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Green Synthesis and Characterization of *Cissus quadrangularis*. L stem mediated Zinc Oxide Nanoparticles

Sumera Nazneen* and Shadan Sultana

Department of Botany, Anwarul Uloom College, Hyderabad, Telangana-India

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Corresponding Author: **Sumera Nazneen** | E-Mail: **(sumeranaazneen2008@gmail.com)**

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ABSTRACT

Nanotechnology is a multidisciplinary approach to scientific research that successfully collaborates with other science disciplines like agriculture, pharmaceuticals, biotechnology, and engineering. The synthesis of nanoparticle methods has also progressed from the classic chemical approach to the green synthesis method. The improved procedure of plant-mediated nanoparticle synthesis has been verified to be less hazardous to the environment, and its inexpensive cost of production adds to its attributes. With the world executing every function at the nanoscale, studying the role, mode of action and impacts of phytotherapeutic preparations combined with nanotechnology is critical. The Cissus quadrangularis stem is also employed in Ayurveda as an alternative, anthelmintic, dyspeptic, digestive, tonic, and analgesic in eye and ear illnesses and irregular menstruation, asthma, and back and spine problems. The current research attempted the green production and characterization of zinc oxide nanoparticles from the plant Cissus quadrangularis L stem. The GC-MS analysis identified the bioactive compounds in the stem of the plant extract. Long-chain fatty acids, phenols, sterols like stigmasterol, alkylating agents, triterpenoids like lupeol, amines, esters and unsaturated hydrocarbons were found in abundance in the zinc oxide nanoparticles extract of C. quadrangularis. The FTIR analysis revealed the presence of several functional groups like alcohol, esters, amines, halo compounds and carboxylic acid. The SEM analysis portrayed the size and shape of the synthesis zinc oxide nanoparticles in the range of 88 nm to 182 nm and was found to be spherical.

Keywords: *Zinc oxide nanoparticles, Cissus quadrangularis, FTIR, GCMS, SEM*

1. Introduction

Nanotechnological principles, when applied to phytotherapeutics, lead to a new arena for the discovery of novel drug delivery systems. Recently, the term "nanomedicine" has been used to describe the use of nanotechnology in biological system monitoring, diagnosis, treatment, and control [1]. Herbal medicines, in the recent past, have proved their efficacy in treating various medical conditions. The quest for new and better pharmaceuticals has experienced a spike in exploring and employing nature, particularly for developing antibacterial, anti-diabetic, and anticancer compounds[2]. Green synthesis is a safe, environmentally benign, and biocompatible way of synthesizing nanoparticles for a multitude of applications, including biomedical aspects. Given its diversity and ease of application, plant-based nanoparticle green synthesis is currently considered the gold standard among green biological approaches [3]. The metal nanoparticles also serve as an excellent nanocarriers for the drugs, thus eliminating the risk of premature degradation of the drug. The benefits of using metallic nanoparticles in drug delivery systems include enhanced drug carrier stability and half-life in circulation, necessary biodistribution, and passive or active targeting into the target region [4]. Native to Sri Lanka, India, and Southeast Asia, *Cissus quadrangularis* is a dicotyledonous flowering plant that has long been used for medicinal purposes [5]. Stem extracts from the plant *Cissus quadrangularis* are frequently used to enhance the healing of bone fractures and are known to have therapeutic efficacy and antioxidant and antibacterial properties. Considering its numerous therapeutic benefits, the plant is regarded as a dynamic medicinal herb in Ayurveda and contemporary drug research fields [6].

The synthesis of Zinc-oxide nanoparticles from the stem extract of *Cissus quadrangularis* is confirmed by UV-VIS spectroscopy. The further characterization of the synthesized nanoparticles is done by Fourier Transform Infrared Spectroscopy. However, the chemical composition and identification of bioactive constituents can be performed by GC-MS technique, which applies the principles of Gas Chromatography and Mass Spectroscopy to detect compounds.

