SYNTHESIS, CHARACTERISATION OF ZIRCONIUM NANOPARTICLES FROM ORANGE -ANTI THEIR AND EXTRACT PEEL AND JUICE ANTIINFLAMMATORY AND OXIDANT TYIACTIV

A.S.Pujar, U.S.Pujeri, M.I.Kumbar and M.S.Yadawe
S.B.Arts and K.C.P. Science College, Bijapur-586 103, India.

Abstract:- Environmental friendly approach synthesis of nanoparticles has become new branch in nanotechnology. Synthesis of zirconium nanoparticles was carried out using Citus sinensis juice and peel extract which acted as a reducing and capping agent. The synthesis was confirmed by UV-visible spectroscopy, SEM analysis, FTIR and XRD spectroscopy. UV-visible spectrum shows that the surface Plasmon broad peak was observed at 282.9nm.SEM image indicated uniformly distributed ZrNPs on the surface with high agglomeration.XRD pattern showed that average size of Citrus sinensis fruit juice and peels aqueous extract obtained around 28nm using Debye-Scherrer equation. In addition, the ZrNPs were screened for in vitro anti-inflammatory, antioxidant activity.

Key words: - Citrus sinensis, SEM.XRD, in vitro anti-inflammatory, antioxidant activity.

Introduction:- The use of environmentally benign materials for the synthesis of nanoparticles nanoparticles offers various benefits in pharmaceutical and biomedical applications [1], as toxic chemical substances are not employed in their synthesis. Natural products have been major sources of bioactive agents and will continue to play a protagonist role in the discovery of new drugs [2]. Citrus sinensis is consumed all over the world as an excellent source of vitamin C, which is a powerful natural antioxidant that builds the body′s immune system [3]. It has been used traditionally to treat ailments like constipation, cramps, colic, diarrhea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, and depression and stress [4].Citrus fruits and derived products from them have been well known to have beneficial effects on human health owing to have bioactive compounds such as phenolic acid, flavonoid, limonoid, carotenoid and fiber[5,6].

Plants have a number of reducing agents such as poly phenols and flavonoids etc, which takes place the reduction of Zr^{4+} ions. These poly phenols and flavonoids ae used as antimicrobial and antioxidant agents by the plants to protect themselves from various pathological conditions. Citrus sinensis var kozan yerly fruit juice has been investigated for phytochemical investigation and it was reported that plant is rich in several poly phenols and other bioactive compounds [7]. In this study we synthesized Zr nanoparticles using fruit extract of Citrus sinensis. The less time processing, well dispersion, spherical, small sized (28 nm) nanoparticles synthesis, efficient anti-

inflammatory and antioxidant properties ensures the study prominent than other biological methods for zirconium nanoparticles synthesis.

Materials and Methods

The Orange Fruits 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 orange fruit was cut into small pieces and 10g of fruit 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.

Antiinflammatory Activity:- 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 cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of new chemical compounds namely Juice and Orange peel extracts to inhibit protein denaturation was evaluated. It was effective in inhibiting heat induced albumin denaturation. The percentage inhibition of protein denaturation of Juice extract was found to be 23 − 57 and

Orange peel extract was found to be 30 - 59. Maximum inhibition was found to be of 57 and 59 % at 500 µg/ml of Juice and Orange peel extract respectively. Aspirin, a standard anti- inflammation drug showed the maximum inhibition 73% at the concentration of 100 µg/ml compared with control.

Antioxidant Activity

DPPH radical scavenging activity

The principle of this assay is based on the reduction of DPPH, a free stable radical by an antioxidant according to the following reaction.

$$DPPH\bullet + AH \rightarrow DPPHH + A\bullet$$

During the reaction, alcoholic solution of DPPH turns from deep violet color to light yellow color. 100 µL of various concentrations of extract (50, 100, 200, 400, 800, 1000 µg/ml) in methanol were added to 100 µL of 0.01% methanolic DPPH solution. The plate was incubated for 30 min in the dark at ambient temperature and the absorbance was recorded at 540 nm using a spectrophotometer. Vitamin C at different concentrations (50, 100, 200, 400, 800, 1000 µg/ml) was used as standard.

