International Journal of Engineering, Management and Humanities (IJEMH)
Volume 5, Issue 2, Mar.-Apr., 2024 pp: 152-164 ISSN: 2584-2145
www.ijemh.com

Anti-Oxidant and Anti-Cariogenic Activity of Synthesized Zinc Nanoparticles Using *Breynia Retusa* Flower Against Dental Caries Pathogens

Vinithkumar V[1], Jeevananthan K[2], Vishnupriyan[3], Mangayarkarasi G[4]
[1,2,3]*UG Student, Department of Biotechnology, Muthayammal Engineering College, Rasipuram, Tamil Nadu, India.*
[4]*Assistant Professor, Muthayammal Engineering College, Rasipuram, Tamil Nadu, India.*

Date of Submission: 31-03-2024 Date of Acceptance: 08-04-2024

ABSTRACT: The biosynthesis of zinc nanoparticles is mostly used for research purposes because of its capability for eco-friendly development. The synthesis of zinc nanoparticles using flower of *Breynia retusa* extract was carried out by antioxidant and anticariogenic activity. The aqueous solution of 2 mM zinc acetate treated with flower extracts separately, was incorporated within one hour. These flower-ZnNPS nanoparticles are identified with fourier transform infrared spectroscopy (FITR), X-ray Diffraction (XRD), Scanning electron microscopy (SEM), UV- Visible spectroscopy, Energy dispersive X-ray spectrometer (EDX). The nanostructure shows the face centered cubic crystal by conformation of XRD result. The synthesized-ZnNPs nanoparticle showed 76.45% at concentration 100 µg/ml while ascorbic acid gave 85.47 % at the same concentration 100 µg/ml. The anticariogenic activity showed maximum inhibition was observed against *Streptococcus sobrinus* (mm) and *Staphylococcus aureus* (mm) for ZnNPs.

Keywords: Breynia Retusa, Zinc Nanoparticles, Antioxidant And Anticariogenic Activity.

I. INTRODUCTION

1.1 GENERAL

Breynia retusa, a plant commonly found in tropical and subtropical regions, has been used in traditional medicine for various ailments. Recent research has focused on the potential of nanoparticles synthesized from different parts of the plant, including the flower, for their biological activities[1]. This study investigates the synthesis of zinc nanoparticles (ZnNPs) from Breynia retusa flowers and explores their antioxidant and anticariogenic properties. Zinc is a crucial mineral for human health and plays a role in various physiological processes. Nanoparticles exhibit unique properties compared to their bulk counterparts, making them promising candidates for biomedical applications[2].

1.2 NANOSCIENCE

Nanoscience is a study of structures and materials on the scale of nanometers. When structures are made small enough in the nanometer range, they can take on interesting and useful properties. The physical and chemical properties of nanomaterials are unique size dependent that make them superior, indispensable in areas of science and technology,research and development[3].

1.3 NANOTECHNOLOGY

The technology that deals with dimensions, tolerances less than that of 100 nm and the manipulation of individual atoms and molecules is named as nanotechnology. At the molecular scale the engineering of functional systems is known as nanotechnology. Many different types of field work are done in nanotechnology. Scatter of light, absorb x-rays, transport electrical current or heat, etc.... are the most special properties enhanced by nanoparticles[4].

1.4 NANOPARTICLES

A microscopic particle which has at least one dimension less than 100 nm is said to be a nanoparticle. Intense scientific research currently covers nanoparticle research in Biomedical field, Optical field, Electrical field; Nanoparticles act as an effective bridge between bulk materials and atomic or molecular structures. Most synthetically produced nanomaterials are called nanoparticles.

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Common production technologies used are milling, gas phase, liquid phase. Nanoparticles and nanomaterials are used in a broad spectrum of applications[5].There are two main processes involved in the synthesis of nanoparticles. They are top-down process and Bottom-up process. The selection of the respective process depends on the chemical composition and the desired features specified for the nanoparticles[6]. Nanoparticles are classified into two types. They are hard nanoparticles and semi-solid or soft nanoparticles[7].

1.5 ZINC OXIDE

Zinc oxide, ZnO is an inorganic compound also known as zincite and occurs rarely in nature, generally in a crystalline form.It usually appears as a white crystalline powder, which is nearly insoluble in water. Most of ZnO which is used commercially is produced synthetically[8]. Zinc oxide has the wurtzite hexagonal crystal structure.In regular zinc oxide these vary between acicular needles and plate shaped crystals.Zinc oxide usually crystallizes in three different forms: hexagonal wurtzite, cubic zinc blende and cubic rock salt[9].

1.6 DENTAL CARIES

Dental caries, a chronic disease, is unique among humans and is one of the most common important global oral health problems in the world today. Dental caries refers to the localized destruction of susceptible dental hard tissues by acidic by products from the bacterial fermentation of dietary carbohydrates[10].

1.6.1 EPIDEMIOLOGY OF DENTAL CARIES

The most recent epidemiological data on the prevalence of dental caries in children indicate a halting of the increasing levels in many developing countries and a continuing decrease in many highly industrialized countries of the world[11].

1.6.2 SIGN AND SYMPTOMS OF DENTAL CARIES

It depends on their extent and location. When a cavity is just beginning and may not have any symptoms at all[12]. As the decay gets larger, it may cause signs and symptoms such as:

I. Toothache
II. Visible holes or pits in teeth
III. Brown, Black or White staining on any surface of teeth
IV. Bad breath and foul tastes
V. Fever, chills, apsces, and trismus.

