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Phytochemical Profiling and Green Synthesis of Zinc Oxide Nanoparticles using Aqueous Extracts of *Cynanchumviminale* (L.) L. (Apocynaceae)

Jinu Mathew*[1], Megha Mariet[1], Sruthy B[2] and Krishnaraj M V[3]

[1]*Department of Chemistry and Centre for Research, Baselius College, Kottayam, Kerala, India*
[2]*Accuratus Scientia Natural Product Research and Development Pvt.Ltd, Attingal, Kerala, India*
[3]*Department of Botany, Baselius College, Kottayam, Kerala, India*

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Corresponding Author: **Jinu Mathew** | E-Mail: **(jinumathew@baselius.ac.in)**

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ABSTRACT

Cynanchumviminale(L.)L. is a traditional medicinal plant that many people use to treat various illnesses. This study identifies phytochemicals in Cynanchumviminale(L.)L supporting its folkloric use and exploring its potential for safe, accessible ZnOnanoparticle synthesis. Carbohydrates. Reducing sugars, proteins, alkaloids, phenolic compounds, saponins, and flavonoids were all found in the initial phytochemical examination. The quantitative study showed a high concentration of alkaloids (9.2581 mg/g), flavonoids (26.2106 mg/g), and total phenolics (32.731 mg/g). Following nanoparticle manufacturing, phytochemical quantification reveals a reduction in the amounts of flavonoids (6.7324 mg/g) and phenolics (21.320 mg/g). UV-Visible spectroscopy, SEM, and EDX analysis validated the NPs' production. A zinc oxide nanoparticle-specific absorption peak was detected at 360 nm in the UV-Vis spectrometer. Scanning Electron Microscope (SEM) analysis revealed the presence of zinc oxide nanoparticles. The Energy Dispersive Spectrum (EDX) data indicate a prominent zinc signal at 1.26 keV, signifying the NPS elemental composition and chemical purity. Stabilising agents derived from plant extract were detected in the EDX spectra, particularly carbon, nitrogen, and other elements.

Keywords: *Cynanchumviminale, Phytochemicals, Aqueous extract, ZnO Nps*

1.Introduction

Plants are extensively used as home remedies and play a significant role in traditional treatments. The WHO supports the use of traditional medicine, and it has been proven to be efficacious and safe. Plants often create these compounds to defend themselves, but new studies showthey can shield people from illness[1]. Understanding these chemical components of plants is important for developing therapeutic agents and identifying new sources of profitable phytocompounds for synthesising complex chemicals and determining the true meaning of traditional medicines. Numerous biochemicals found in plant extracts, including alkaloids, polysaccharides, vitamins, amino acids, proteins, enzymes, tannins, phenolic acids, saponins, and terpenoids, have been identified in studies as potential contributors to the green synthesis of nanoparticles[2–6]. These phytochemicals impart significant medicinal and biological activities to nanoparticles.

Cynanchumviminale(L.)L. isa xerophytic leafless plant of the Apocynaceae family, the fifth most valuable medicinal plant family among Angiosperms[7]. Locally, it is known as 'Somalatha'. Traditionally, the plant is used to prepare "somarasas" (rejuvenating drink) used by Aryans[8]. Previous literature showed that this plant has been used by ethnic communities in India[8]. Recent studies give evidence that aqueous extract of the stem of the *Cynanchumviminale* (L.)L.shows pharmacological effects such as anxiolytic, antipsychotic, anti-inflammatory, hepatoprotective, and CNS inhibitory [9].

The plant's medicinal properties are due to its phytochemical compounds, which affect the mind and body. As a result, phytochemical screening might discover a variety of significant substances that could be employed as the basis of current medications for treating various ailments. spontaneous reducing and capping capacities have attracted significant attention towards green nanomedicine. CuO NPs synthesised from the stem extract of *Sarcostemmaacidum*were found to have better anti-inflammatory activity[10].

Silver nanoparticles (SNPs) synthesised by xerophytic plants, *Cynanchumviminale* and *Cynanchumsarcomedium* [10] exhibited a maximum absorbance at 500 nm in the UV-Vis spectrum for *C. viminale* synthesised SNPs.SEM revealed that*C. Viminale* produced agglomerated nanoparticles with an average diameter of about 60–68 nm. Additionally, *C.sarcomedium* extract was used to synthesize spherical nanoparticles with an average size of roughly 60-85 nm. SNPs produced by both lants showed a strong silver signal at 3keV according to EDAX analysis.