2. Materials and Methods

2.1 Preparation of Plant extract

To remove surface impurities and debris, 10gm of the fresh stem of *Cissus quadrangularis L.* is cut and carefully cleaned under running water. After that, it is wholly disinfected using pure alcohol to sterilize it. The stem is divided into small pieces, put in a beaker with 100ml of distilled water, and cooked for around 8 minutes at 70°C. After the plant extract has cooled, it is streamed through Whatman filter paper No. 1 to create a filtrate. To create zinc nanoparticles, keep the filtrate chilled to 4 degrees Celsius [7].

2.2 Synthesis of Zinc Oxide Nanoparticles

One mM zinc acetate was dissolved in 50 ml Milli-Q water and stirred for 1 hour. After that, 20 mL of NaOH solution was gradually added to the Zinc acetate solution, followed by 25 mL of plant extract. After 1 hour of incubation, the colour of the reaction mixture was changed. The solution was stirred for three hours. The appearance of a yellow color after incubation period verified the production of ZnO Nps.

Centrifugation at 8000 rpm at 60 degree Celsius for 15 minutes separated the precipitate from the reaction solution, and the pellet was recovered. The pellet was dried in an 80 °C hot air oven for 2 hours and stored in airtight bottles for further investigation [7].

2.3 GC-MS Analysis

At the Central Analytical Facility, University College of Technology, Osmania University, the silver nanoparticle stem extract was analyzed using the conventional GC-MS methodology. The equipment used for the GC-MS analysis is a SHIMADZU type GC-2010, MS-QP2010. The carrier gas used in this process is helium, flowing at a rate of one ml per minute. With a 30 mm length, 0.32 mm internal diameter, 0.25 mm film thickness, and a temperature range of -60 to 325 degrees Celsius (350 degrees Celsius), the HP5 column is employed. The duration of the movie GC was 35 minutes. From 70 to 280 degrees Celsius, the oven's temperature was raised at a rate of 8 degrees per minute. One liter of sample is injected through the injector. At 70eV, the MS was conducted. The name, molecular weight, and structure of unknown compounds were detected by comparing the spectra of unknown chemicals to the spectrum of known compounds in the NIST Library.

2.4 SEM Analysis

On the Hitachi S-3700N SEM, synthesized ZnONPs were analyzed using scanning electron microscopy (SEM). Before analysis, the ZnONPs powder was sonicated for 5 minutes, and a drop of the correctly diluted sample was applied to a copper grid covered with carbon. The shape and size of the nanoparticles are determined via magnifying effect caused by this.

2.5 Fourier transform infrared spectrophotometer Analysis

With the help of a Fourier transform infrared spectrophotometer (FTIR), the functional groups present in the plant extract components are detected. The annotated spectrum provides the wavelength of absorbed light, which is indicative of the chemical bond. Chemical bonding can be identified by analyzing infrared absorption spectra. The silver nanoparticles were spun at 12,000 rpm for 30 minutes in preparation for FTIR analysis. Following that, 25 ml of de-ionized water was used to rinse the pellets three times [8].

The dried powder of *Cissus quadrangularis L.'s* silver nanoparticles is used in the investigation. By encasing 10 mg of the sample in 100 mg of KBr pellet (Perkin Elmer), a transparent sample disc is produced. Then, an FTIR spectroscope was used to analyze the nanoparticle extract from the stem of *Cissus quadrangularis L.*

3. Results and Discussions

3.1 GCMS Analysis

Some chemicals from the GC fractions of the silver nanoparticle extract of the stem of the *Cissus quadrangularis L.* plant were seen as peaks on the GC-MS spectrum analysis chromatogram. The observations showed the presence of bioactive compounds like Trifluoroacetoxypentadecane, Carbon dioxide, Carbamic acid, monoammonium salt, Methane, nitroso, DL-Methyltartronic acid, Butanal, 3-methyl, alpha. -D-Galactopyranoside, methyl 3,6-anhydro, Acetic acid, dichloro, Piperazinecarboxamide, N, N-diethyl-4-methyl, Methylamine, N-[4-(1-pyrrolidinyl)-2-butynyl],Butane, 2,2,3-trimethyl,Ethyl Acetate,Furan, tetrahydro,Oxirane, ethyl, Butanoic acid, 2-hydroxy-2-methyl, methyl ester and Toluene. The chromatogram is shown in Figure 2, and Table 2 lists the chemical compounds' names, molecular weight, peak area percentage, and retention times. According to reports, the bulk of these substances have antibacterial, anti-mycobacterial, anticonvulsant, antiviral, anticancer, anti-inflammatory, and antioxidant activities. Gentisic acid (GA) is a phenolic acid connected to effects on human health that include anti-inflammatory, antigenotoxic, hepatoprotective, neuroprotective, and primarily antioxidant [9].