The DPPH radical scavenging activity (%) was calculated as follows:

DPPH scavenging activity (%) = $[(Ac - As) / Ac] \times 100$

Results and discussion

UV-Visible Spectra Zirconium nanoparticles

The synthesis of green ZrNPs had been confirmed by measuring the UV-Vis spectrum of colloidal solution which has absorbance peak at 282.9 and 281.6 nm; and the expanding of peak indicated that the particles are mono-dispersed as shown in Figure 1&2. UV − Visible spectroscopy is a simple and fast way to confirm the formation of zirconium nanoparticles. Synthesis of nano sized particles with antibacterial and antifungal properties is of great interest in the development of new pharmaceutical products. Among the different types of extracts prepared, Citrus *sinensis* juice and fruit peel extracts showed good antibacterial and antifungal activity.

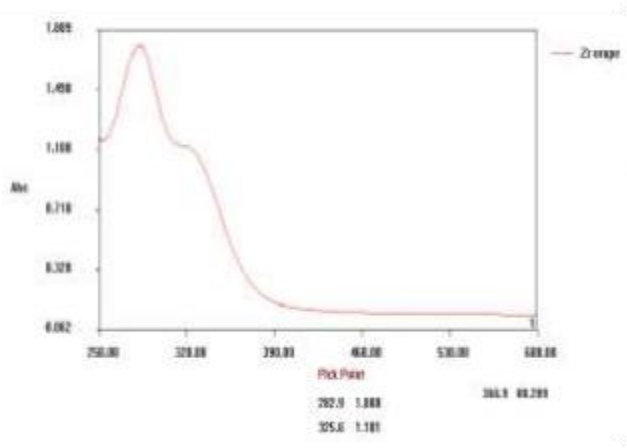


Fig.1.Citrus sinensis juice extract

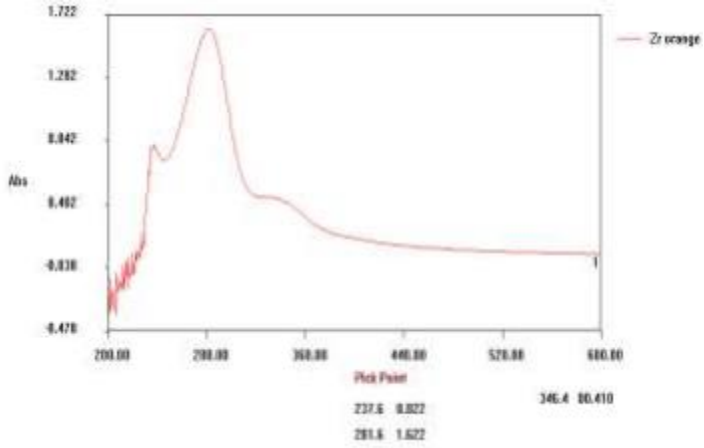


Fig.2.Citrus sinensis peel extract

FTIR Spectra Zirconium nanoparticles

FTIR analysis (Fig.3&4) indicated the possible naturally occurring organic molecules, taken part in stabilization and capping of the Zirconium nanoparticles FTIR spectrum shows absorption bands at 3370, 2810, 1590, 1240 and 1040 cm^{-1}. The absorption bands at 3370 cm^{-1} and 2810 cm^{-1} represent the OH group stretching and sp^2 C-H (alkane) vibration respectively. The absorption at 15800 cm^{-1} was attributed to the C—C═C symmetric stretching vibration of aromatic rings. The absorption band at 1190 $cm^{-1}$was attributed to the C—N stretching vibration. The absorption band at 1040 cm^{-1}might is contributed by the C—O group of the polysaccharides in the fruit juice of *Citrus sinensis var. kozan yerly*. It is assumed that −OH group present (indicated in FTIR) in the *Citrus sinensis var. kozan yerly* is responsible for the reduction of zirconium ions to elemental silver nanoparticles through the oxidation of alcohol to aldehydic group.