Studies on the synthesis of copper oxide nanoparticles in the30-70nm diameter range and polymer functionalized copper oxide nanoparticles in the diameter range of 27-32nm were also reported. The reports revealed maximum in vitro antidiabetic activity of polymerised copper nanoparticles compared to biosynthetic copper nanoparticles from *Cynanchumviminale* stem extract[11].

The present research aims to quantify some of the important phytochemicals present in the aqueous extract of the aerial part of *Cynanchumviminale*(L.)L.(somalatha) ,which the rural people widely use to treat various diseases and to study the effect of phytochemicals on the synthesis of zinc oxide nanoparticles.

2. Materials and methods

2.1. Plant collection

The aerial part of *Cynanchumviminale*(L.)L. were collected in August 2023 from the Thiruvananthapuram district of Kerala, India. The plant material was shade-dried, powdered, packed in closed containers, and stored for phytochemical studies.

2.2. Preparation of plant extract

30g of plant material was extracted using a hot extraction method using 900 mL water. It is boiled for 5-6 hours in a heating mantle to prepare aqueous extracts. Subsequently, it is filtered through a muslin cloth and preserved at around $4^{o}C$ for future utilization.

2.3. Phytochemical screening

2.3.1. Qualitative analysis of phytochemicals

The principal phytochemical components of aqueous extract were qualitatively tested using the conventional methods outlined by Harborne et al[12]. The primary metabolites analysed included carbohydrates, reducing sugars, starch, and proteins, while the secondary metabolites comprised alkaloids, phenolic compounds, saponins, and flavonoids.

2.3.2.Quantitative analysis of phytochemicals

2.3.2.1. Total alkaloid content

The total alkaloid content of the aqueous extract was determined spectrophotometrically using Atropine as a standard, as described by Selvakumar S with slight modification[13].1mL of the aqueous extract of the sample is evaporated to dryness. It is then dissolved in 2N HCl and filtered 1 mL into a separating funnel. Wash three times with 10mL chloroform. Shake thoroughly after adding 0.1N NaOH, 5ml of Bromocresol green and 5mL of phosphate buffer. Extract using 1,2,3,4 ml of chloroform while vigorously shaking. Collect in a10 mL volumetric flask and dilute with chloroform. The absorbance was measured at 470nm. Atropine was used to plot a calibration curve and the total alkaloid content was expressed as Atropine equivalents.

2.3.2.2. Total flavonoid content

Estimation of flavonoids was done using the $AlCl_3$ method, using Quercetin as a standard as described by Madhavi et.al. with slight modification[14].1mL of the plant extract and 4 mL of water were taken. After 5 minutes,0.3 mL of 5% sodium nitrite and 0.3 mL of 10% $AlCl_3$ were added to it. After 6 minutes of incubation at room temperature, 2mL 1M NaOH was added to the reaction mixture. It was made up to 10mL with distilled water. Absorbance was measured at 510nm against a blank. The calibration was drawn using Quercetin as a standard.

2.3.2.3 Total phenolic content

The total phenolic content of aqueous extract of the sample was determined spectrophotometrically according to the Folin-Ciocalteu method with modification[15]. Different concentrations of the aqueous extracts of the *Cynanchumviminale*(L.)L.was mixed with 0.4 ml Follin-Ciocalteu reagent (diluted 1:10v/v). 4 mL of sodium carbonate solution was added after five minutes. The final volume is made up to 10 ml with distilled water which is then let to stand at room temperature for 90 minutes (about one and a half hours). Absorbance was measured at 750 nm. A calibration curve was created with catechol solution as a standard, and total phenolic content was expressed in mg catechol/ g of dry weight,using the standard graph.