1.6.3 PREVENTION AND CONTROL

Personal hygiene care consists of proper brushing and flossing daily. Proper brushing and flossing is to remove and prevent the formation of plaque or dental biofilm. Professional hygiene care consists of regular dental examinations and professional prophylaxis(cleaning). Minimizing snacking is recommended, since snacking creates a continuous supply of nutrition for acid-creating bacteria in the mouth. Chewy and sticky foods tend to adhere to teeth longer, brushing the teeth after meals is recommended. For children, ADA and the EAPD recommend limiting the frequency of consumption of drinks with sugar, and not giving baby bottles to infants during sleep[13].

1.7 BREYNIA RETUSA

Herbal medicines are a valuable as well as a precious gift from nature. They were existing even before human beings made their appearance on the earth[14].Wherever We are born we have around us herbs,Shrubs and plants useful to us. *Breynia retusa* is a shrub with spreading branches.It grows abundantly in Bangladesh.

1.7.1 CHEMICAL COMPONENTS

Chemical composition/kay active constituents various phytochemicals reported from this species such as reducing sugars,phenolics (most abundant phenols are gallic acid, ellagic acid, cummaric acid, ferulic acid and vanillic acid). Alkaloids, tannins, glycosides, flavonoids and saponins, proteins[15].

1.7.2 MEDICINAL USES

Macerated Breynia retusa leaf juice is used to cure body pain, skin inflammation, hyperglycaemia, diarrhoea and diuretic. The fruits have been used for dysentery and twigs used for toothache[16].

1.7.3 PHARMACOLOGICAL ACTIVITY

Breynia retusa leaves have maximum antioxidant activity and anti- arthritic activity and it could be natural, Diabetic, antioxidant activity and anti-arthritic source and thus could be useful as therapeutic agents in preventing these diseases[17]. Further studies are needed for their active principle to elucidate. Breynia retusa bark has been used traditionally in Indian medicine for the treatment of a broad spectrum of disorders[18].

II. MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIAL

The flower of *Breyniaretusa* has been gathered in the month of December from Mullipatti, Pudukottai, Tami Nadu, India[19].

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2.2 ETHANOLIC EXTRACT OF PLANT PREPARATION

The flower of *Breynia retusa* plant were collected and washed with fresh water. After, it was dried in a shade region for 35-40 days and made into fine powder. In a dry beaker, an absolute amount of powder and few amounts of ethanol were added. These solutions were shifted into another beaker, stirred with glass rod and closed them with watch glass. Boil this solution for 20-30 minutes; the colour changes from slight green into dark green in colour and cooled at room temperature for 1 hour. The extract was filtered using Whatman No.41 filter paper in a clean beaker. Finally, these stock solutions were transferred into a brown bottle and then stored in as cooled place for further study[20]**.**

2.3 PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT FROM *BREYNIA RETUSA*

The phytochemical screening was performed using a modified procedure of Yadav *et al.*, 2014 [21].

TEST FOR TANNINS

2 ml of extract was added to 2 ml of distilled water in a test tube and then filtered. Add a few drops of 0.1% ferric chloride to the filtrate. Green precipitate was regarded as the presence of tannins.

TEST FOR PHLOBATANNINS (PRECIPITATE TEST)

To 2 ml of extract, 2ml of 1% HCL was added and boiled. Red precipitate was regarded as positive for the presence of phlobatannins.

TEST FOR SAPONINS

To 5ml of the extract was added with the 5 ml of water in a test tube. The solution was shaken vigorously and observed for stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed in the formation of an emulsion.

TEST FOR FLAVONOIDS

A few drops of 10% lead acetate solution were added to 1 ml of extract. A yellow coloration indicates the presence of flavonoids.

TEST FOR STEROIDS (SARKOWSKI TEST)

To 2 ml of extract, 2 ml of chloroform and two drops of concentrated sulfuric acid were added. Presence of steroids was identified by the appearance of a reddish brown ring.

TEST FOR TERPENOIDS (SARKOWSKI TEST)

To 2 ml of extract, 2 ml of chloroform concentrated sulfuric acid were added. Presence of terpenoids on the interface was indicated by a reddish brown ring.

TEST FOR CARDIAC GLYCOSIDES

To 1 ml of extract, 2 ml of glacial acetic acid, 1 ml of ferric chloride and 1 drop of concentrated sulfuric acid was added. Formation of violet or brown rings was regarded as the presence of cardiac glycosides.

TEST FOR LEUCOANTHOCYANIN

To 1 ml of extract, 1 ml of isoamyl alcohol was added to the observation of the organic layer into the red and was regarded as positive for the presence of leucoanthocyanin.

TEST FOR ANTHOCYANINS

To 2 ml of extract, 2 ml of HCL and 1 ml of Ammonia solution was added, Color changes from pinkish red to bluish violet coloration was regarded as positive for the presence of anthocyanin.

TEST FOR ANTHRAQUINONE

To 2 ml of extract, 1 ml of benzene and 2 ml ammonia solution was added. Pink, violet or red coloration was regarded as positive for the presence of anthraquinone.

TEST FOR PROTEINS (XANTHOPROTEINS TEST)

To 1 ml of extract, 1 ml of concentrated sulfuric acid was added and boiled. White precipitate was regarded as positive for the presence of protein.

TEST FOR COUMARINS

To 2 ml of extract, 3 ml of 10% sodium hydroxide was added. Yellow color was regarded as positive for the presence of coumarin.

