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RESEARCH ARTICLE

GREEN SYNTHESIS OF HYDROXY APATITE NANOPARTICLE BY SOL - GEL METHOD USING PAPAYA LEAF (CARICA PAPAYA) AND INDIAN NETTLE LEAF (ACALYPHA INDICA) AS SOLVENTS

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Abstract

The HAP Nanoparticles were produced from Sol-Gel method by adding 1M of calcium hydroxide, 0.6M of orthophosphoric Acid were prepared using papaya (*Carica papaya*) leaf and Indian nettle (acalypha indica) leaf extract as the solvents. Sol-Gel method was not sensitive regarding the preparation conditions, easy to operate and implement, which controlled the structure, morphology, and particle size of nanomaterials. The HAP materials synthesized by sol-gel process were efficient to improve the contact and stability at the artificial/natural bone interfaces in both in vitro and in vivo environment.Hydroxyapatite was the dominant inorganic phase in natural bone. Synthetic hydroxyapatite particles, films, coatings, fibers and porous skeletons were used extensively in various biomedical applications. The presence of HAP nanoparticles were studied by various analytical techniques such as UV, XRD, FTIR and SEM. HAP had excellent bioactivity and biocompatibility along with strong antimicrobial activity that make them as potential materials for tissue engineering, orthopaedic and dental applications.

Introduction:-

Hydroxyapatite (HAP) or pentacalcium hydroxide triphosphate, is a inorganic compound with hexagonal structure and It is an white crystalline solid [1, 2]. Molecular weight of HAP is 1004.6 g/mol, molecular formula of HAP is Ca10(PO4)2OH2. The fundamental element of hydroxyapatite is calcium and phosphorus with stoichiometries calcium and phosphorus ratio of 1.667 [3-6]. Hydroxyapatite is the widely accepted biomaterial for the repair and reconstruction of bone tissue imperfections. HAP has outstanding properties, such as bio-active, biocompatible, osteoconductive, grate bonding property, non- toxic, noninflammatry, non-immunogenic properties. It is widely used in bone and teeth implantation treatment [7-11]. Because it is very effective in biological performance due to the similar structure and character with original bone and teeth become main criteria and innovation of future synthetic bone [12, 13].

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Martials and Methods:-

The HAP Nanoparticles were produced from Sol-Gel method by adding 1M of calcium hydroxide, 0.6M of orthophosphoric Acid were prepared using papaya (Carica papaya) leaf and Indian nettle (acalypha indica) leaf extract as the solvents. HAP Nanoparticles were synthesised by the following steps.

Collection of Leaves:

The carica papaya is a small and fast growing but short- lived plant, one of the 22 accepted species in the genus carica of the family caricaceae. Its origin is in the tropics of the Americas, Central America [14]. b)The Acalypha indica (Indian acalypha, Indian mercury, Indian copper leaf, etc,) family of Euphorbiaceae. The fresh leaf samples of carica papaya and Acalypha indica were washed with distilled water then cut into pieces. The leaf samples were dried at room temperature for 4days. After a drying, the dried leafs were ground into fine powder using electric blender and stored in separate container.

Preparation of Leaf Extract:

About 1.5g of papaya (carica papaya) and Indian nettle (acalypha indica) leaf powders kept in two separate beaker containing 250ml of double distilled water. Then the leaf powders containing solution mixture were stirred well with using glass rod. After stirring, the beakers were closed with watch glasses. And then beakers were marked as A and B and placed on hotplate. The solution mixtures were boiling for 2 hours then extract were cooled down in room temperature, filtered, and stored at 4oc for further use.

