GREEN SYNTHESIS CHARACTERIZATION AND BIOLOGICAL POTENTIAL OF METEL OXIDE NANOPARTICLES USING GRAPE SEEDS

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ABSTRACT :

Zinc oxide nanoparticles are well known metal oxide nanoparticles having numbers of applications in the field of cosmetology, medicine, and chemistry. Numerous chemical and physical techniques were used to prepare ZnO-NPs, but biological techniques, which are considered "green," have the potential to be more environmentally friendly than chemical and/or physical techniques when applied to various substrates (such as bacteria, enzymes,microorganisms, and plant extracts). There have been reports of therapeutic advantages for zinc oxide nanoparticles. Zinc oxide nanoparticles have been shown in several studies to have antibacterial, anticancer, antioxidant, and immunomodulatory properties. Zinc oxide nanoparticles were also added to sunscreens, Furthermore, it has been claimed that zinc.

KEYWORDS : Zin oxide, Nanoparticles, Anticancer, Antioxidant.

INTRODUCTION :

The term "nanotechnology" described the capacity to precisely engineer materials at the nanoscale. This is actually how the term is currently understood; it now refers to the design, characterization, production, and application of materials, with the scope having expanded to include devices and systems in addition to materials.(N.Taniguchiet al., 2005).Therefore, the design and manufacture of materials, tools, and systems with control at nanoscale dimensions is referred to as nanotechnology.Therefore, size and control are fundamental to nanotechnology. Some people prefer to refer to them as "nanotechnologies" in plural due to the wide range of applications; however, they all have control at the nanoscale scale in common.(Jeremy J. et al.,2005).

Nanoparticles have gotten relatively less attention than other planetary materials. This is mostly due to the fact that their structures are challenging to examine using standard techniques. The careful synthesis of homogeneous materials, in-depth experimental investigations, and sophisticated computational modeling are necessary for the analysis of nanoparticle reactivity. Numerous issues that were previously virtually unsolvable are now solvable thanks to recent advancements in techniques and strategies. The earth science community is paying more and more attention to nanoparticles due to growing awareness of

their unique and significant physical, chemical, magnetic, and optical properties—a subset of which only "emerge" at the nanoscale. (Moore et al.,1998).

Zinc oxide nanoparticles are highly significant among metal nanoparticles because they are used in drug delivery systems, gas sensors, biosensors, cosmetics.In terms of how they are made, ZnO NPs can be made chemically using a variety of techniques, including the hydrothermal process, vapor transport method, and precipitation method. These days, it is also typical to produce ZnO NPs through biogenic synthesis utilizing various plant extracts. Compared to chemical synthesis, this green synthesis is much safer and more environmentally friendly.(Sidra Sabir et al., 2014).

Grape seeds are among the food waste and extract of it was being utilized in the synthesis of nanoparticles.Rich in secondary metabolites and polyphenolic compounds, grape seed extract (GSE) exhibits strong antibacterial properties against a variety of pathogens, including both Gram-positive and Gram-negative bacteria. (Shahad K. et al.,2021).

MATERIALS AND METHODS :

Preparation of grape seed extract :

Grape seeds were collected from Thoothukudi. The collected seeds were washed 3-4 times using distilled water. Then they were being dried in the shade for 7-14 days. The well dried seeds were made into powder by using mortar and pestle. The collected powder was stored in an airtight container. 1 gram of grape seed powder was dissolved in distilled water and boiled for 5 to 10 minutes at 60-70 degree centigrade. The solution was filtered by using Whatman no. 1 filter paper. The filtered extract was collected and stored in 4°C for further use.

Synthesis of zinc oxide nanoparticles :

Figure 1 : Synthesis of Zinc oxide nanoparticles using grape seed extract

CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES :

UV visible spectroscopy :

The UV-Visible spectroscopy is a characterization technique that is inexpensive, straightforward, quick, and sensitive (Rajeshkumar, et al., 2018). It is used to assess the optical properties, stability, formation, absorbance, and stability of bio-nanoparticles in solution (Talamet al., 2012,).

Fourier transform infrared spectroscopy (FTIR) :

This method uses nanoparticles to identify the structural characteristics and functional groups of biological extracts (Gangadooet al., 2014,). The study of the vibrational characteristics of the nanoparticles is made possible by FTIR, which compares the wavelength of light with the infrared intensity (Heeraet al., 2015,).