2.4. Green synthesis of zinc oxide nanoparticles

To 25 ml of 0.5 M zinc acetate dihydrate,2.5 ml of plant extract was added. To continue maintaining the mixture's pH at 8, 2 M NaOH was added gradually. The mixture was subsequently agitated at 70°C for 30 minutes to achieve full reduction and form a white precipitate. Powdered ZnO nanoparticles were produced by collecting the resulting material through decantation, washing it with distilled water to eliminate residues, and then oven-drying it overnight at 70 °C. For further analysis and uses, the dried sample was kept in an airtight jar at ambient temperature. The yield % was sub sequently determine dusing the following formula[16]:

Yield (%) = (Experimental weight of ZnO/Theoretical weight of ZnO) × 100

2.5. Qualitative and quantitative analysis of phytochemicals in the aqueous extract of *Cynanchumviminale*(L.)L after the synthesis of ZnO Nanoparticles

2.5.1. Qualitative analysis of Phytochemicals

Major phytochemical constituents of aqueous extracts, like carbohydrates, reducing sugars, starch, protein, amino acid, alkaloids, phenolics, saponins and tannins, were qualitatively analyzed according standard protocols outlined by Harborne et al.

2.5.2. Quantitative analysis of phytochemicals

2.5.2.2. Total flavonoid content

Estimation of flavonoids was done using $AlCl_3$ method, which used quercetin as a standard[14].1mL of the plant extract and 4 mL of water was taken. After 5 minutes,0.3 mL of 5% sodium nitrite and 0.3 mL of 10% $AlCl_3$ were added to it. After 6 minutes of incubation at room temperature, 2mL 1M NaOH was added to the reaction mixture. It was made up to 10mL with distilled water. Absorbance was measured at 510nm against a blank. The calibration was drawn using quercetin as a standard.

2.5.2.3. Total phenolic content

The total phenolic content of aqueous extracts of the sample was determined spectrophotometrically using the Folin-Ciocalteu method with slight modification[15]. Different concentrations of the aqueous extract of the *Cynanchumviminale* were mixed with 0.4 mL Follin Ciocalteu reagent (diluted 1:10v/v). After 5 minutes, 4 mL of sodium carbonate solution was added. The final volume is made up to 10 ml of distilled water and allowed to stand for 90 minutes (about one and a half hours) at room temperature. Absorbance was measured at 750 nm. A calibration curve was constructed using catechol solution as a standard, and total phenolic content was expressed in mg catechol/ g of dry weight using the standard graph.

2.6. Characterisation of ZnO NPs

Using a double-beam UV–visible spectrophotometer (T70/T80 series UV/Vis Spectrophotometer) in the 200–800 nm range, the synthesised ZnO NPs from the aqueous extracts of *Cynanchumviminale* were confirmed and characterised in order to see the distinctive peak verifying ZnO NPs formation.

Using a scanning electron microscope (JEOL JSM5800) with an accelerated voltage of 5-20 kV the sample's surface morphology was recorded.

3. Results and discussion

3.1. Phytochemical screening

3.1.1. Qualitative analysis of phytochemicals

Preliminary phytochemical screening of the aqueous extract revealed the presence of primary and secondary metabolites. The results are shown in Table 1.

Table 1: Preliminary screening for primary and secondary metabolites of aqueous extracts of Cynanchumviminale (L.)L.

Phytochemical constituents	Test	Result
Carbohydrates	Molisch	+
Reducing sugars	Fehling's	+
Starch	Iodine	+
Protein	xanthoproteic	+
Amino acid	Ninhydrin	-
Alkaloids	Dragendroff's	+
Phenolic compounds	Ferric chloride	+
Saponins	Froth	+
Tannins	Gelatin	-

3.1.2. Quantitative analysis of phytochemicals

Based on the qualitative analysis of phytochemicals, quantitative analysis was also done for major phytochemicals such as alkaloids, flavonoids, and phenolic compounds. Phenolics and flavonoids are known for their antioxidant properties, which enable them to neutralize harmful reactive oxygen species (ROS) within the plant cells. So, detecting phenolic and flavonoid compounds in plants is crucial to their potential to enhance our body's antioxidant capacity, thereby offering cardioprotective, anti-cancer, anti-diabetic, anti-ageing, and neuroprotective benefits.

3.1.2.1 Total alkaloid content

Alkaloids are nitrogen-containing secondary metabolites used as medications to treat chronic diseases such as cancer, diabetes, and neurological disorders. Aqueous extract of *Cynanchumviminale(L.)L.*contains 26.21063 mg/g of the dried sample. It is expressed using atropine as a standard.