TEST FOR GLYCOSIDES (LIBERMANNS TEST)

To 2 ml of extract, 2 ml of chloroform and 2 ml of acetic acid were added. The color changes from Violet to blue and then to green color was regarded as positive for glycosides.

TEST FOR PHENOLS

To 1 ml of extract, few drops of ammonia solution were added. Reddish orange precipitate formation was regarded as positive for the presence of phenols.

TEST FOR ALKALOIDS (HAGERS TEST)

To 2 ml of extract, few drops of Hager's reagent were added and shaken gently to extract the alkaloids base, yellow precipitate was regarded as positive for the presence of alkaloids.

TEST FOR XANTHOPROTEINS

To 1 ml of extract, four drops of ferric chloride were added. Blue black coloration was regarded as positive for the presence of xanthoproteic.

TEST FOR EMODIN

To 2 ml of extract, 2 ml of ammonium hydroxide and 3 ml of benzene was added, red color was regarded as the presence of emodin.

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TEST FOR CARBOHYDRATES

To 2 ml of extract, 2 ml of distilled water, 2 drops of ethanolic alpha naphthol and 2 ml of concentrated sulfuric acid were added; appearance of reddish violet ring was regarded as the presence of carbohydrates.

2.4 QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS PRESENT IN PLANT

The presence of flavonoids, terpenoids, alkaloids, saponins and phenol in mg\g was shown by phytochemical analysis of *Breynia Retusa*[22].

ALKALOIDS

The alkaloids are determined using the spectrophotometer technique. 2g of dried powder is placed in a beaker with 9 ml of 10%acetic acid in ethanol solution. The temperature of the combination is kept at room temperature. The above-mentioned mixture is then filtered. The filtrate from the beaker is collected and deposited in a water bath. The filtrate had been reduced to its original volume. After a few minutes, the beaker is allowed to cool before adding more concentration ammonium hydroxide solution till precipitation occurs. The solution is discarded once the precipitation occurs, and the precipitate is collected, washed with ammonium hydroxide solution, and filtered. The residue is placed in a watch glass and allowed to dry at room temperature. It is weighed once it has dried.

PHENOLS

The 4g of material is collected as a dried powder. For ten minutes, the sample is heated in ten millilitres of diethyl ether. After boiling, pour 2ml of the mixture into a beaker and add 1 ml of pure water. After a few minutes, a little amount of concentrated ammonium hydroxide solution is added, followed by 1 ml of concentrated amyl alcohol. Because it reacts to color development, the combination is left to sit for a few hours. The color is generated and cured at room temperature and the sample is then measured in a spectrophotometer at a wavelength of 505 nanometers.

TERPENOIDS

The material is weighed at 4g and dissolved in 10 millilitres of alcohol for one day. Whatman no.1 filtrate paper is used to filter the sample. After that, petroleum ether is used to dissolve the filtrate. After measuring the dried condition with a spectrophotometer, the filtrate is dried to preserve room temperature.

TANNINS

To determine the tannin solution, a spectrophotometer is utilized. Weigh a 0.5g powder sample and place it in a beaker. 10 ml of distilled water was added to the mixture, which was agitated for a few minutes. The above-mentioned solution is then filtered. The filtrate is placed in a test tube and 1ml of 0.1M ferric chloride in 0.1M hydrochloric acid isadded, as well as 0.008M potassium ferrocyanide.The combination is kept at a constant temperature of room temperature Spectrophotometer technique was used to weigh the dry powder.

FLAVONOIDS

Weigh 4g of the powder sample and place it in a beaker. At room temperature, the solution is dissolved in methanol.The filtrate from the above said solution was then collected in a beaker. The beaker is placed in a water bath, and the solution is dried after a few minutes.The sample is then weighed with a spectrophotometer.

SAPONINS

The saponins are measured using a spectrophotometer. The 4g sample powder is dissolved in 2 ml of ethanol solution in a conical flask and placed in a water bath with constant stirring. The solution is kept at room temperature after the water bath. The solution is filtered and the residue is dissolved in ethyl alcohol before being deposited in a water bath to reduce volume. The solution is then transferred to a separation funnel, where 5 ml of diethyl ether is added and violently agitated. The kept in a few minutes after shaking is not disturbed. The two layers are then removed and discarded. Separate the aqueous layer and a few drops of n-butanol and a few drops of 5% Nacl before placing it in hot water once more. After the solvent has evaporated, the sample is dried in an oven and then weight is calculated.

2.5 SYNTHESIS OF ZINC NANOPARTICLES

The weighed 2mM Zinc acetate dissolved in 100 ml of distilled water. From the prepared solution, 50ml of 2mM Zinc acetate and 5ml ethanolic extract of flower with constant and continuous stirring. The mixture reacts to an environmental condition and Zinc gets reduced in Zn+ ion. After 2 hours the colour changes are observed. The colour changes from transparent white to Yellow and Green colour. That shows the formation of Zinc Oxide. The formation of Zinc

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Oxide, that is Zn+ ion, was confirmed by the UV-spectral analysis[23].

2.6 CHARACTERIZATION TECHNIQUES

Characterization of synthesised Nanoparticles were carried out to study the characteristic wavelength and functional group bound to Zinc oxide NPs by UV-VIS Spectra and FTIR and its size, crystalline nature and elemental composition using SEM, XRD and EDAX.

2.6.1 UV-Visible analysis:

The optical properties of ZnO Nanoparticles were characterized using UV-VIS Spectra. The synthesized zinc oxide nanoparticles were analyzed and the colour change observed from transparent white to light sandal indicated the presence of ZnO Nanoparticles. The absorbance was recorded between 350-500 nm by UV after 24 hours of addition.