Scanning electron microscope (SEM) :

A concentrated beam of high-energy electrons is used by the scanning electron microscope (SEM) to produce a range of signals at the surface of solid specimens. The signals that result from interactions between the electrons and the sample provide details about the sample, such as its external morphology.(Claflin, Y. et al., 2004).

X-ray diffraction method :

X-ray diffraction is a flexible, non-destructive analytical technique that can be used to identify and quantify different crystalline forms, or "phases," of a compound that are present in powder and solid samples. When waves interact with a regular structure whose repeat distance is roughly equal to the wavelength, diffraction occurs. The phenomenon is widespread and occurs on a wide variety of scales in the natural world.

Antibacterial activity - well diffusion method :

ZnO-NPs were tested for their antibacterial activity against Gram positive bacteria, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. In order to prepare stock cultures, the strains were inoculated onto Tryptone Soya Agar (TSA) and then incubated for 24 hours at 35 °C. Until the working cultures were ready, these cultures were maintained at 4 °C. A loopful from the stock culture was transferred into 5 ml and incubated for 24 hours at 35 °C to obtain working cultures.(Duffy. L et al.,2018).

Antioxidant activity :

Antioxidant activity was carried out by DPPH assay. The chemicals like 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid was purchased from Sigma-Aldrich. Phosphate

buffer (pH- 7.4) and Methanol. All the chemicals and solvents used were of analytical grade. (Blois, M.S et al.,1958).

DPPH radical scavenging activity :

DPPH scavenging activity was carried out by the method of Blois (1958). DPPH solution (0.004 %), sample extracts and standard (vitamin C) was prepared in methanol. Sample extract and standard (vitamin C) solution were prepared in different concentrations 20, 40, 60, 80 and 100 µg/ml. 0.5ml of different concentrations of standard solution or sample extracts was taken in different test tubes and then 0.5 ml of DPPH (0.004 %) solution was added and kept in dark for 30 min.

And absorbance was recorded at 517 nm. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It was visually noticeable as a colour change from purple to yellow. (Majo et al., 2008) The percentage inhibition activity was calculated using the formulae below:

% DPPH free radical scavenging = (Absorbance of control – Absorbance of test) X 100.(Blois, M.S et al.,1958).

Anti – inflammatory :

Diclofenac sodium was used as Standard for anti-inflammatory activity. SD fine grade and the solvents methanol of SD fine grade were used in this experiment.

Animal used

Adult Wistar albino rats of either sex weighing between 150 and 180 gm were used. The selected animals were housed under standard environmental conditions (temperature of 22±10° C) in a 12 hours light- dark cycle. The animals were fed on standard laboratory animal diet (Amruth animal feed company, Sangli, Maharashtra) and the animals were fasted overnight before the experiment (Blois, M.S., et al 1958).

Experimental study

Anti – inflammatory testing

Anti – inflammatory activity was assessed by the method suggested by Winters et al., (1962) using carrageenan as phlogistic agent. The adult Wister albino rats of either sex weighing between 150 & 180 gm were housed in groups of four animals each. They were starved overnight during the experiment but had free access to water. The volume of paw of each animal was determined before giving any drug. Animals are divided into four groups each consisting of four animals. Group I served as control which received normal saline [1ml/Kg, (p.o)], Group II received standard drug Diclofenac sodium [10 mg/Kg (p.o)], Group III

received 400mg/kg p.o. Acute inflammation was produced by sub plantar injection of 0.1 ml of 1% suspension of carrageenan in normal saline, in the right hind paw of the albino rats. One hour after oral administration of the drugs, the paw edema was measured with the aid of a standard plethysmometer (Inco, Chennai) at 1, 2, 3 and 4 hours after the injection of carrageenan. The difference between the readings at time 0 minutes and the different time intervals was taken as the thickness of edema volume. Percentage inhibition of paw edema was calculated by comparing the control for each dose at different hours as given below.

Percentage inhibition= 1 – Vt/Vc* 100

Where,

Vt = volume of paw edema in treated animals.

Vc= volume of paw edema in control animals.

Wound healing activity

Ointment formulation (british pharmacopoeia, 1993)

A control ointment base was formulated without any drug content. Creams was formulated by using 10% extract (10 gm of sample I was incorporated in 100gm of cream base). The standard drug for screening wound healing activity is Povidone iodine ointment (5%w/w) which was bought commercially.