Table 2: Absorbance of standard at different concentrations

Concentration of standard atropine (mg/ml)	Absorbance(nm)
0.2	0.1489
0.4	0.2154
0.6	0.3254
0.8	0.4568
1	0.5245
Concentration of alkaloids(mg/g)	Absorbance(nm)
9.2581	0.1862

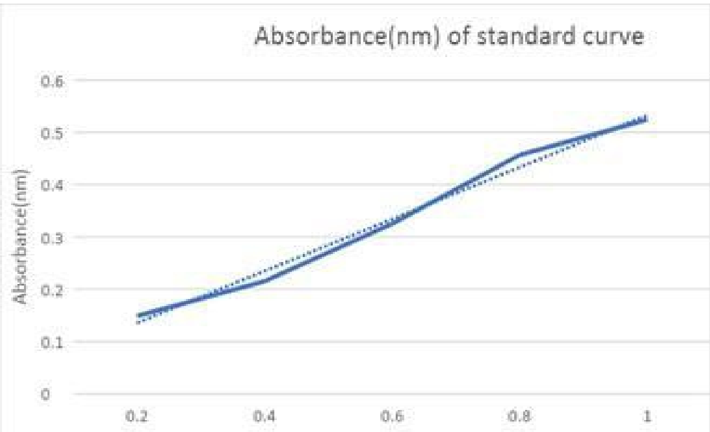

Fig 1: Calibration curve for the quantification of the and absorbance of plant extract for alkaloid determination. total alkaloid content using Atropine as a standard

3.1.2.2. Total phenolic content

Phenolic compounds in the plants are essential because of their group scavenging abilities. They are better known for their antioxidant activities,so quantification is important. Aqueous extract of *Cynanchumviminale*(L.)L contains 32.731mg/g of dried sample. It is expressed by using Catechol as a standard.

Table 2: Absorbance of standard at different concentrations

Concentration of standard catechol(mg/ml)	Absorbance(nm)
0.2	0.0984
0.4	0.1654
0.6	0.2415
0.8	0.3256
1	0.4215
Concentration of phenolic compounds (mg/g)	Absorbance(nm)
32.731	0.445

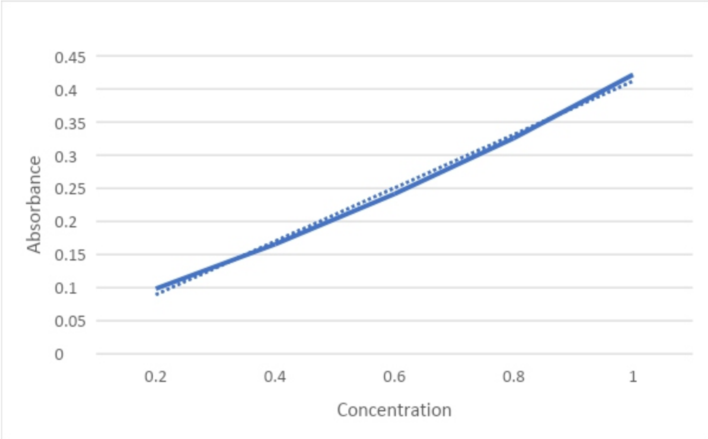

Fig 2: Calibration curve for the quantification of and the absorbance of plant extract for determination of total total phenolic content using Catechol as a standard phenolic content

3.1.2.3 Total flavonoid content

Flavonoids are significant because of their anti-inflammatory, anti-microbial, and enzyme-inhibiting properties. Aqueous extract of *Cynanchumviminale* (L.) L contains 26.2106mg/g of the dried sample. It is expressed by using Quercetin as a standard.