2.6.2 FTIR analysis:

Fourier Transform Infrared Spectroscopy is also known as FTIR spectroscopy or FTIR analysis. The synthesized ZnO Nanoparticles can be scanned by infrared spectroscopy and it absorbs within the range of 400-4000 cm-1. Multiple functional groups may be absorbed at a particular frequency and it gives rise to different characteristic absorptions .

2.6.3 XRD analysis:

The structure of nanomaterials (range of 1-100 nm) can be studied by using X-ray Diffraction (XRD) analysis. The position of values of the product (amorphous or crystallinity nature) can be observed by XRD. The fingerprint regions of relative intensity are found in XRD analysis with respect to d-spacing values.

2.6.4 SEM analysis:

Scanned Electron Microscope (SEM) analysis of synthesized ZnO Nanoparticles was performed to evaluate the surface morphology of nanoparticles. ZnO.NPs were prepared and dried to eliminate the moisture content and images were taken by using FEI Quanta 250 SEM operating at 10 Kv.

2.6.5 EDAX analysis:

Analysis through Energy Dispersive X-ray Spectrometer proved that ZnO Nanoparticles are present. The horizontal axis shows energy in keV whereas the vertical axis shows the number of X-ray counts.

2.7 THE ANTI-CARIOGENIC ACTIVITY OF SYNTHESIZED ZINC NANOPARTICLES USING *BREYNIA RETUSA*

2.7.1 COLLECTION OF TEST PATHOGENS

The anticariogenic activity of synthesized zinc nanoparticles from flaxseed was exhibited against *Streptococcus mutans*(MTCC 890), *Streptococcus salivarius*(MTCC 13429), *Streptococcus sobrinus* (MTCC 33479) and *Staphylococcus aureus* (MTCC 25923) were prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

2.7.2 DETERMINATION OF ANTI-CARIOGENIC ACTIVITY BY DISC DIFFUSION METHOD

The disc diffusion method is used to determine the anti-cariogenic activity of the synthesized ZnO Nanoparticles of *Breynia Retusa*. The obtained nanoparticles are allowed to bind with the paper disc for some time. 25 ml of Mueller-Hinton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organisms followed by various concentrations of isolated compounds of 60,80 and 100 mg/ml. Filter paper disc loaded with 10µl of amoxicillin was used as positive control. Negative control was prepared using ethanol as solvent. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimeters[24].

2.8 DETERMINATION OF MIC AND MBC

Take multiple test tubes and add 2 ml 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

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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)[25].

III. RESULT AND DISCUSSION

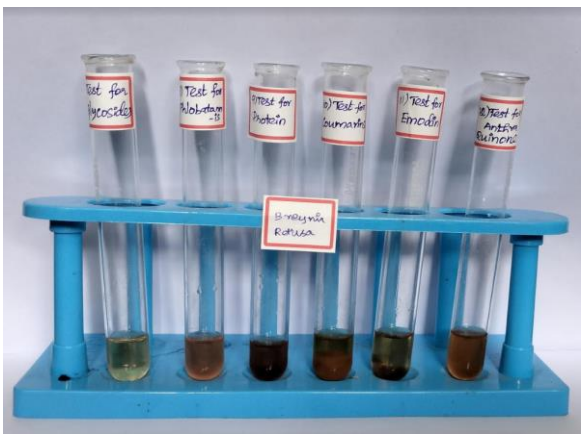
3.1 QUALITATIVE ANALYSIS OF PHYTOCOMPOUNDS FROM THE *BREYNIA RETUSA* FLOWER EXTRACT

Phytochemical constituents are found in the flower extract of *Breynia retusa* as seated in the table:3.1**.** Various assays are used to identify phytochemical compounds such as phenol, terpenoids, xanthoprotein, Flavonoids, cardiac glycosides, saponins, leucoanthocyanin, tannins, carbohydrate, alkaloids, anthocyanins, steroids, protein, glycosides, coumarins and anthraquinone are strongly present in the flower extract (Figure 3.1).

Test No	Test for	Observation	Result
1	Terpenoid	Reddish brown	+++
2	Flavonoids	Yellow colour	+++
3	Saponins	Formation of froth	+++
4	Tannin	Green precipitate	+++
5	Alkaloids	Yellow colour Precipitate	+++
6	Steroids	Reddish brown ring	+++
7	Glycosides	Violet into blue into Green colour	++
8	Phlobatannins	Red precipitate	+++
9	Proteins	White precipitate	+++
10	Coumarin	Yellow precipitate	+++
11	Emodin	Red colour	+++
12	Anthraquinone	Pink, Violet, Red Colour	++
13	Anthocyanin	Pinkish red to bluishViolet colour	+++
14	Carbohydrate	Reddish violet ring Formation	+++
15	Leucoanthocyanin	Organic layer into Red	+++
16	Cardiac glycosides	Formation of violet Browning	+
17	Xanthoprotein	Blue black colour	+++
18	Phenols	Reddish orange Colour	+++

A – Absence, + - Trace, ++ - Moderate, +++ - Strong

Table 3.1: Qualitative analysis of phytochemical compounds from the ethanolic extract of *Breynia retusa*