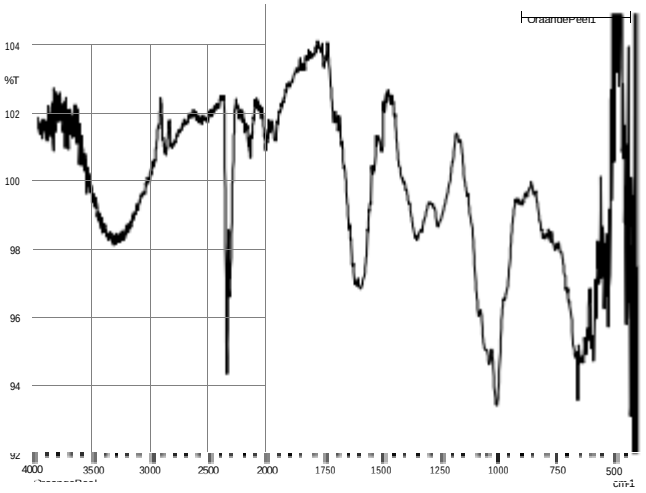


Fig 3.FTIR of Zirconium nanoparticles with Citrus sinesis juice.

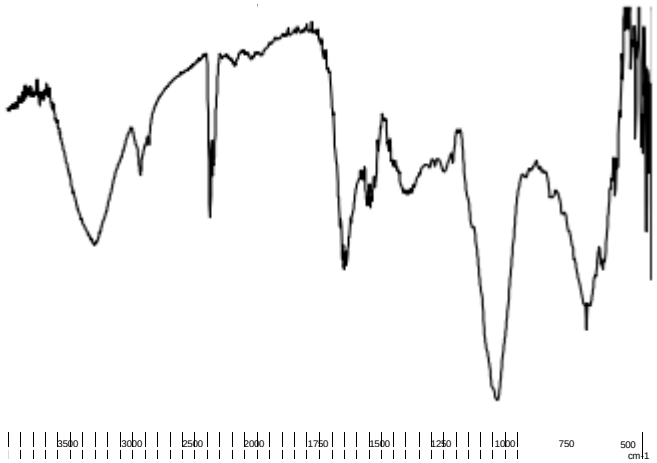


Fig 4.FTIR of Zirconium nanoparticles With citrus sinesis peel extract.

SEM of Zirconium nanoparticles

SEM is shown in Figure 5a was employed to analyze the structure and morphology of the nanoparticles to give further insight into the features of the ZrNPs obtained from the proposed biogenic synthesis method, the image showed relatively spherical shape of the formed nanoparticles. The SEM analysis confirmed the presence of nano-size metal particles. They were spherical and cubical in shape and the diameter of the particle was 100 nm(both juice & peel extract). The SEM image of both types of SNPs showed the presence of polydispersed ZrNPs.