Table 3: Absorbance of standard at different concentrations

Concentration of standard quercetin (mg/ml)	Absorbance (nm)
0.2	0.5478
0.4	0.9874
0.6	1.3241
0.8	1.7895
1	2.1245
Concentration of flavonoid (mg/g)	Absorbance(nm)
26.2106	1.88

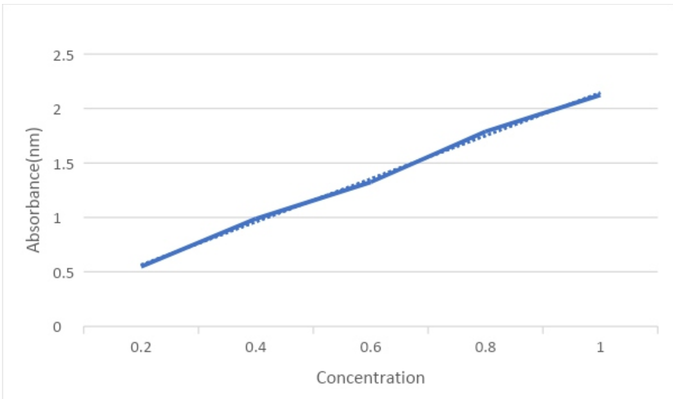

Fig 3: Calibration curve for the quantification of and the absorbance of plant extract fordetermining flavonoid content using Quercetin as a standard. Flavonoid content

3.2. Green synthesis of ZnO Nanoparticles

This study developed a cost-effective, eco-friendly method for green ZnO NP synthesis from zinc acetate dihydrate using aqueous *Cynanchumviminale* extracts as reducing and capping agents. The aqueous extract is considered eco-friendly over organic extracts and found more appropriate for the green synthesis of ZnO NPs[17, 18]. Further, ZnO NPs are studied using instrumental techniques such as UV-Visible and SEM analysis. Several phytochemicals present in aqueous extracts of *Cynanchumviminale*, such as flavonoids, phenolics, and aromatic compounds, exhibit antioxidant properties, and consequently, can play an important role as reducing and/or capping agents in nanoparticle synthesis.

Quantitative analysis of important phytochemicals, such as flavonoids and phenolic compounds, which act as significant reducing and capping agents for nanoparticle synthesis, was performed. It was observed that phenolics and flavonoids in the plant extract can trigger the reduction of zinc acetate[19].

3.2.1Qualitative analysis of Phytochemicals

Phytochemical screening of the aqueous extracts of *Cynanchumviminale* was done after the synthesis of ZnO nanoparticles. The results were shown in Table 4

Table 4: Preliminary screening for primary and secondary metabolites in aqueous Cynanchumviminale extract after the synthesis of Nps.

Phytochemical constituents	Test	Result
Carbohydrates	Molisch	+
Reducing sugars	Fehling's	±
Starch	Iodine	-
Protein	xanthoproteic	-
Amino acid	Ninhydrin	-
Alkaloids	Dragendroff's	-
Phenolic compounds	Ferric chloride	±
Saponins	Froth	++
Tannins	Gelatin	-

Where; + Positive, ± Trace, - Negative

3.2.2. Quantitative analysis of Phytochemicals

3.2.2.1. Estimation of total phenolic content after the synthesis of ZnO nanoparticles

The total amount of phenolics as determined using the regression equation of calibration curve ($y=0.0806x+0.0086$, $R^2=0.9953$) and expressed in catechol equivalent was 21.3920 mg/g of plant extract. The standard calibration curve of catechol is given in Figure 4. Table 5 shows the total phenolics in aqueous extracts of *Cynanchumviminale* determined by the Folins-Ciocalteau method.

Table 5: Absorbance of standard at different concentrations

Concentration of standard catechol(mg/ml)	Absorbance(nm)
0.2	0.0984
0.4	0.1654
0.6	0.2415
0.8	0.3256
1	0.4215
Concentration of phenolic compounds (mg/g) after the synthesis of ZnONps	Absorbance(nm)
21.320	0.270

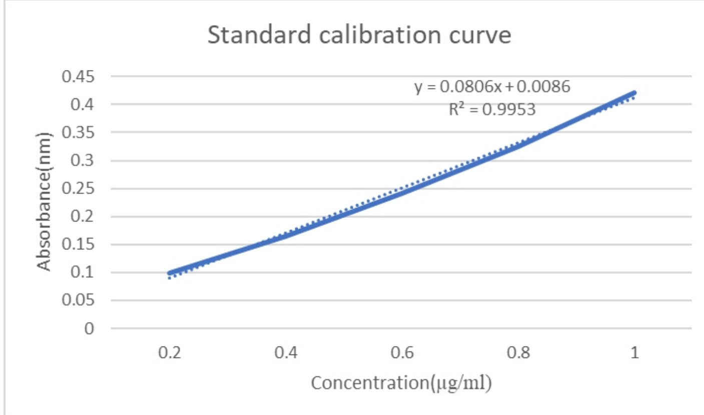

Fig 4: Standard calibration curve for total Phenolics and absorbance of plant extract for the determination content of standard Catechol of Phenolic compounds after the synthesis of ZnO Nps

