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Biosynthesis of Zirconium nanoparticles with (Ocimum sanctum) Tulasi Leaves extract and its anti-inflammatory activities

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ABSTRACT:-The field of nanotechnology is the most active area of research in modern science. In this present study zirconium nanoparticles were synthesized from aqueous zirconium nitrate (1mM) through a simple and eco-friendly route using leaf broth of Ocimum sanctum as reductant and stabilizer. The bioreduced zirconuium nanoparticles were characterized by UV-Vis spectrophotometer, Fourier transmission infra-red (FTIR), Transmission electron microscope(TEM) and X-ray diffraction(XRD)).The mean particle of synthesized nanoarticles as found to be 10 nm as confirmed by TEM. Tulasi leaves extracts (ZrNPs) were found to have enhanced anti-inflammatory activity.

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I. INTRODUCTION

In recent years researchers in the field of nanotechnology are finding that nanoparticles have all kinds of previously unexpected benefits. They find applications in various fields like medicine electronics energy saving environment, textile, and cosmetics. Owing to their applicability in such wide sectors, their demand is increasing at an overwhelming rate. Researchers are continuously developing newer methods of synthesis of highly monodispersive ZrNPs which are efficient in terms of synthesis rate as well as energy usage. The chemical constituents of this herbs includes ursolic acid euggenol, oleanalic acid, rosmarinic acid linalool and carvacol.. The high euganol contain in this plant helps to act like a pain killer. These functions are often attributed to tulasi'shigh content of phenolic compounds and antioxidant properties with Krishna tulsi having a higher phenolic content and antioxidant capacity than white Vanatulsi [1]. Synthesis of metal nanoparticles has gained significant interest in last twenty years because of their unusual properties and prospective applications in optical, electronic, catalytic magnetic materials, thermal properties with corresponding bulk metals. A number of methods have been developed for the preparation of metal nanoparticles such as photo catalytic reduction, radiolytic reduction, solvent extraction reduction, micro emulsion technique, polyol process and alcohol reduction [2].

The most common and important medicinal properties i.e.Ocimum sanctum L, which have different medicinal properties such as anticancer, antimicrobial, cardio-protective, antidiabatic, analgesic antiplasmodic, antiemetic, hepatoprotective, antifertility, adaptogenic,and diaphoretic actions. The active constituent of leaves of Ocimum sanctum L is Eugenol(1-hydroxy-2-methoxy-4-allylenzene) which is mainly responsible for the therapeutic potentials of cardiovascular system, urinary system, reproductive system, immune system gastric system blood biochemistry, central nervous system, blood biochemistry, central nervous system and also significance in various ailments in modern medicine[3-5].To the best of our knowledge, the use of Ocimum sanctum leaf extract at room temperature for the green synthesis of zirconium nanoparticles has not been reported.. Hence the present study was carried out to synthesize and characterizethe zirconium nanoparticles using Ocimum leaf extract.

II. MATERIAL AND METHODS

The Tulasi Leaves were washed with sterile distilled water and the outer covering of the fruit was peeled off and fleshy part of orange was washed with sterile distilled water. The Tulasi Leaves were cut into small pieces and 10g of Tulasi Leaves was ground using mortar and pestle with distilled water. The extraction was filtered using muslin cloth and then Whatmann No.1 filter paper. Zirconium nitrate (0.1M) was used as precursor for synthesis of zirconium nanoparticles. The mixture was incubated at 37^0C. Then the mixture was filtered using watmann filter paper. It was followed by redispersion of the precipitate in deionized water to get rid of any uncoordinated biological molecules.

Characterization of Zirconium nanoparticles

UV-Vis Spectra analysis: Ultraviolet visible spectrophotometer (UV-Vis) refers to absorption spectroscopy in the UV-Visible spectral region. This means it uses light in the visible and adjacent (near UV and near- infrared (NIR) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Ultraviolet visible spectrophotometer (UV-Vis) is procured from Systronics. A small aliquot of the sample was taken for UV-Vis spectrum analysis (200-800nm).

Fourier Transform Infrared: Dried powder of the ZrNPs was subjected to analyze the presence of possible functional groups for resulting in formation of ZrNPs using Fourier transform infrared (ATR schimadzu Japan) spectroscopy.