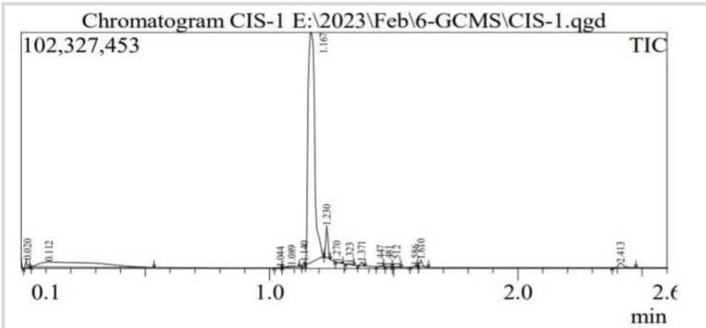

Figure:1 GC-MS chromatogram of the ZnONPs of the stem of Cissus quadrangularis L.

Table:1 Phytocompounds from GC-MS analysis of ZnONPs of the stem of Cissus quadrangularis L.

S.No.	RT time	Name of the compound	Formula	MW	Peak Area (%)	Nature of Compound.
1.	0.020	Trifluoroacetoxypentadecane	C17H31F3O2	324	0.60	Terpenoid
2.	1.045	Carbon dioxide	CO2	44	0.02	Inorganic gas
3.	1.045	Carbamic acid, monoammonium salt	CH6N2O2	78	0.02	Carboxylic acid
4.	1.140	Methane, nitroso-	CH3NO	45	0.30	Organic gas
5.	1.140	DL-Methyltartronic acid	C4H6O5	134	0.30	Carboxylic acid
6.	1.165	Butanal, 3-methyl-	C5H10O	86	76.99	Alcohol
7.	1.165	alpha. -D-Galactopyranoside, methyl 3,6-anhydro-	C7H12O5	176	76.99	Carbohydrate
8.	1.230	Acetic acid, dichloro-	C2H2Cl2O2	128	3.36	Organochlorine compound
9.	1.270	Piperazinecarboxamide, N,N-diethyl-4-methyl-	C10H21N3O	199	-0.03	Carboxylic acid
10.	1.270	Methylamine, N-[4-(1-pyrrolidinyl)-2-butynyl]-	C9H16N2	152	-0.03	Amine

11.	1.370	Butane, 2,2,3-trimethyl-	C7H16	100	0.42	Alkane
12.	1.445	Ethyl Acetate	C4H8O2	88	0.09	Acetate ester
13.	1.510	Furan, tetrahydro-	C4H8O	72	0.06	Heterocyclic organic compound
14.	1.510	Oxirane, ethyl-	C4H8O	72	0.06	Cyclic ether
15.	1.610	Butanoic acid, 2-hydroxy-2-methyl-, methyl ester	C6H12O3	132	0.73	Carboxylic acid
16.	2.415	Toluene	C7H8	92	1.16	Aromatic compound

3.2 SEM Analysis

In *C. quadrangularis* extract treated with zinc sulfate, surface-formed zinc oxide nanoparticles can be observed clearly at greater magnification. Images from scanning electron microscopy (Fig. 5) show that the zinc nanoparticles in the stem of *Cissus quadrangularis L*. were spherical in shape and uniform in size. The nanoparticles seem to be uniformly distributed and aggregated. The synthesized nanoparticles had a diameter that varied from 88.7 to 182 nm.