3.2.2.2. Estimation of total flavonoid content after the synthesis of ZnO nanoparticles

The total flavonoid content was quantified using the calibration curve's regression equation($y=0.3956x+0.168$, $R^2=0.9975$) and expressed in quercetin equivalent was 6.7324 mg/g of plant extract. The standard calibration curve of Quercetin is given in Figure 5. Table 6 shows the amount of total flavonoids in aqueous extracts of *Cynanchumviminale* determined by the $AlCl_3$ method after synthesis of ZnO nanoparticles.

Table 6: Absorbance of standard at different concentrations

Concentration of standard quercetin (mg/ml)	Absorbance (nm)
0.2	0.5478
0.4	0.9874
0.6	1.3241
0.8	1.7895
1	2.1245
Concentration of flavonoid (mg/g) after the synthesis of ZnO NPs.	Absorbance(nm)
6.7324	0.606

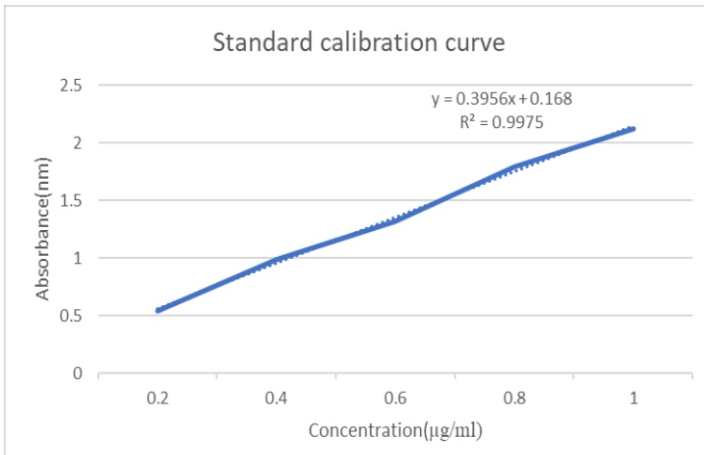

Fig 5: Standard calibration curve for total flavonoid and the absorbance of plant extract for the determination content of standard quercetin of flavonoids after synthesising ZnO NPs.

3.3. Characterisation of ZnO NPs

3.3.1. UV–Visible spectral analysis

Secondary metabolites in plants convert zinc ions in solution into zinc oxide. The plant extract functions as both a reducing agent and a stabilizing agent. This was validated by UV-visible spectrum analysis in the region of 280nm-800nm. The produced ZnO NPs were dispersed in deionized water and subjected to sonication for 10 min to detect the UV–visible spectra. The UV-Vis spectra confirmed the successful formation of ZnO NPs using *Cynanchumviminale* aqueous extract, with a strong absorption peak at 360 nm(Fig. 6). For ZnO nanoparticles, the absorbance peak is reported between 310nm and 360 nm [20].

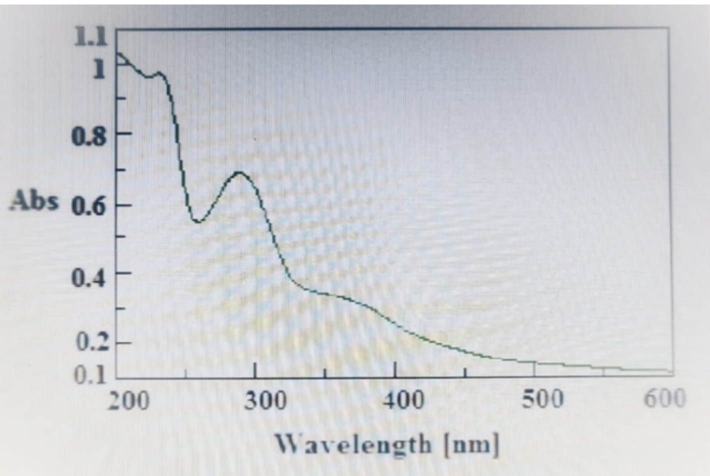

Fig 6: UV-Visible Spectra of ZnONps.

