureus	**60**	**40**
Streptococcus sobrinus	**40**	**60**
Streptococcus salivarius	**40**	**40**

CONCLUSION:

The ZnO NPs have been successfully synthesized from the ethanolic extract of flower from *Ziziphus mauritiana* and formation of ZnO NPs was confirmed by UV-Visible spectroscopy and the functional groups and chemical properties were analysed using FT-IR. Also characterisation techniques such as SEM, EDAX and XRD were performed and the SEM result revealed that the

By performing antioxidant activity by DPPH assay it was confirmed that the synthesised ZnO NPs showed good inhibition percentage of 84.821% at higher concentration of 100µg/ml. Also anticariogenic activity through disc diffusion method of ZnO NPs was exhibited against Streptococcus mutans, Staphylococcus aureus, Streptococcus sobrinus and Streptococcus salivarius. The synthesized ZnO NPs from flower extract of *Ziziphus mauritiana* showed that streptococcus sobrinus had maximum inhibition zone of 13.5mm radius at concentration 100µg/ml which is more efficient. Therefore, ZnO NPs synthesised from ethanolic extract of flower of *Ziziphus mauritiana* is an effective antioxidant agent and anticariogenic agent against dental caries.

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