III) Preparation of HAP Nanoparticle:

1M of calcium hydroxide (Ca(OH)2), 0.6M of orthophosphoric Acid (H3PO4) were prepared using papaya (Carica papaya) leaf extract as the solvent. 1M calcium hydroxide (Ca(OH)2), 0.6M of orthophosphoric Acid (H3PO4) of prepared solution was stirred for 30 minutes separately, with using magnetic stirrer at room temperature. The estimated molar ratio of Ca:P was adjusted in the range between 1.67 and 2.00. The orthophosphoric acid solution are added in drops to the calcium hydroxide solution. After adding it the precipitated solution was vigorously stirred for an hour. The pH value of the solution was controlled and maintained around 11 by addition of ammonia solution drop wise. The solution was aged for 24 hours. Subsequently, the solution was filtered and washed with distilled water several times before drying process. The precipitate was dried in oven for

6 h less than 200°C to remove the water completely. The reaction of mixing both (Ca(OH)2), (H3PO4) solutions. Finally HAP Nanoparticle synthesized using carica papaya leaf extract. The same procedure was repeated using Indian nettle (acalypha indica) leaf extract.

Characterization of Hydroxyapatite Nanoparticle I) UV-visible spectroscopy:

The UV–visible absorption spectra of the both HAP (using Carica papaya, Acalypha indica leaf extract) nanoparticle samples were recorded in the wavelength 255nm using UV spectrometer.

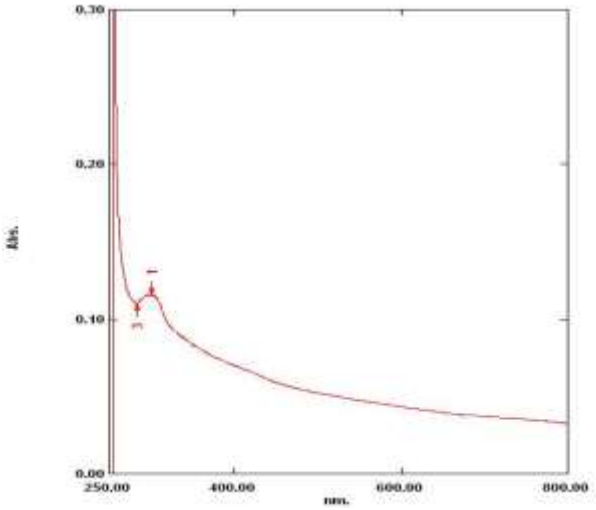

Fig 1:- UV-spectrum of synthesized HAP (using Carica papaya leaf extract) Nanoparticle.

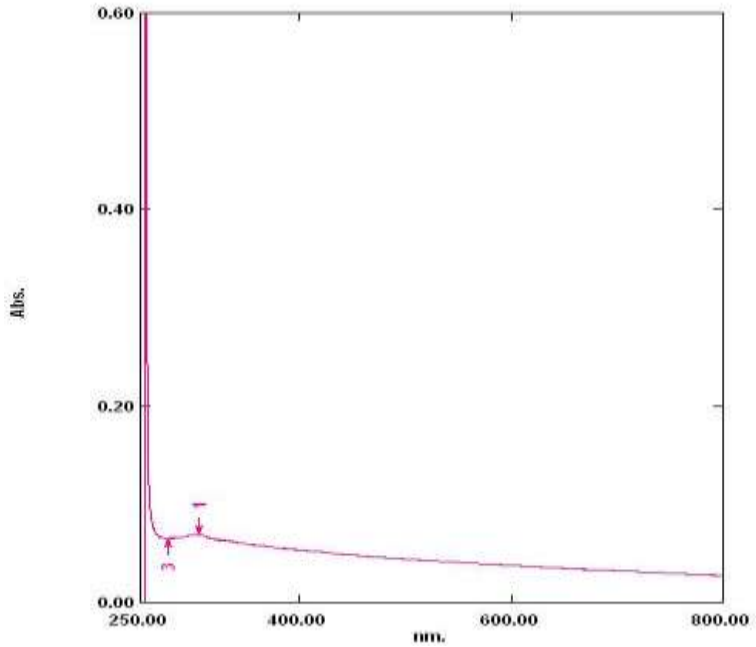

Fig 2:- UV-spectrum of synthesized HAP (using Acalypha indica leaf extract) Nanoparticle.