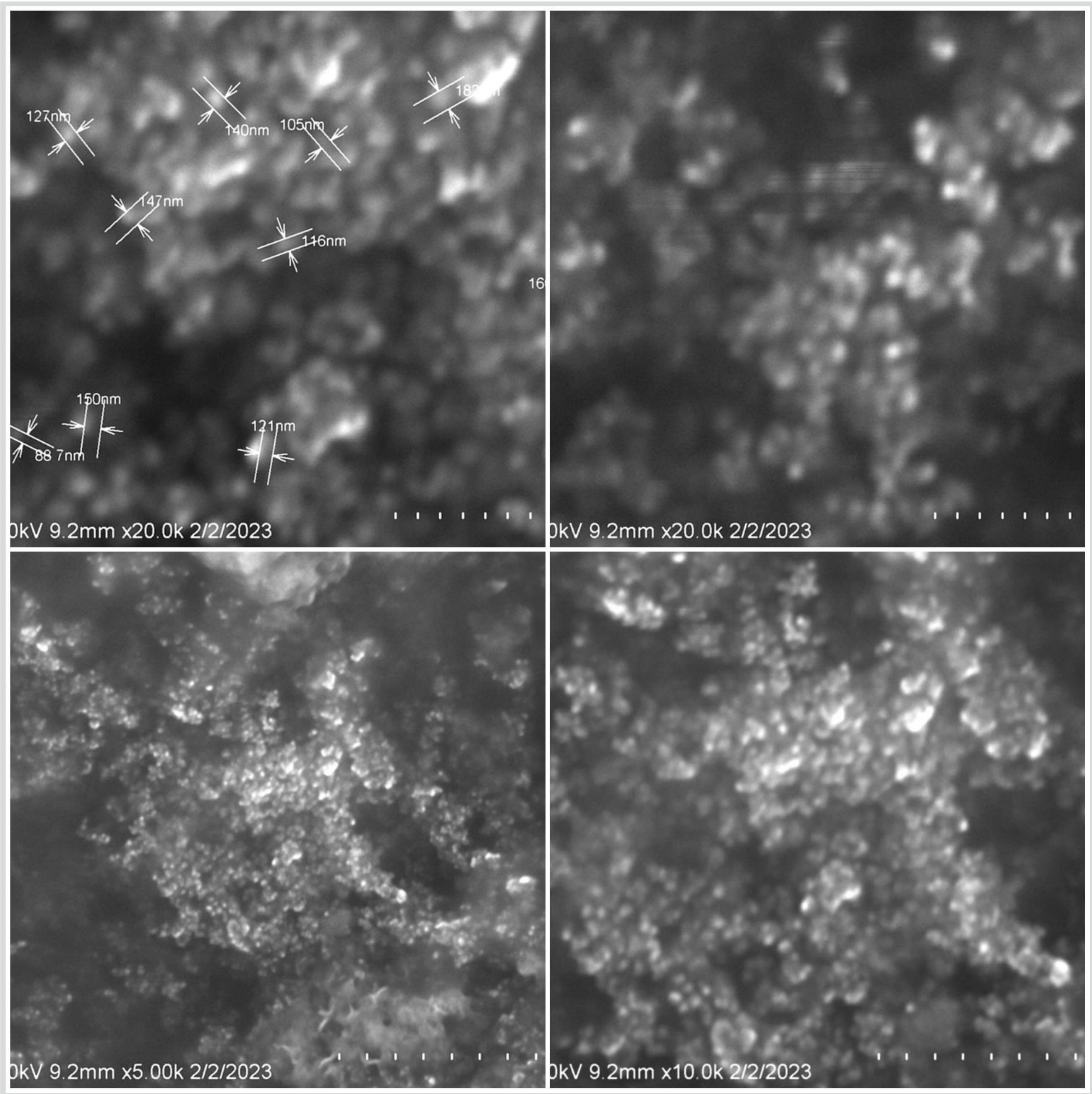

Figure:2 SEM Micrographs showing the size of ZnONPs of the stem of Cissus quadrangularis L.