SEM Analysis of Zirconium Nanoparticles: Scanning Electron Microscope (SEM) analysis was done using (JEOL Model JSM - 6390LV) SEM machine. The films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid.

X-Ray Diffraction Analysis: To determine the nature and size of the synthesized ZrNPs, X-ray diffraction (XRD) was performed using on an Bruker,D-8 Advance, Germany, which was operated at a voltage of 40 kV and current of 40mA with Cu-Ka radiation.

The anti-inflammatory activity of new compound was studied by using Inhibition of protein (albumin) denaturation technique according to Mizushima et al and Sakat et al [6,7]2. The reaction mixture (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% aqueous solution) and 0.05 ml of new compound (100, 200, 300, 400 & 500 µg/ml of final volume).pH was adjusted at 6.3usingasmallamountof1NHydrochloricacid.Thesampleswereincubatedat37°Cfor 20 min and then heated at 51°C for 3 min. Aftercoolingthesample,2.5mlofphosphate buffer solution was added into each test tube. Turbidity was measured spectrophotometrically at 660 nm for control tests; 0.05 ml of distilled water was used instead of new compound while product control tests lacked bovine serum albumin. The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated using following formula

Percentage inhibition = (Abs Control – Abs Sample) X 100/ Abs control

III. RESULTS AND DISCUSSION

UV-Visible Spectra : Uv visible spectra of colloidal zirconium nanoparticles shown in fig.1.It shows well defined surface Plasmon resonance at about 328 nm. The UV-Visible spectra also show that particle are uniformly distributed and round in nature. The SPR characteristic peak is due to oscillation of conduction band electron of zirconium. The broadening peak is due to wide size distribution in solution. The zirconium nanoparticle suspended in DMF and UV-Visible peak of this solution was observed at 328nnm wavelength. The position and shape, of Plasmon absorption of zirconium metal nano cluster is strongly dependant on size, shape, dielectric medium, surface adsorbed species and surrounding matrix.

It is known that all ZrO_2polymorphs are very similar in vibration structure and a minor variation in their band frequencies or intensities infers small differences in the Zr^{4+} distribution in Zr-O sites and the oxygen vacancies and their structural defects [8].

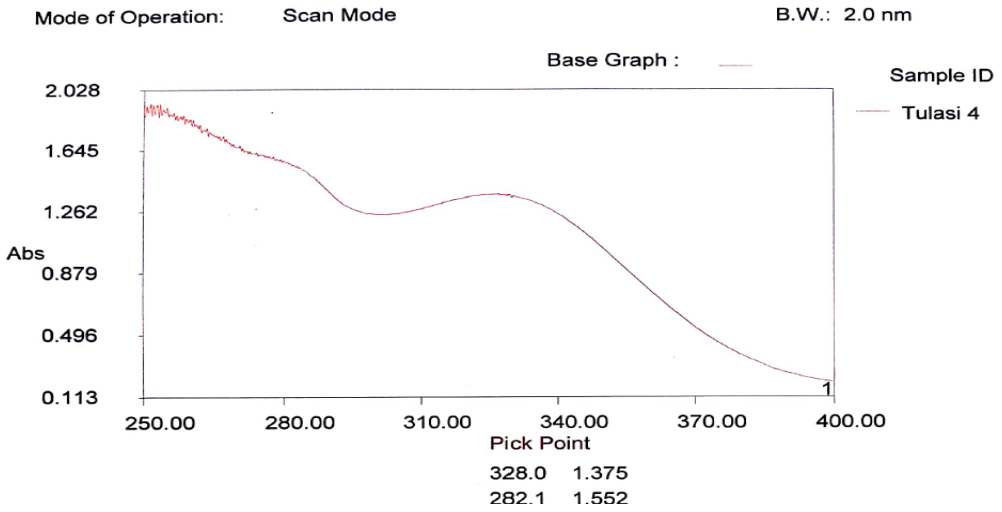


Fig.1.