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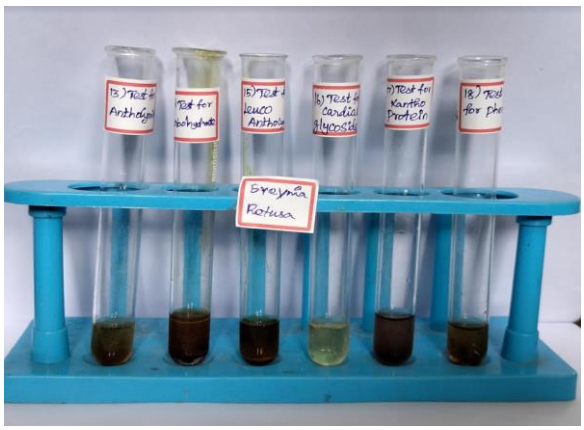

Figure 3.1: Qualitative analysis of phytochemical compounds from the ethanolic extract of *Breynia retusa* flower extract

3.2 QUANTITATIVE INVESTIGATION OF *BREYNIA RETUSA* EXTRACT

Quantitative analysis of *Breynia retusa* was found to possess Tannin , Saponin,Alkaloids, Terpenoid, Flavonoids, Phenols when compared to grain extract and is represented in table 3.2.

The phytochemical constituents such as Tannins (0.028mg/g), Saponins (0.019mg/g), Alkaloids (0.05mg/g), Terpenoids (0.013mg/g), Flavonoids (0.006mg/g) and Phenols (0.023mg/g) are present in the *Breynia retusa* grain extract (Figure 3.2).

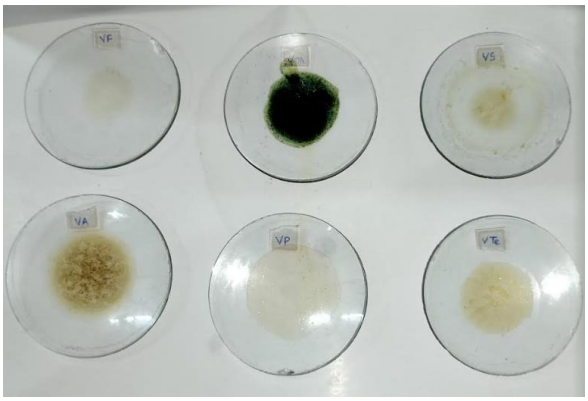

Figure 3.2: Quantitative Analysis Of Secondary Metabolites From *Breynia Retusa* flower Extract

S.NO	Phytochemical Constituents	*Breynia retusa* flower extract(mg/g)
1	Saponins	0.019
2	Alkaloids	0.050
3	Flavonoids	0.006
4	Phenol	0.023
5	Terpenoids	0.013
6	Tannins	0.028

Table 3.2: Quantitative Analysis Of Secondary Metabolites From *Breynia Retusa* flower Extract

3.3 SYNTHESIS OF ZNO NANOPARTICLES

The magnetic stirrer method is used for synthesis of ZnO nanoparticles. When 30 mL of 1mM zinc acetate solution was added to 8 mL of ethanolic extract and subjected to magnetic stirring, zinc acetate was reduced to zinc oxide. This reduction was observed as a reddish brown precipitate, indicating the presence of Zno nanoparticles (Fig. 3.3).

Figure 3.3 Synthesis of ZnO nanoparticles

3.4 CHARACTERIZATION TECHNIQUES

3.4.1 UV SPECTROSCOPY

Absorption spectra of synthesized ZnO NP at different concentration and temperature were analyzed by UV visible spectroscopy. A single

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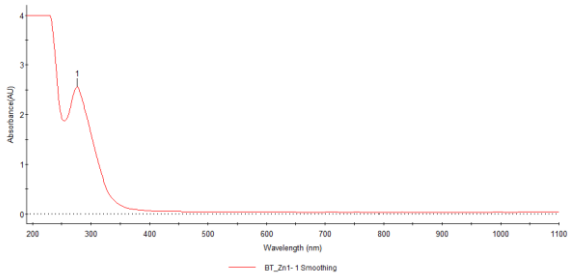
peak of absorption band at 2.555 AU height and 276.2 nm wavelength was obtained without any deviation, indicating the absence of impurities and presence of phenolic group (Figure 3.4).



Name	No.	Peak(nm)	Peak(AU)	No.	Valley(nm)	Valley(AU)
BT_Zn1-	1	276.2	2.555			

Figure 3.4: UV-Visible spectroscopy of ZnO NPs

3.4.2 FTIR SPECTROSCOPY

Functional group and chemical properties of synthesized zinc NPs were analyzed by FTIR techniques. The FTIR spectrum of ZnO NPs synthesized was recorded in the range of 4000 to 400 cm-1, the observation showed that the bands of ZnO NPs at 3436.27 cm-1 and 2078.03 cm-1 were attributed to stretching vibrations of the primary and secondary amines. observed at 1384.35 and 1014.50 cm-1 bands have been assigned to alcohols and phenolic group.The alcoholic groups-c-,OH,NH2 show vibration, secondary amine show due to the bond formed by 1638.12 cm-1 show conjugation of aromatic ring. The hydroxyl of flavonoids and phenolic compounds represents the stretching vibration of C-O bond and C-H curve was observed at 658.92 cm-1.