Fourier-Transform Infrared Spectroscopy (FTIR):-

FTIR was carried out in order to assign the functional groups present in the synthesized Hydroxyapatite Nanoparticle. Figure 3, 4 illustrated the FTIR spectra of the nHAP synthesized by Sol-Gel method using Papaya leaf (Carica papaya) extract and Indian nettle leaf (Acalypha indica) extract as a solvent.

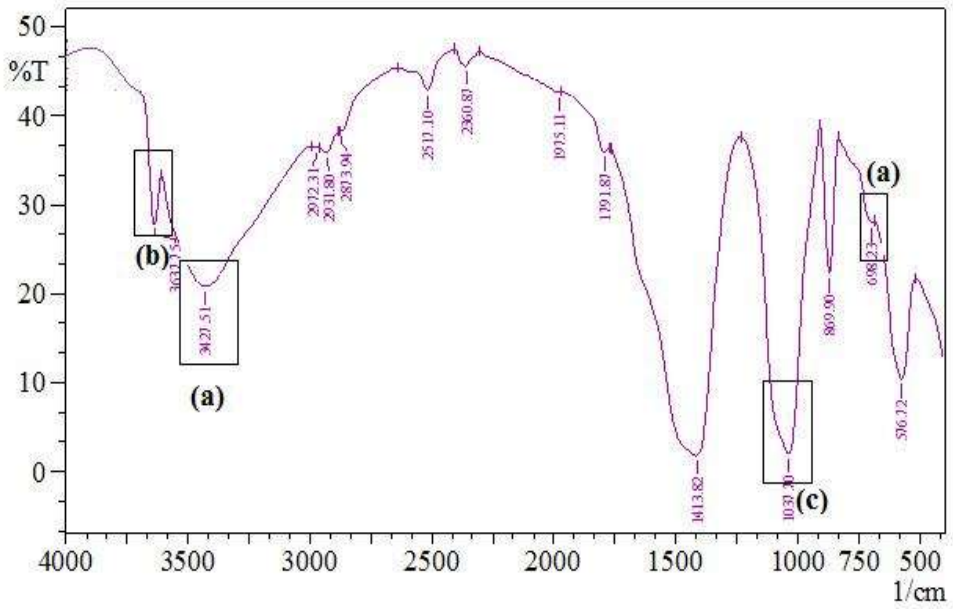

Figure 3:- FTIR Spectra of HAP using Carica papaya extract as solvent.

In that figure 3 (a) represents the confirmatic peak of HAP i.e., the hydroxyl peaks were detected at 3427 cm-1 and 698 cm-1 as stretching and pending modes. The lattice water was also found at the peak 3607 represented by (b). The peak at 1037 represented by (c) shows the presence of Phosphate group. The above said peaks reveal the formation of mixed phases of calcium phosphate.