3.3.2. Scanning Electron Microscope

SEM images of ZnO nanoparticles biosynthesised by *C. viminale* extract were obtained, but the precise shape of the nanoparticles could not be predicted. The particle size was 100nm(Fig.7). The hydrogen bonding and electrostatic attraction between bioorganic capping molecules and NPs have resulted in their aggregation [21].

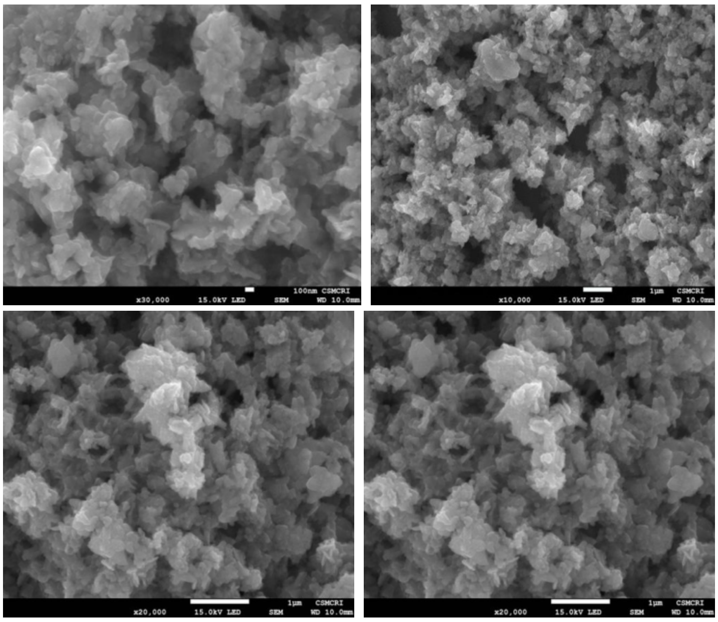

Fig 7: SEM image of ZnO NPs at different magnifications

The elemental composition of ZnO NPs was obtained from EDX analysis. EDX analysis of ZnO NPs generated with *C.viminale* extract revealed a significant zinc signal at 1.26 keV, indicating the nanoparticles' elemental composition and chemical purity. ZnO production is indicated by high Zn and O content. Stabilising agents derived from plant extract were detected in the EDX spectra, particularly carbon, nitrogen, and other elements.

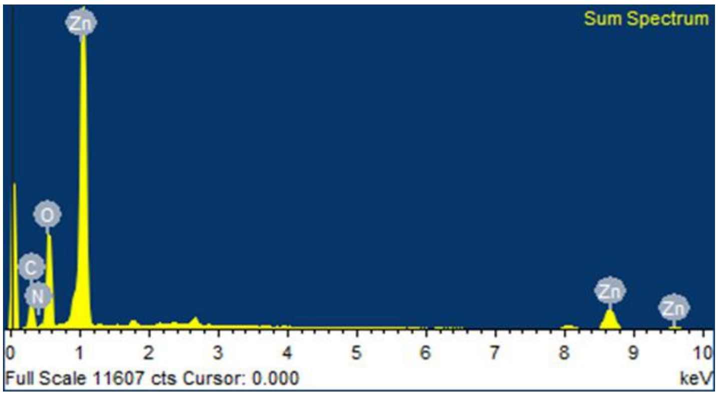

Fig 8: EDX spectrum of ZnONps

Discussion

The phytochemical examination of *C.viminale*'s aqueous extract revealed the presence of carbohydrates, reducing sugars, starch, protein, amino acids, alkaloids, phenolic compounds, and saponin. The quantitative analysis of three identified phytochemicals (alkaloids, flavonoids, and phenolics) revealed a high amount of phenolics (32.731 mg/g), followed by flavonoids (26.2106 mg/g) and alkaloids (9.2581 mg/g). For therapeutic purposes, phenolics are of utmost significance. Diaz et al. also reported that medicinal plants' anti-inflammatory and antioxidant activities were correlated with the levels of phenolic and flavonoid compounds[22]. Tukun et al. reported that phenolic content is significantly connected to antioxidant activity[23]. Also, phenolic phytochemicals play various protective roles against abiotic stresses, such as UV light, and abiotic stresses, namely predator and pathogen attacks.