FTIR Spectra: The result of FT-IR analysis of Zirconium particles is depicted in Figure 3. The spectra of zirconium particles showed transmission peak at 3400, 3000, 1610, 1150 and 1100 cm^{-1}.The absorption band located around 3400$cm^{-1}$is associated with the O-H stretching vibration of absorbed water and hydroxyl group while the absorption band at 1610 cm^{-1} is due to the bending mode of associated water [9].The peak at 1100 indicate saturated alkanes, the peak at 1150 indicate alcohol, phenol. The peak at 1700 indicates amide, the peak 2500 indicates carboxylic acid, and the peak at 3400 indicates the hydrogen bonded alcohol and phenol. Figure 3 shows that the FT-IR spectra of biosynthesized zirconium nanoparticles and carried out to identify the possible interaction between protein and zirconium nanoparticles. The result of FT-IR study showed sharp absorption peak located at 1100 and 3000 cm^{-1}.Anothher important absorption band can be observed at 470 cm $^{-}$1, which is related to the vibration of the Zr-O bond in ZrO_2 [10].

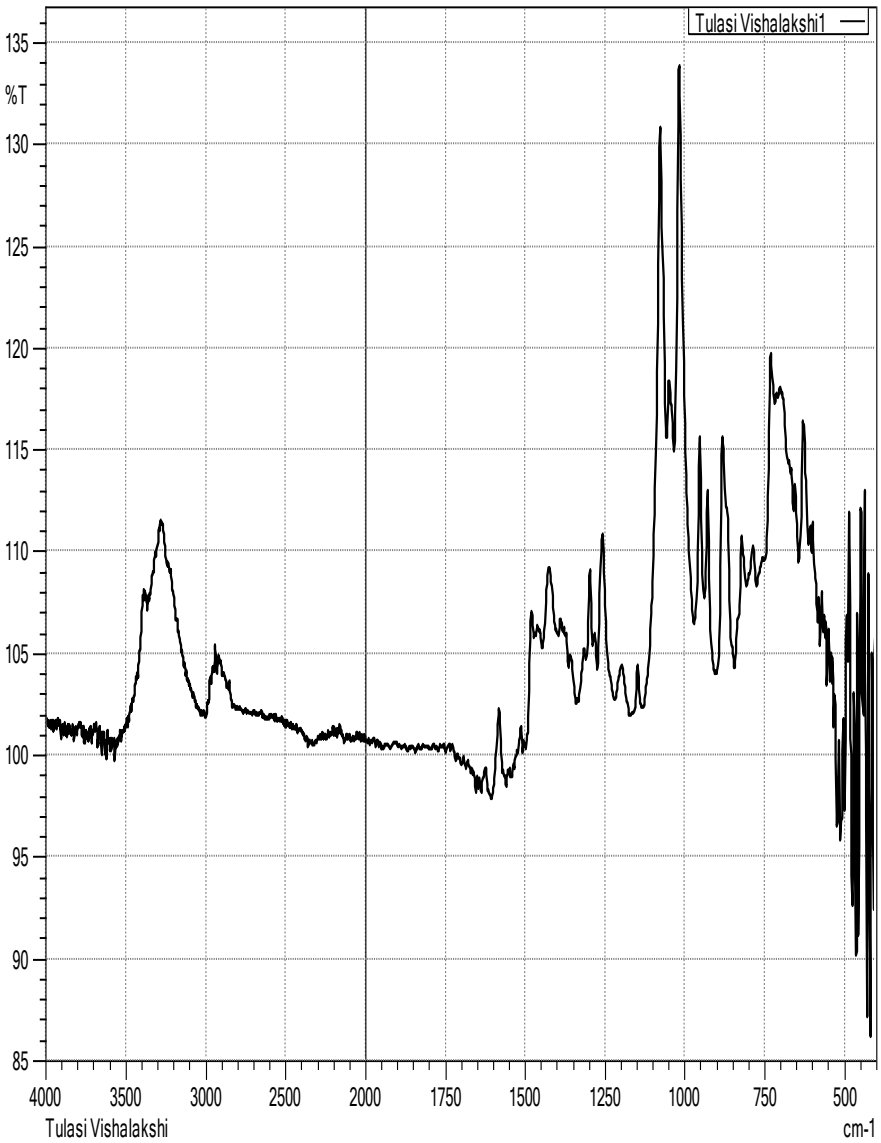


Fig.2 IR Spectra of zirconium nanoparticles with Tulsi leaves extract

PXRD Analysis: XRD analysis

Analysis through x-ray diffraction was carried out to confirm the crystalline nature of zirconium nanoparticles. The dry powders of zirconium nanoparticles were used for XRD analysis (Figure 3). The diffracted intensities were recorded from 20 to 80 ^{o}C at 2 theta angles. The comparison of our XRD spectrum with the standard confirmed that zirconium nanoparticles form were in the form of nano crystals as different diffraction lines were observed at 2 theta angle 15, 16, 24.5 and 28 respectively. The average particles size of the zirconium nanoparticles synthesized by present bio synthesis method can be calculated by using

Debye-Scherer's equation, the average particle size of synthesized zirconium nanoparticle is 17.42 nm.

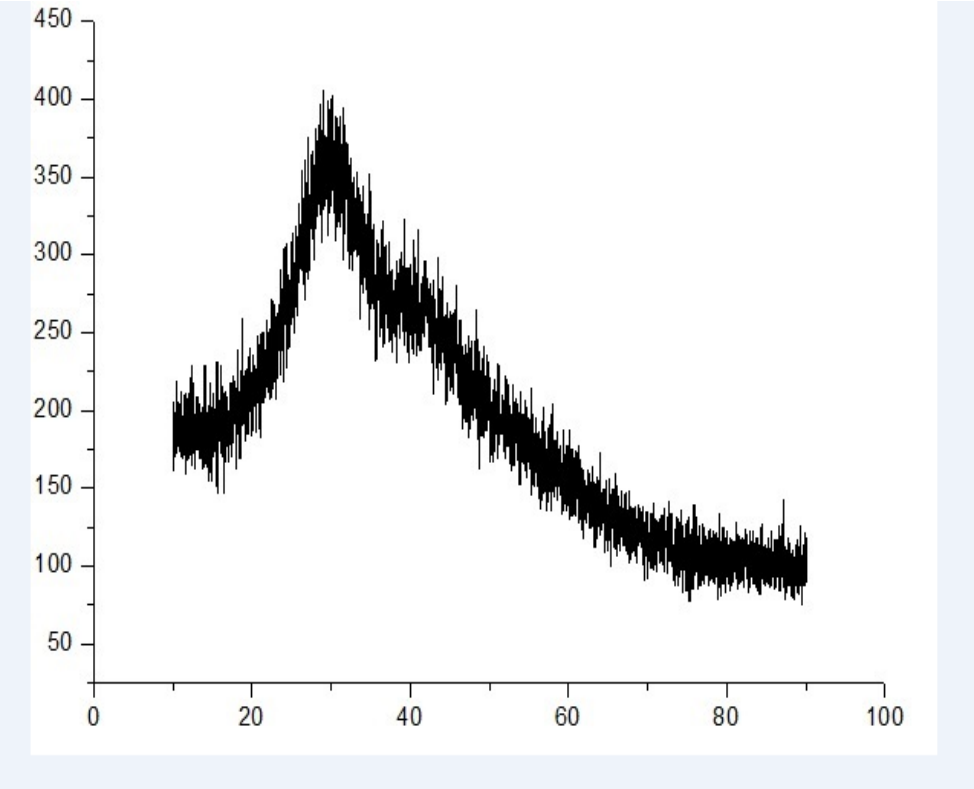
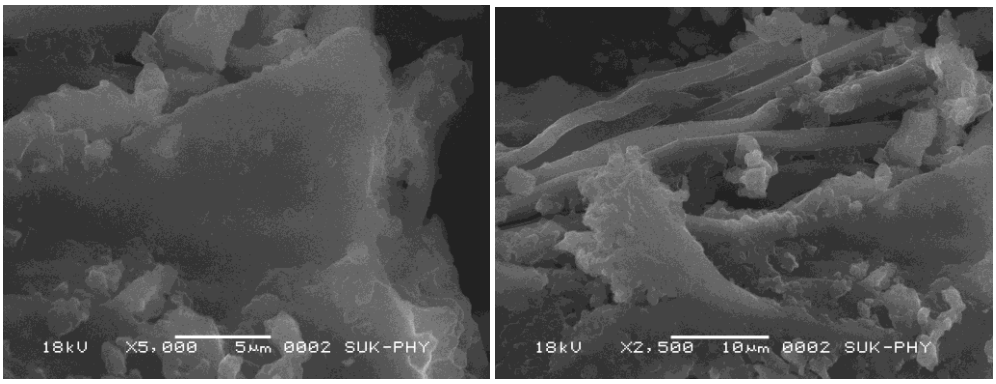