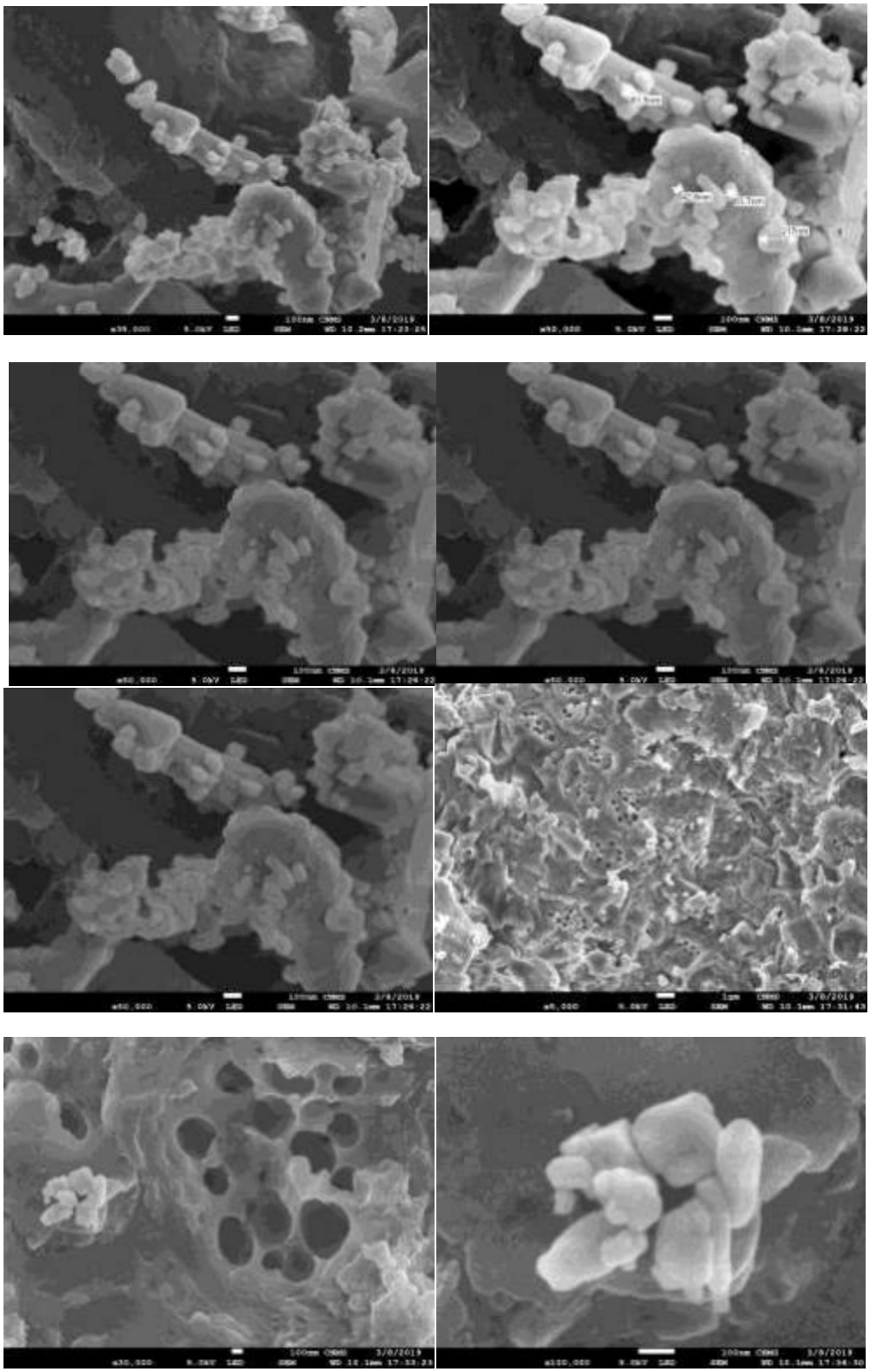


Fig.5 a-f ZrNPs from Citrus sinensis juice extract

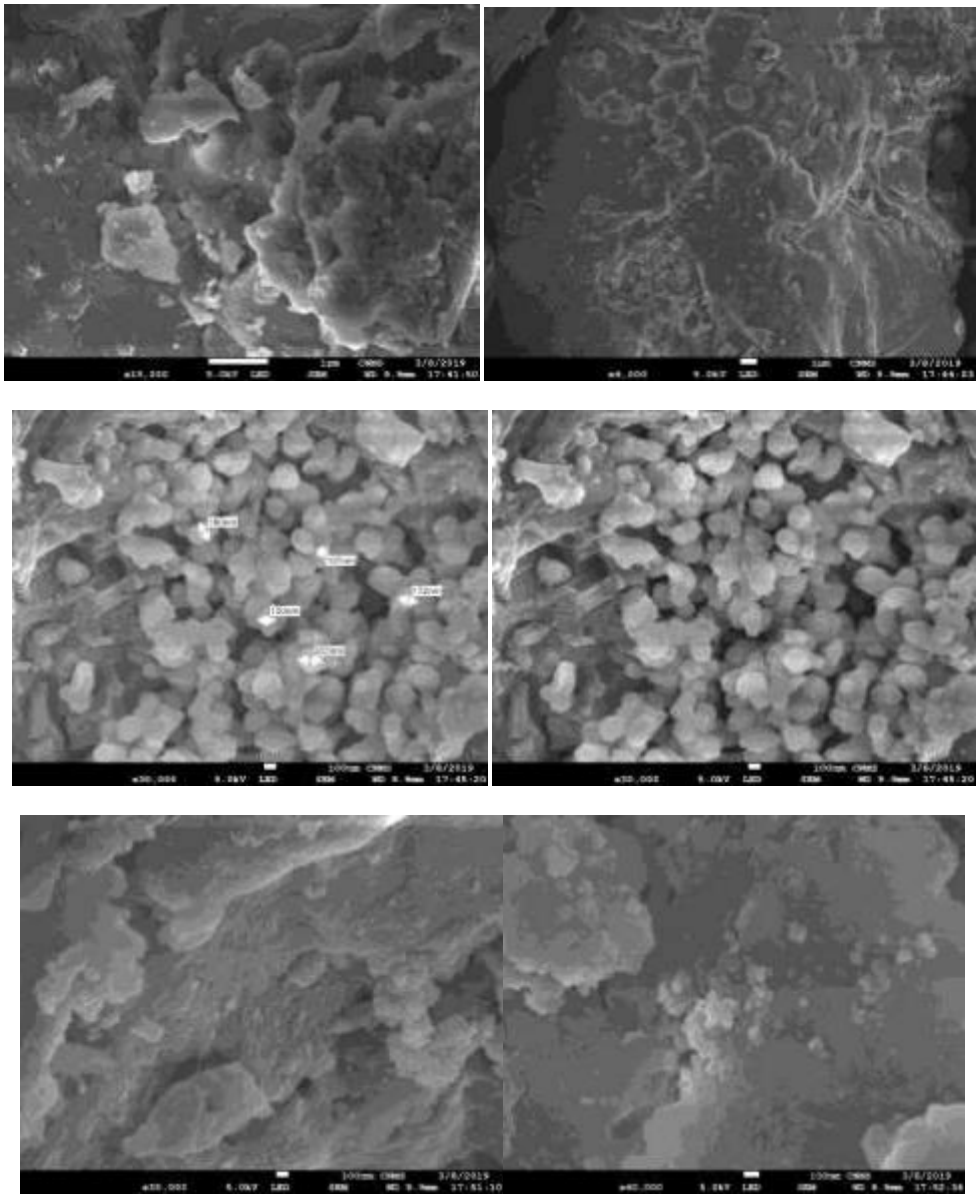


Fig.6 a-f ZrNPs from Citru sinesis peel extract.

PXRD Spectra of ZrNPs from citrus sinesis juice and peel extract

The XRD pattern of the dried Zirconium nanoparticles synthesized using *citrus sinesis juice and peel* extract is shown in figure 7 & 8. The four diffraction peaks were observed at 37.9^0,, 44.1^0,, 64.3^0, and77.2^0, in the 2theta range $20–80^0$, can be ascribed to the (111), (200), (220) and (311) reflection planes of