Animal model

Albino rats (150-250gm) of either sex were procured from animal house used for the present study. The Albino rats were divided into four groups of six rats. Group I rats were treated with simple ointment base (control). Group II rats were treated with a reference standard Povidone iodine ointment. Group III rats were treated with 10% sample I ointment respectively.

Wound healing activity

The excision wound healing activity was studied by the method described by Luisa A DiPietro., (2003) . The skin area on the dorsal thoracic region of the mice was removed by using a suitable depilatory (Anne French hair removing cream) one day prior to the experiment. Alcohol (70%) was used as antiseptic for the shaved region before making the wound. The surgical procedures were carried out under sterile conditions. The experimental animals were anesthetized with anesthetic ether. After successful anesthesia mice were fixed in a dorsal posture on a surgery table. Circular, full thickness surgical wounds with diameters of 5mm, 1 cm away from the backbone were made using 5mm biopsy punch. Using this excision wound method, the epidermal, dermal, hypo-dermal and panniculus carnosus layers were removed completely. After making surgical wounds, all mice were randomly marked

using a non-toxic colour. The animals were divided into the following four groups of six animals each (both male and female) and were treated as given below

Group I – Normal control group received petroleum jelly

Group II – Standard group received Povidone iodine ointment

Group III – Drug treated group received 10% w/w of Sample

Incision wound model :

Animals were anaesthetized and para vertebral incisions (2.5-3.0 cm long) were made through the entire length of skin. After the incision was made, the parted skin was kept together and stitched with nylon thread at 0.5 cm apart with curved needle (No. 11). The two test formulations, cream base and povidone iodine ointment were applied on wound once daily for 7 day. The sutures were removed on day 8 and wound tensile strength was measured on day 10 by using constant water flow technique.

RESULT AND DISCUSSION:

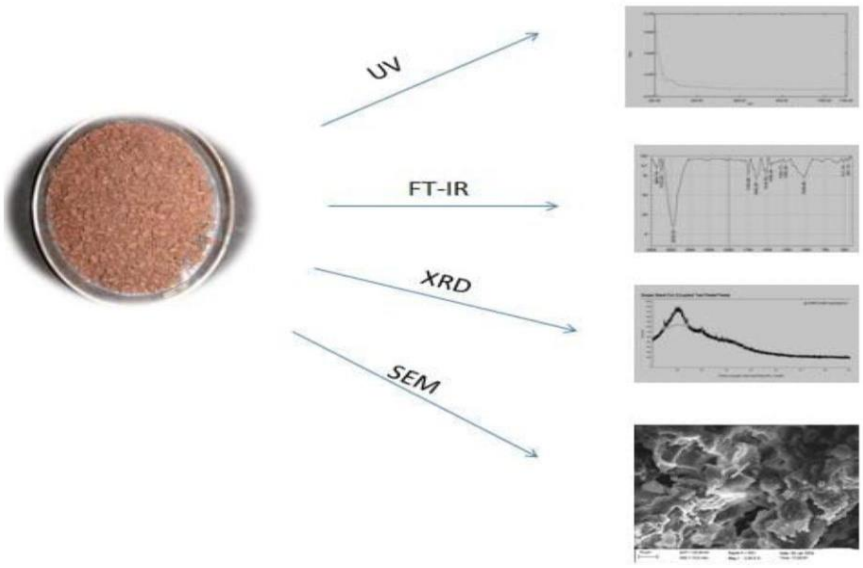

Figure 2 : characterization of Zinc oxide nanoparticles

Antibacterial – Well diffusion method

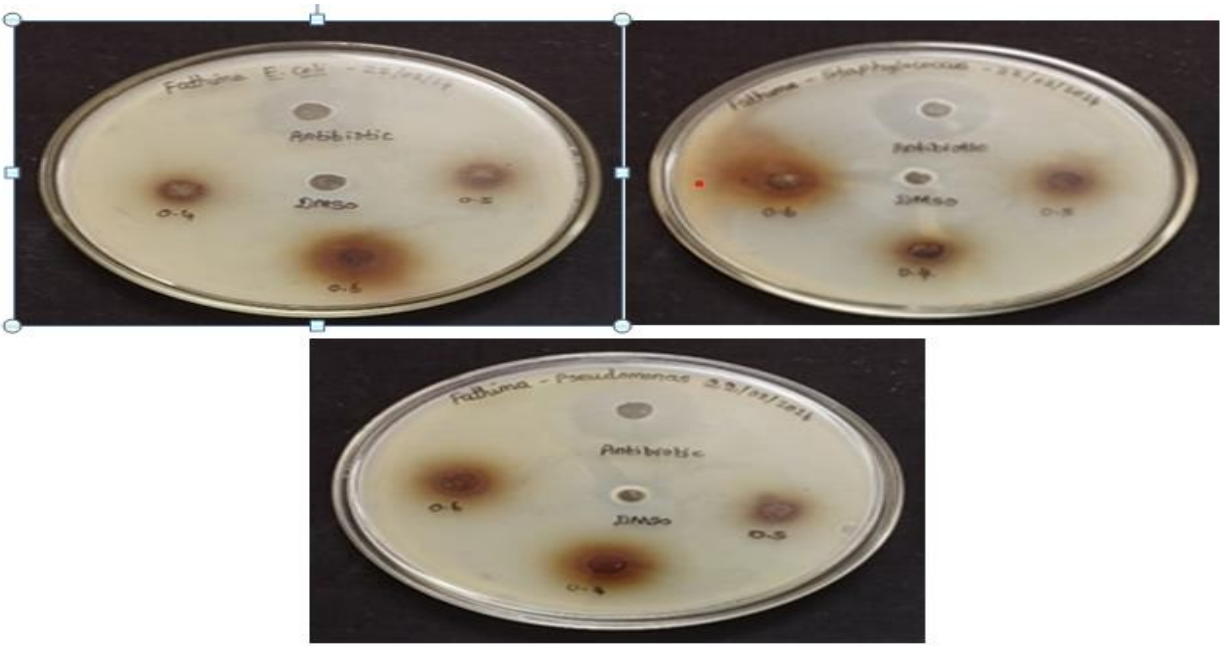

Table:1 Antibacterial activity of Synthesized ZnO nanoparticles by Well Diffusion

S.NO	Bacterial Strain	Concentration (mg)	Zone inhibition (mm)	
			Synthesized ZnO nanoparticles	Streptomycin Control
1	*E.coli*	0.4	1.5	7
		0.6	3	7
		0.8	5	7
2	*S.aureus*	0.4	5	5
		0.6	6	5
		0.8	3	5
3	*P.aeruginosa*	0.4	2	8
		0.6	3	8
		0.8	2	8

Method

Antioxidant Activity :

Table 2: Anti oxidant activity of synthesized ZnO nano particles by DPPH Assay

Treatment	Dose (µg/ml)	Absorbance @517 nm	% activity against DPPH radicals
Control	----	DPPH control= 0.993	----
Vit C	100	0.188	80.36
Sample	20	0.486	50.35
Sample	40	0.420	57.70
Sample	60	0.365	62.54
Sample	80	0.298	69.20
Sample	100	0.226	76.54

Treatment	% wound contraction in excision wound model		
	0 day	8th day	16th day
Group I Control (simple ointment)	359.04±2.08	326.12±2.20 (9.16 %)	186.35±2.28 (48.09%)
Group II Standard	378.06±2.22	312.14±2.00 (17.43%)	48.14±0.65 (87.23%)
Group III Sample -10%	413.24±2.16	326.12±2.24 (21.08%)	130.24±1.24 (68.48%)

Anti-inflammatory Activity

Table: 3 Anti inflammatory activity of ZnO Nanoparticles

Wound healing activity

Table: 4 (a) Effect of Topical Application of Sample on Incision Wound Model

Table : 4 (b) Effect of Topical Application of Sample on Incision Wound Model

Treatment	Tensile strength (gm)
Group I Control (simple ointment)	138.82±0.24
Group II Standard	108.22±0.26
Group III Sample -10%	122.24±0.16

CONCLUSION :

The present study estimated the Zinc oxide nanoparticles are well known metal oxide Nanoparticles having numbers of applications in the field of cosmetology, medicine, and Chemistry. Grape seed extract (GSE) is a natural source of polyphenolic compounds and Secondary metabolites, which have been tested for their possible antimicrobial activities.Synthesized from grape seed extract may lead to opportunities for the discovery ofcheaper and more beneficial therapy for woundHealing. However, the wound healing effects of ZnO NPs need to be supported by in vivo Findings.

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