Fig 3 PXRD of ZrNPS from tulasi extract:

SEM of ZrNPS from Tulasi extract:

SEM analysis provided the morphology and size details of the nanoparticles. Fig.4(I,II,III &IV) shows that high density of nanopartices synthesized by plant extract of ocimum sanctum, the interaction such as hydrogen bonding and electrostatic interaction between the bioorganic capable to form molecular bond is a reason for synthesis of nickel nanoparticles using plant extract. The zirconium nanoparticles are spherical in shape with uniform distribution. However the average size of an individual particle is estimated to be 1-10 μm. Due to the surface Plasmon resonance, the zirconium nanoparticles show the absorption peaks of higher counts. SEM images revealed that the synthesized zirconium oxide nanoparticles were aggregated as irregular sphere shapes with rough surfaces.



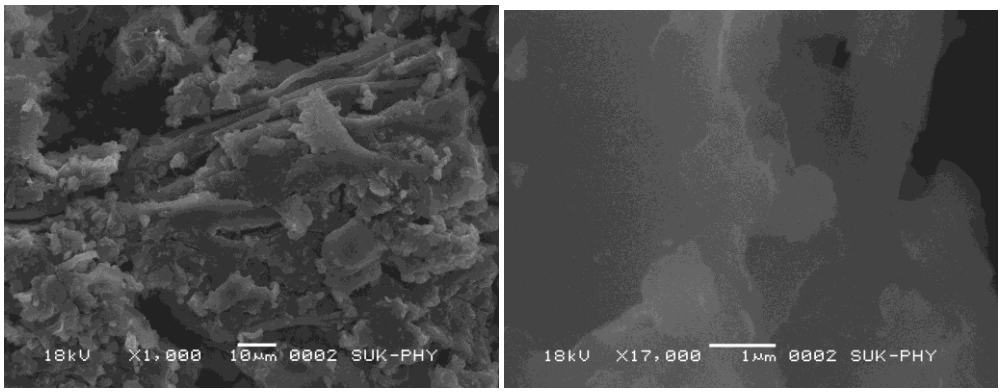


Fig 3.I,II,III,IVSEM images of Zr NPs from Tulasi extract:

Effect of new chemical entity on Protein (albumin) Denaturation:

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented because of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of new chemical compoundto inhibit protein denaturation was evaluated. It was effective in inhibiting heat induced albumin denaturation. The percentage inhibition of protein denaturation of test compound was found to be 25 –52. Maximum inhibition was found to be of 52 at 500 µg/ml of new compound. Aspirin, a standard anti- inflammation drug showed the maximum inhibition 75% at the concentration of 100 µg/ml compared with control. The results are tabulated in Table - 1.

Table 1: Anti-inflammatory effect of new compound on heat induced inhibition protein denaturation

Sl. No.	Concentration(µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
1	Control	0.40 ± 0.02	---
	New compound		
2	100	0.30 ± 0.01*	25
3	200	0.26 ± 0.01**	35
4	300	0.24 ± 0.02**	40
5	400	0.22 ± 0.01**	45
6	500	0.19 ± 0.02**	52
7	Aspirin 100	0.10 ± 0.02**	75

Values are mean ± SEM, n=3,*Significant values, $p<0.01$ and $p<0.001$ compared to control.

Statistical analysis

The data obtained from the above findings were subjected to statistical analysis following one-way ANOVA followed by Tukey's Kramer Multiple Comparison Test to assess the statistical significance of the results using Graph pad prism software.

IV. CONCLUSION

The present work indicated the green synthesis of ZrNPs using Tulasi leaves extract and use as an anti-inflammatory agent. The results confirmed that orange juice and peel plays an important role in reduction and stabilization off zirconium. The outcomes of this study illustrate a broad range of applications in medical field.

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