face-centred cubic (fcc) structure of ZrNPs phases. Two diffraction peaks observed at 37.8^0, and 43.9^0, in the 2 theta range $20–80^0$, of peel extract can be ascribed to the(111) and (200) reflection planes of face-centred cubic (fcc)structure of Zr phases. Two diffraction peaks observed at 38^0, and 44.4^0, in the 2 theta range $20–80^0$, of sample can be ascribed to the (111) and (200) reflection planes of face-centred cubic (fcc) structure of zirconium phases.

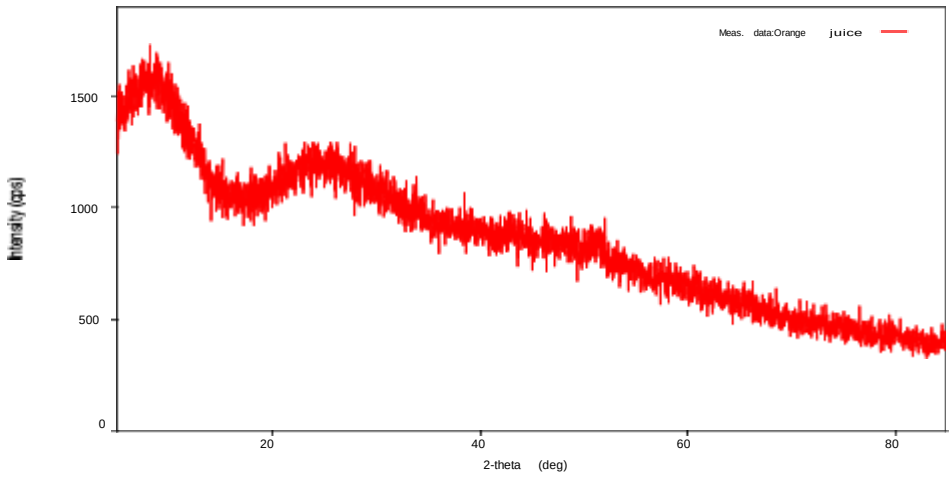


Fig.7.PXRD Spectra of ZrNPs from citrus sinesis juice extract.