Flavonoids are polyphenolic chemicalsprotecting plants from UV radiation, pathogens, and oxidative stress. According to Okoli and Okere, flavonoids are powerful water-soluble super antioxidants and free radical scavengers that protect cells from oxidative damage, have significant anticancer properties, and suppress tumour development[24]. ROS are free radicals that serve as messengers inside cells, but when present in high concentrations, they can harm proteins, RNA, and DNA. Cells have developed numerous ways to fix the damage caused by these species, which is why antioxidants could be helpful as medicine. Due to their structure, flavonoids can function as reducing agents in various processes, providing an antioxidant effect. Flavonoids' underlying methods include scavenging ROS, blocking oxidases that produce the superoxide anion, chelating trace metals, and activating antioxidant enzymes. The high phenolic and flavonoid content supports the traditional use of *C.viminale* as an anti-inflammatory agent.

Plants are the most popular green and easy method for creating nanoparticles because they provide the large-scale manufacturing of stable nanoparticles in various sizes and forms. Using natural plant extracts is economical, easy, and environmentally beneficial. Numerous studies have shown how to use various plant extracts to create zinc oxide nanoparticles in an environmentally friendly manner[17, 25–29]. However, the *C.viminale* plant has not been reportedly used for ZnO NPs synthesis. The green method synthesized zinc oxide NPs with a preferred size of 100nm. The fundamental mechanism for the green synthesis of ZnO nanoparticles remains incompletely elucidated.

Free amino and carboxylic groups of proteins, alkaloids, phenolics, or flavonoids found in plant extracts may bind to zinc (Zn^{2+}) surfaces, causing the production of ZnO NPs. Protein amides and the C=O, C=O–C, and C=C groups of heterocyclic compounds may have stabilising properties[30]. The flavonoid and phenolic compounds present in the extract have been used up for the synthesis of ZnO NPs, as evidenced by the quantitative determination of phenolics(21.320 mg/g) and flavonoids (6.7324mg/g) after the synthesis of NPs.
Future research will examine the use of ZnO NPs in various domains where their anti-cancer, anti-bacterial, anti-microbial, anti-tyrosinase, and anti-biofilm properties are employed; size and shape are crucial factors.

Conclusion

The therapeutic value of medicinal plants is determined by their phytoconstituents, either separately or in combination. In addition to its central nervous system inhibitory properties, *Cynanchumviminale* is utilised as an antioxidant, antibacterial, anti-inflammatory, antifertility, anti-ulcer, anxiolytic, and antipsychotic. The results show that the plant's aqueous extracts contain carbohydrates, reducing sugars, starch, protein, amino acids, alkaloids, phenolic compounds, and saponins. Quantitative examination of three identified phytochemicals (alkaloids, flavonoids, and phenolics) revealed a high amount of phenolics (32.731 mg/g), followed by flavonoids (26.2106 mg/g) and alkaloids (9.2581 mg/g). Secondary metabolites in plants convert zinc ions in solution into zinc oxide. The plant extract acts as both a reducing and stabilising agent. The quantitative study of the aqueous extracts post-synthesis of ZnONps indicated a reduction in the levels of significant secondary metabolites, specifically phenolics (21.320 mg/g) and flavonoids (6.7324 mg/g). This finding suggests that they are depleted throughout the ZnONps production process. UV-visible spectral analysis verified this, revealing a 360 nm peak characteristic of ZnO nanoparticles. SEM examination validates the size and structure of ZnO, whose purity and composition were confirmed by EDX. This study suggests that an ecologically benign method for producing metallic nanoparticles will considerably aid in developing an important synergy between material science and biology. The physical properties of the produced nanoparticles were also proved, and the current work is also an efficient pilot research to manufacture ZnO nanoparticles using an environmentally benign method.

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