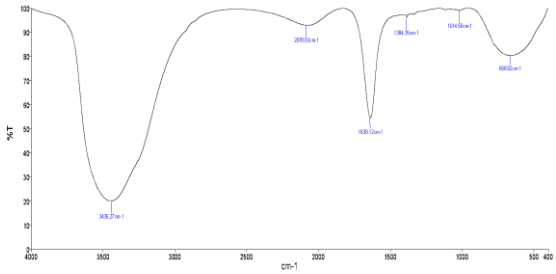


Figure.3.5 FTIR spectroscopy of ZnO NPs

3.4.3 SCANNING ELECTRON MICROSCOPE (SEM)

The surface morphology of synthesized ZnO NPs was evaluated using the SEM image. The ZnO NPs synthesized were within the range of 200 nm.The peaks in range of 73.75-93.83 nm. From (Figure 3.6) the obtained SEM image of the synthesized ZnO NPs was revealed to be Crystals in shape.

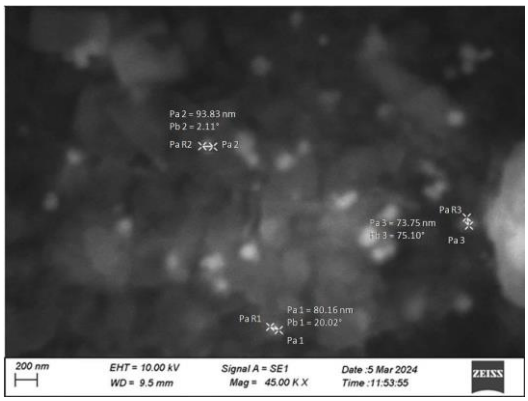


Figure 3.6: SEM image of ZnO NPs

3.4.4 ENERGY DISPERSIVE X-RAY SPECTROMETER (EDAX)

Energy dispersive X-ray (EDX) spectrometer analysis was performed to confirm the elemental signal of ZnO NPs. Presence of sub-peaks of oxygen (O) and Zinc (Zn) in the EDAX spectra of the as-synthesized ZnO nanoparticles (NPs). The weight of zinc in ZnO NPs reduced by extract was found to be 67.65%.

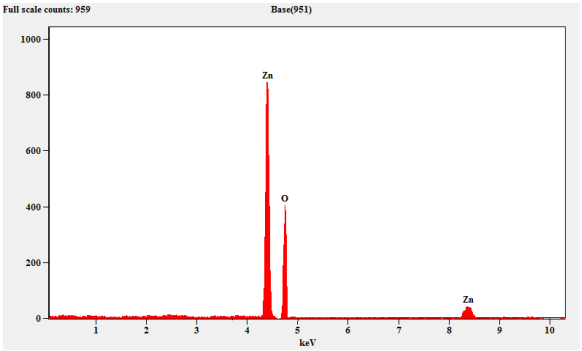


Figure 3.7: EDAX analysis of ZnO NPs

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Element Line	*Weight %*	*Weight % Error*	*Atom %*
O K	32.24	± 0.24	52.67
Zn K	67.65	± 1.82	47.30
Zn L	---	---	---
Total	100.00		100.00

Table 3.3: Elemental composition of *sample* mediated ZnO NP.

3.4.5 X-RAY DIFFRACTION (XRD)

In X-ray crystallography the crystalline nature of zinc oxide nanoparticles (Table 4.4). The synthesized ZnO nanoparticles pattern performed by XRD was represented in Figure 4.8. The X-ray diffraction peaks obtained at 31.2787°, 32.2734°, 45.8706°, 49.0132°, 54.7171° and 66.9235° corresponded to the lattice plane of (100), (002), (101), (102), (110), (112) suggested the face-centered cubic crystal structure of the nanoparticle. Joint Committee on Powder Diffraction standards (JCPDS) was used as a reference to assign the lattice planes according to the peaks obtained. Powder Diffraction standards (JCPDS) was used as a reference to assign the lattice planes according to the peaks obtained.

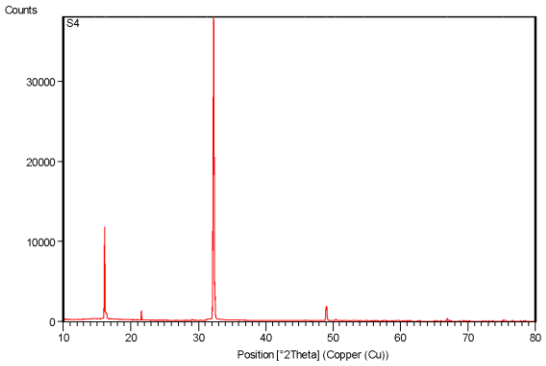

Figure 3.8: XRD analysis of synthesized Zinc nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
14.6094	148.91	0.7872	6.06341	0.39
16.1192	11631.53	0.1181	5.49872	30.74
16.4000	794.66	0.0787	5.40519	2.10
21.5471	1206.90	0.1181	4.12426	3.19
29.0970	128.08	0.1181	3.06902	0.34
31.2787	140.18	0.1968	2.85976	0.37
32.2734	37843.14	0.1968	2.77385	100.00
45.8706	58.63	0.1968	1.97833	0.15
49.0132	1619.18	0.2755	1.85859	4.28
50.4193	228.97	0.1181	1.81000	0.61
54.7171	61.56	0.1181	1.67756	0.16
66.9235	389.85	0.0960	1.39704	1.03
75.3268	141.83	0.0960	1.26067	0.37

Table 3.4: XRD patterns for synthesized ZnO NPs4.5 Anticariogenic Activity Of Synthesized Zinc Nanoparticles From Samples

The substandard quality of oral cavity health leads to chronic diseases in humans. The species which are prone to oral health includes *Streptococcus mutans, Streptococcus salivarius, Streptococcus sobrinus* and *Staphylococcus aureus*. The anticariogenic activity of synthesized ZnNPs against these bacteria was investigated and results were tabulated in the (Table 3.5). The zone of inhibition of these bacterial strains was observed against *Streptococcus sobrinus* (7 mm) and *Staphylococcus aureus* (5 mm) for ZnNPs (Figure 3.9).