3.3 Fourier transforms infrared spectrophotometer Analysis (FTIR)

The goal of the FTIR analysis was to identify the biomolecules that become trapped on nanoparticle surfaces and are in charge of reducing metal salts to the matching nanoparticles [10]. The sorts of chemical linkages that are present in the silver nanoparticle extract of the stem of the *Cissus quadrangularis L.* plant are shown in Fig. 3 by the Fourier transforms infrared spectrophotometer. When the extract was run through the FT-IR, the functional groups of the components were divided based on the peak ratio [11].Table 3 depicts theoccurrence of the alcohols,primary amine, aliphatic primary amine, secondary amine, carboxylic acid, amine salt,alkyne, alkene,alkane, aldehyde, thiol, aromatic compound, conjugated aldehyde, imine/oxime, conjugated alkene, amine, nitro compound, sulfate, sulfonyl chloride, phenol, sulfonate, aromatic amine, aromatic ester, alkyl aryl ether, ester, halo compound and 1,2,4-trisubstituted at their respective frequency range of the peaks. Some functional groups may vanish due to bio reduction during the formation of ZnONPs.

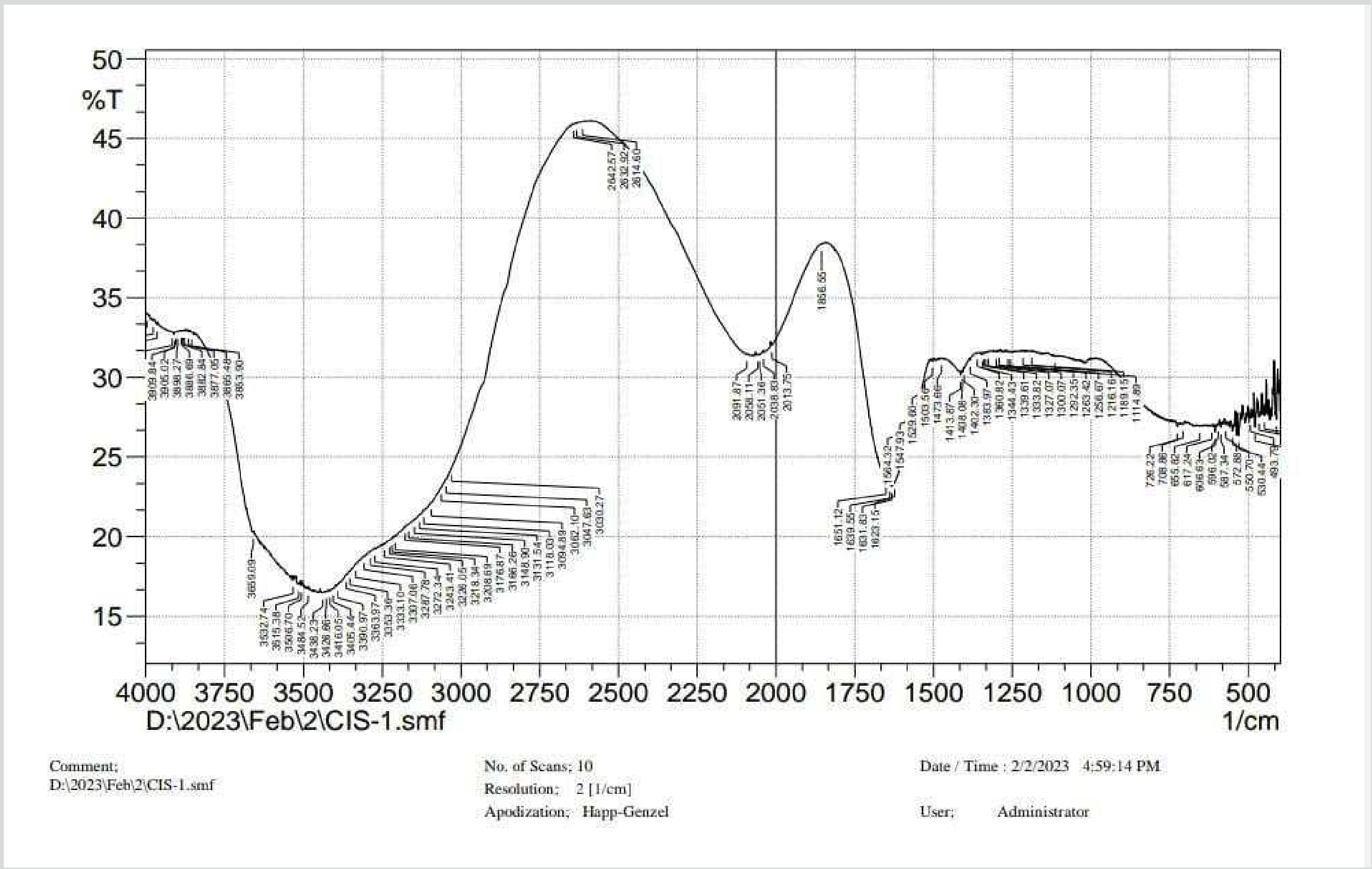

Figure:3 FTIR Spectrum of ZnONPs of the stem of Cissus quadrangularis L.

Table:2 Peak values and functional groups of ZnONPs of the stem of Cissus quadrangularis L.

Peak	Compound Class	Group	Appearance
3532.74- 3208.69	Alcohol	O-H stretching	strong, broad
3506.7 - 3353.36	Amines	N-H stretching	medium
3307.06	carboxylic acid	O-H stretching	strong, broad
3272.34-3333.1	alkyne	C-H stretching	strong, sharp
3030.27-3118.03	alkene	C-H stretching	medium
2091.87-2642.57	aldehyde	C-H stretching	medium
2614.6	thiol	S-H stretching	weak
1651.12-2013.75	aromatic compound	C-H bending	weak
1503.56-1547.93	nitro compound	N-O stretching	strong
1413.87	sulfate	S=O stretching	strong
1383.97	phenol	O-H bending	medium
1292.35-1339.61	aromatic amine	C-N stretching	strong
1256.67-1300.07	aromatic ester	C-O stretching	strong
550.7-726.22	halo compound	C-Cl stretching	strong
530.44-655.82	halo compound	C-Br stretching	strong
530.44-596.02	halo compound	C-I stretching	strong

4.Conclusion