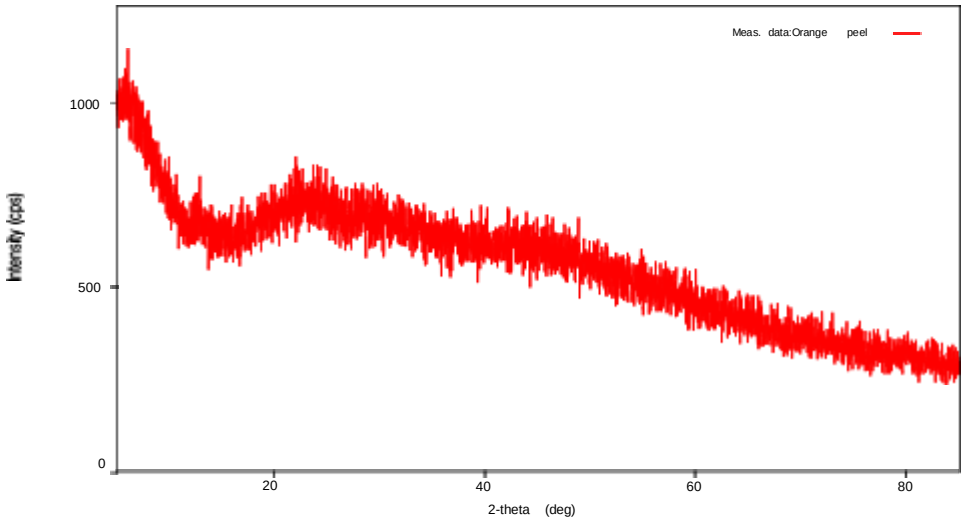


Fig.8.PXRD Spectra of ZrNPs from citrus sinesis peel extract

In-vitro anti-inflammatory activity of ZrNPs from citrus sinesis juice and peel extract.

Effect of new chemical compound on Protein 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 cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of new chemical compounds namely Juice and Orange peel extracts to inhibit protein denaturation was evaluated. It was effective in inhibiting heat induced albumin denaturation. The percentage inhibition of

protein denaturation of Juice extract was found to be 23 − 57 and Orange peel extract was found to be 30 - 59. Maximum inhibition was found to be of 57 and 59 % at 500 µg/ml of Juice and Orange peel extract respectively. Aspirin, a standard anti- inflammation drug showed the maximum inhibition 73% at the concentration of 100 µg/ml compared with control. The results are presented in table 01.

Table 01: Effect of Juice and Orange peel extract on heat induced protein denaturation

Sl. No.	Concentration (µg/ml)	Absorbance at 660 nm	% inhibition of protein denaturation
1	Control	0.42 ± 0.03	---
	Juice Extract		
2	100	0.32 ± 0.02*	23
3	200	0.28 ± 0.01**	33
4	300	0.26 ± 0.02**	38
5	400	0.24 ± 0.01**	42
6	500	0.18 ± 0.02**	57
	Orange peel extract		
7	100	0.29 ± 0.02*	30
8	200	0.26 ± 0.01**	38
9	300	0.23 ± 0.02**	45
10	400	0.22 ± 0.01**	47
11	500	0.17 ± 0.02**	59
12	Aspirin 100	0.11 ± 0.02**	73

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 [8,9].

Antioxidant Activity

Nitric oxide scavenging activity

Nitric oxide was generated by sodium nitroprusside and measured by Griess reaction. Sodium nitroprusside (5 mM) in standard phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of ethanol extract (50, 100, 200, 400, 800, 1000 µg/ml), Vitamin C as reference standard (50, 100, 200, 400, 800, 1000 µg/ml) and dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 250°C for 5 hr [10-11]. Control experiments without the test compounds but equivalent amounts of buffer were conducted in an identical manner. After 5 hours, 0.5 ml of incubation solution is removed and diluted with 0.5 ml of Griess reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylene diamine was read at 546 nm. All the determinations were performed in 6 replicates.