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Samples	Concentrati on (µg/ml)	Organisms/ Zone of Inhibition (mm)			
		Streptococcus mutans B1	*Streptococcus salivarius B2*	*Streptococcu s sobrinus B3*	*Staphylococcus Aureus B4*
Synthesized flower-ZnNPs	60	3	2	3	2
	80	4	3	6	3
	100	5	4	9	5
Standard (Amoxicillin)	10	8	7	17	10
Ethanol	10	0	0	0	0

Table 3.5: Anticariogenic activity of Synthesized ZnNPs from sample

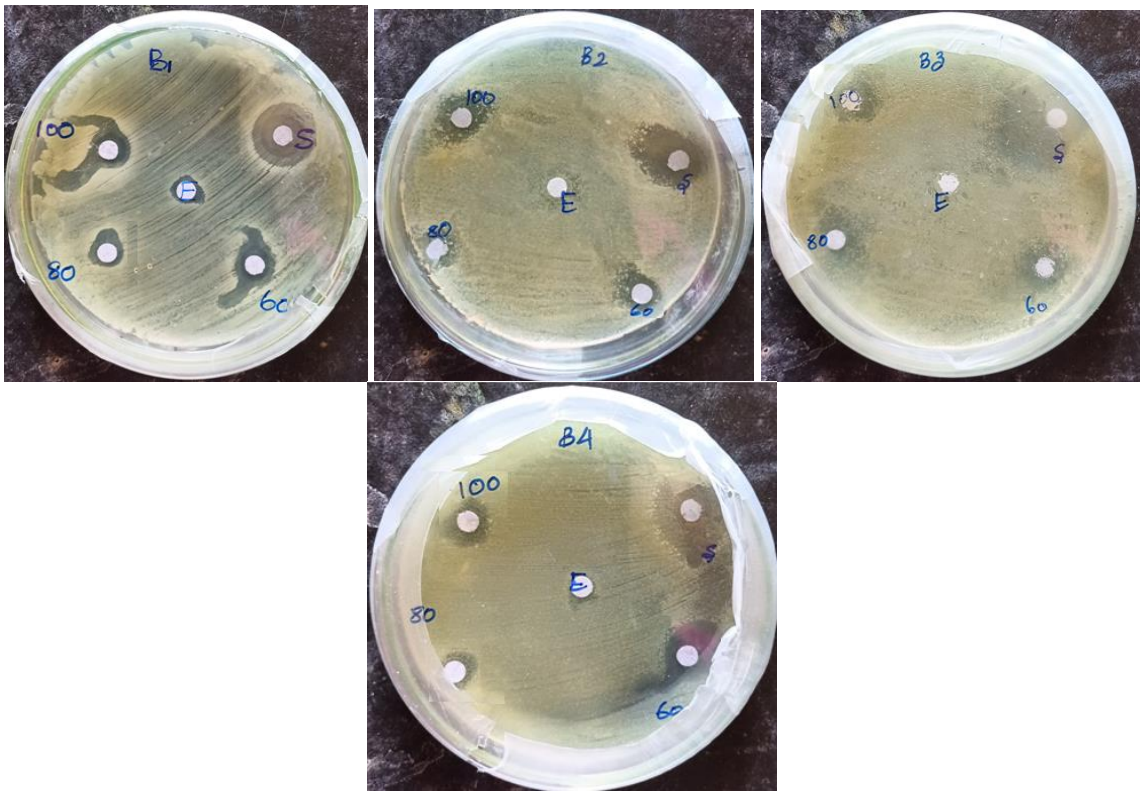

Figure 3.9: Anticariogenic Activity of Synthesized Zinc Nanoparticles

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

The minimum inhibitory concentration (MIC) for the biosynthsised ZnO NPs was found and minimum bactericidal concentration was evaluated which was presented in (Table 3.6).

From table 3.5, 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 synthesized zinc nanoparticles showed different anticariogenic activities.

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Table 3.6: Results of MIC and MBC of synthesized zinc nanoparticles

BACTERIA	MIC (µg/ml)	MBC (µg/ml)
Streptococcus mutans B1	20	40
Staphylococcus aureus B4	60	40
Streptococcus sobrinus B3	40	20
Streptococcus salivarius B2	40	60