Cissus quadrangularis L. has been a part of ancient medicine and used in a variety of forms and formulations in the treatment of a broad spectrum of diseases. The utilization of herbal drugs in the form of nanomedicine has proved to enhance its efficacy. The green synthesis of nanoparticles employed in this experiment turned out to be eco-friendly, non-toxic, and required fewer chemicals than the physical and chemical methods. The presence of phytochemical components in the leaf extract aids in the formation of metal oxide nanoparticles by promoting oxidation and reduction reactions. The functional groups- esters, amines, aromatic compounds, and halo compounds- that stimulated the formation of zinc oxide nanoparticles were commonly detected. The SEM analysis confirmed the formation and size of nanoparticles. The GCMS analysis revealed the presence of some bioactive constituents which possess some pharmaceutical and pharmacological properties.

The pharmacological activities of the synthesized Zinc oxide nanoparticles both in-vitro and in-vivo could provide a more clear evidence supporting its utilization in the treatment of health disorders. Green synthesis is a quick and inexpensive procedure that eliminates the formation of any sort of by-product in the nucleation and synthesis of nanoparticles. It results in the creation of well-dispersed nanoparticles of regulated shape and size [12]. Due to their distinct characteristics and higher surface to volume ratio than their bulk counterparts, metallic nanoparticles are very valuable in both industrial and scientific domains [13]. Zinc oxide its unique conductive, electrical, and pyroelectric qualities, zinc oxide may be a unique material with a wide range of uses in coatings, paints, ultraviolet (UV) light emitters, electricity devices, chemical sensors, spin electronics and transparent electronics [14]. Nanotechnology in combination with other domains of science can be used in further exploration of the therapeutic properties of the medicinally significant plants. As stability enhancers, they can be applied to targeted drug administration to increase the bioavailability, biodistribution, and accumulation of treatments, preferably in the targeted disease region[15].

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