Percentage inhibition of nitric oxide radical was calculated as follows:

Nitric oxide scavenging activity (%) = [(Ac − As) / Ac] × 100

Fig.9.Free radical scavenging activity of extract by DPPH method.

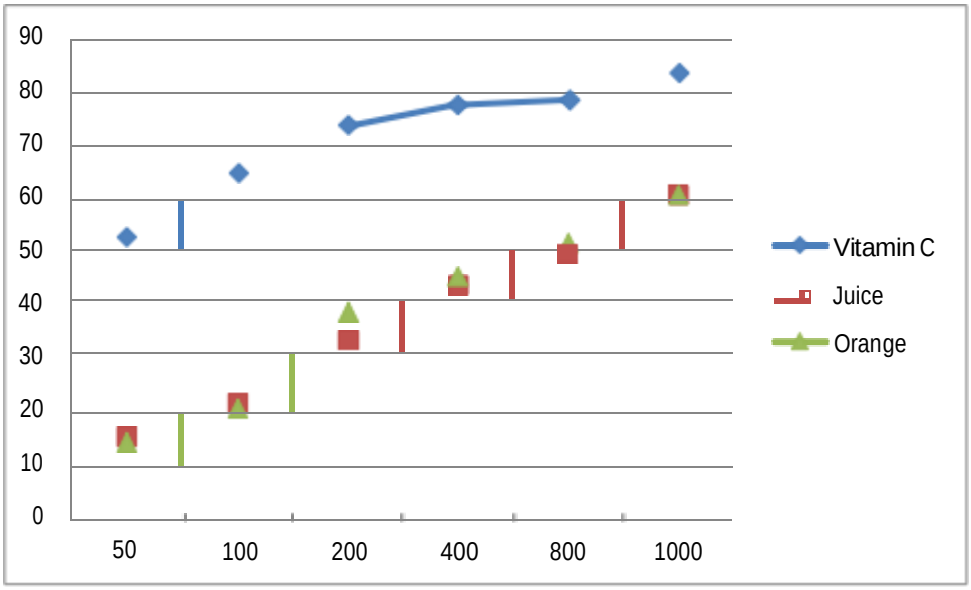
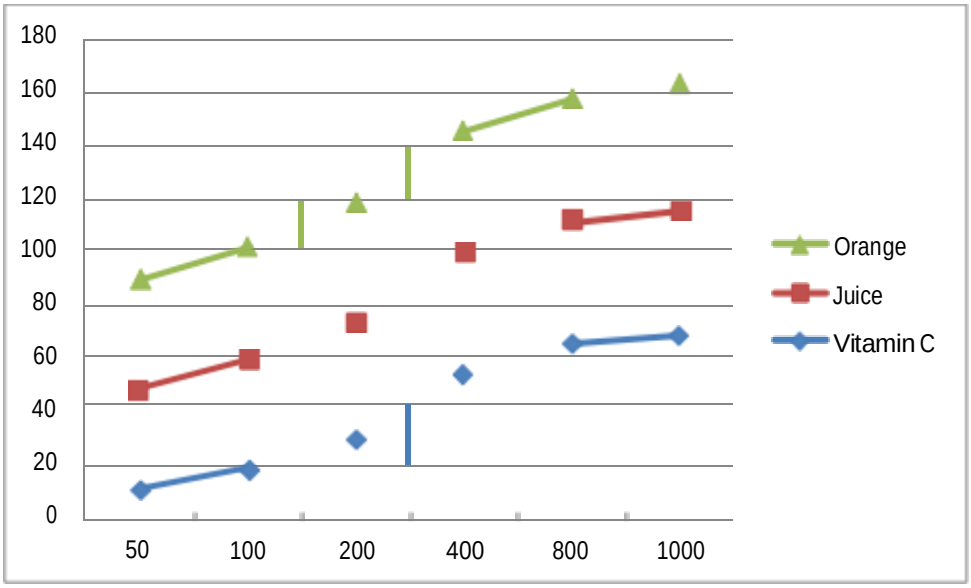


Fig.10.Free radical scavenging activity of extract by Nitric oxide method.



Conclusion

The present work indicated the green synthesis of ZrNPs using orange juice and peel extract and use as an anti-inflammatory and antioxidant 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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