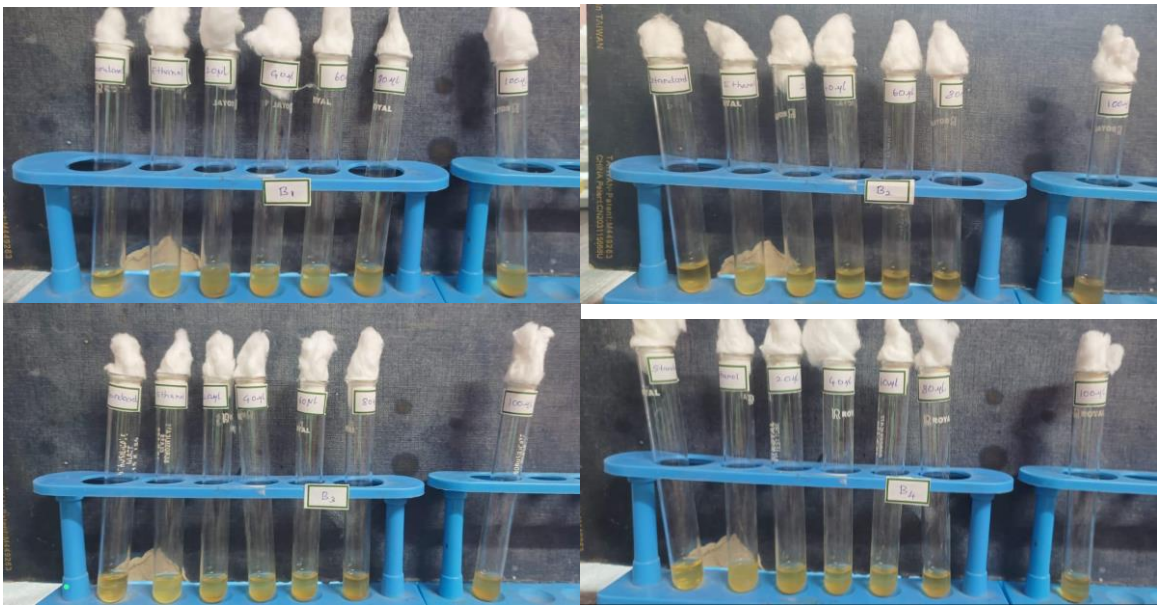

Figure 3.10: Minimum Inhibitory Concentration of Synthesized Zinc Nanoparticles

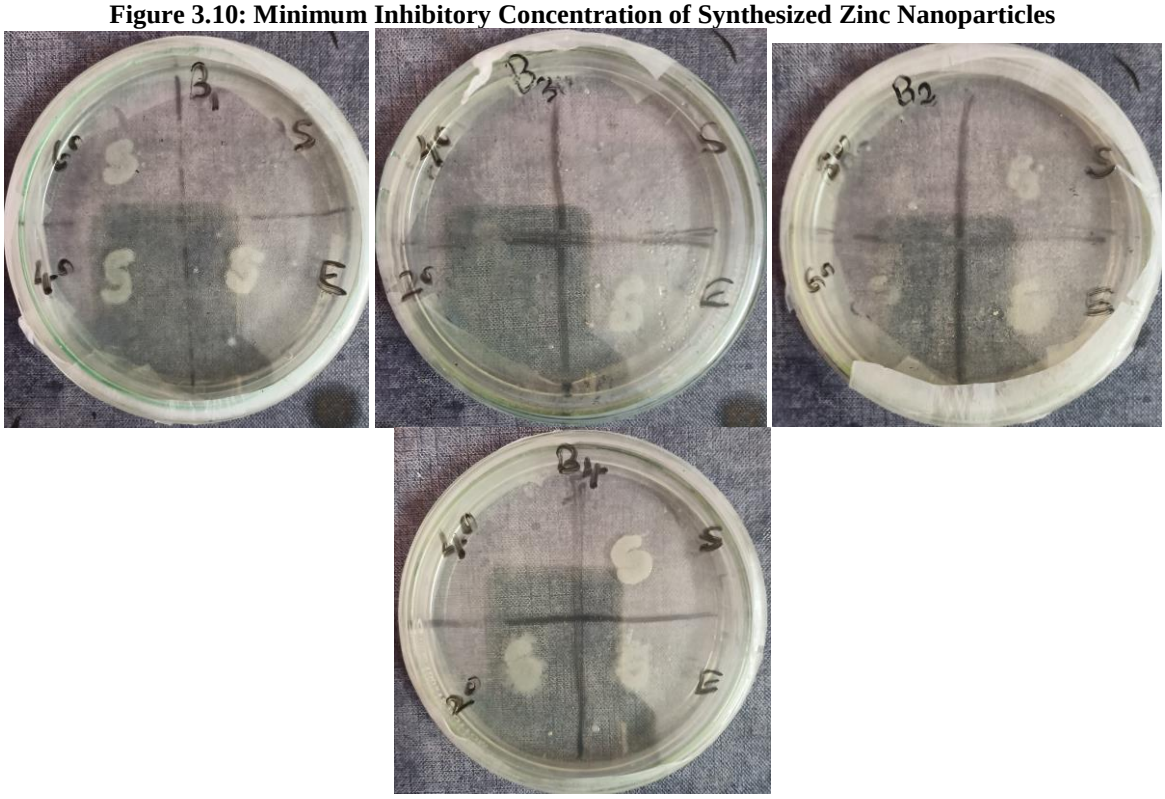

Figure 3.11: Minimum Bactericidal Concentration Of Synthesized Zinc Nanoparticles

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IV. CONCLUSION

The zinc nanoparticles synthesis was successfully performed by green synthesis method using zinc acetate and the *Breynia retusa* extract as the reducing agent. The method does not require any chemicals. The method used was simple, safe, inexpensive, eco-friendly and non-toxic. The ethanol extract of flowers of *Breynia retusa* showed great capability to synthesize the zinc nanoparticles separately. The surface plasmon Resonance band in the UV –Visible spectrum represents the absorption peak at flowers-ZnNps at the range of 333.15 nm which clearly indicates the formation of zinc nanoparticles. The list of phytochemical compounds present in the *Breynia retusa* identified by qualitative method. The XRD pattern represents the synthesized silver nanoparticles of FCC structure. This study suggests that it is free from the requirements like high energy, extended preparation and special equipment for the synthesis. The anticariogenic activity showed maximum inhibition was observed against *Streptococcus sobrinus* (mm) and Staphylococcus aureus (mm) for flowers-ZnNPs.

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