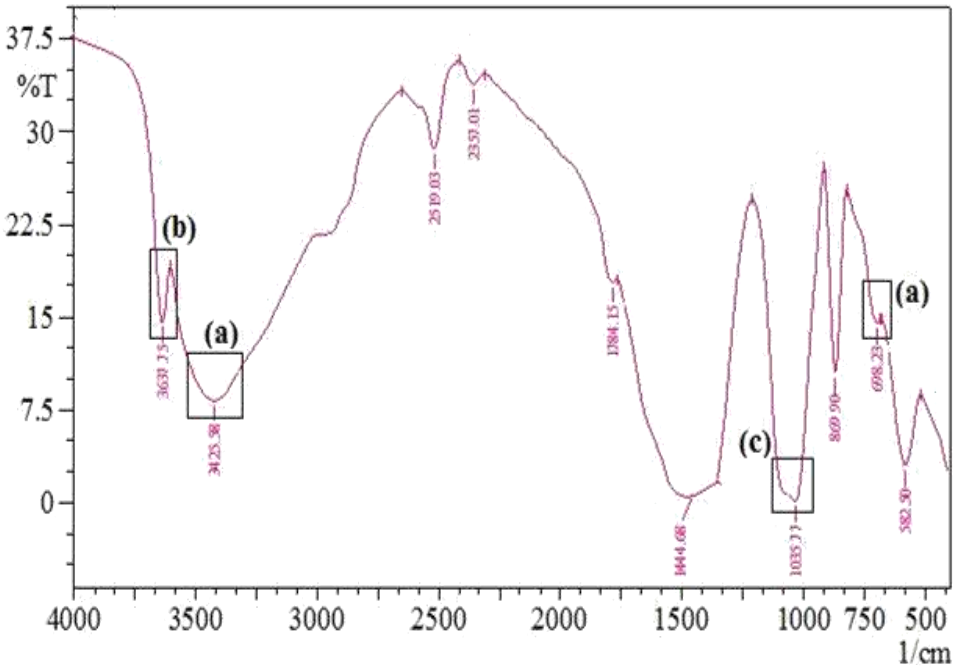

Figure 4:- FTIR Spectra of HAP using Acalypha indica extract as solvent.

In that figure 4 (a) represents the confirmatic peak of HAP i.e., the hydroxyl peaks were detected at 3425 cm-1 and 698 cm-1 as stretching and pending modes. The lattice water was also found at the peak 3637 represented by (b). The peak at 1035 represented by (c) shows the presence of Phosphate group. The above said peaks reveal the formation of mixed phases of calcium phosphate.

X-Ray Diffraction Studies:

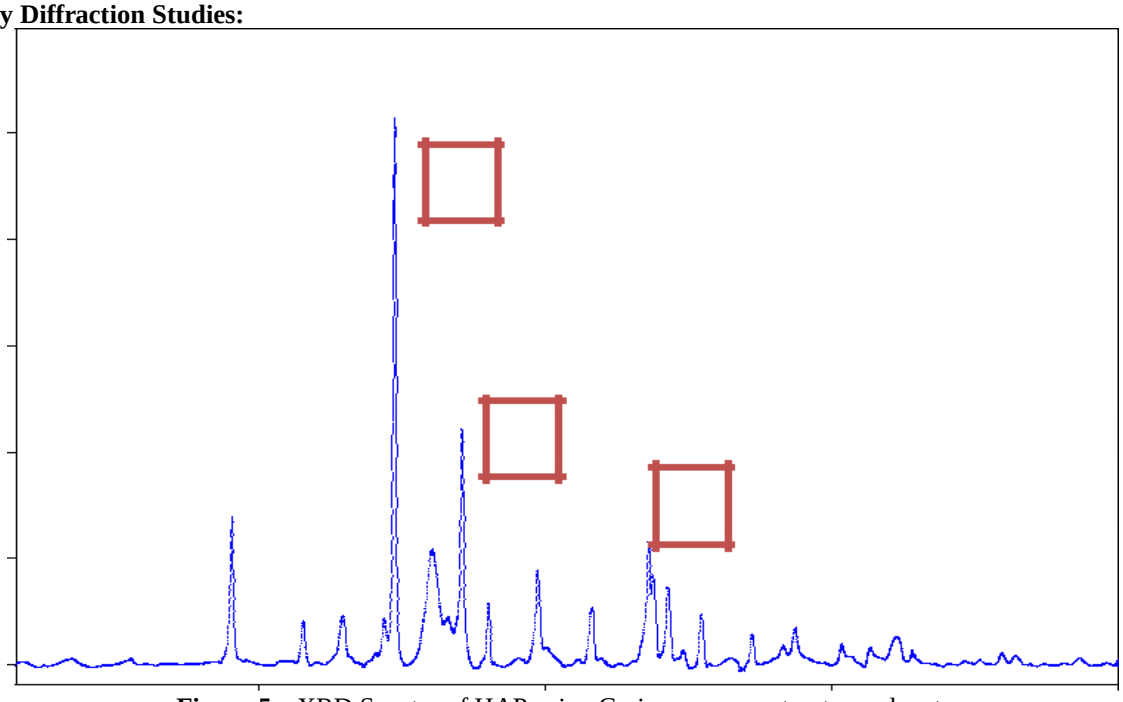

Figure 5:- XRD Spectra of HAP using Carica papaya extract as solvent.

S.No	Peak	2Θ(deg)	FWHM(deg)	D(nm)

1	A	34.077	0.282	9.6091
2	B	35.98	0.230	6.2988
3	C	46.76	0.33	5.5102

Table 2:-

S.No	Peak	2Ɵ (deg)	FWHM (deg)	D(nm)
1	a	34.102	0.267	9.9457
2	b	35.97	0.226	6.4103
3	c	46.76	0.18	10.1053

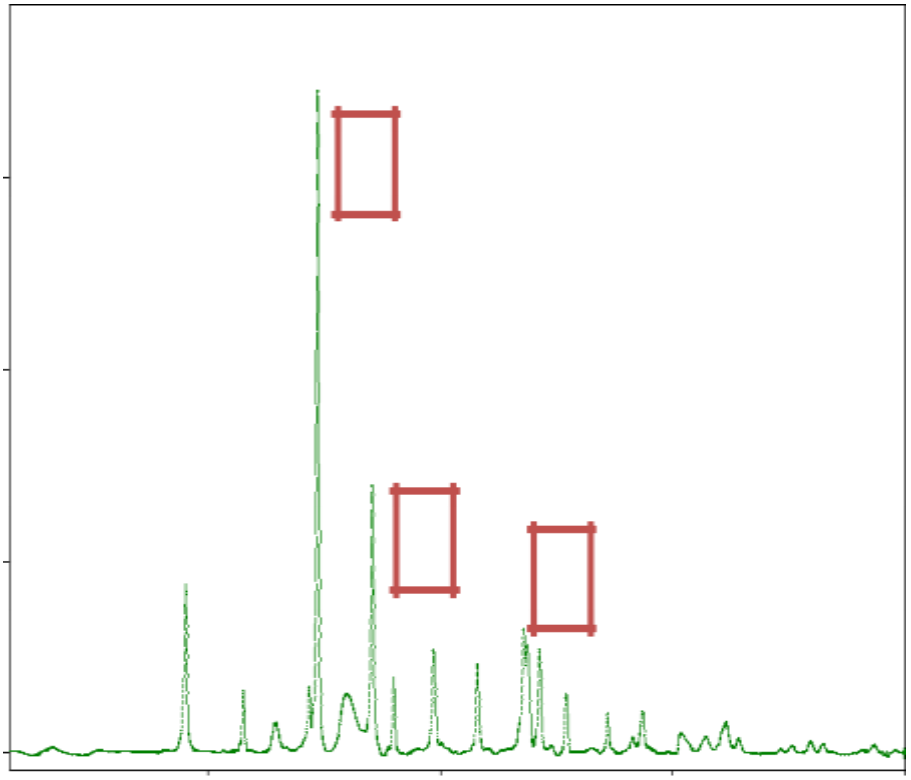

Figure 6:- XRD Spectra of HAP using Acalypha indica extract as solvent.

The XRD pattern for the HAP sample synthesized from 2 different leaf extract as shown in the Fig. 5, Fig. 6, reveals the presence of Nano particle. The average crystallite size of the synthesized HAP sample was calculated from the XRD line broadening measurement using the Debye- Scherrrer's equation and it lies between the Nano particle sizes i.e., 2nm – 100nm.

Field Emission Scanning Electron Microscopic Results:

Fig 7:- FESEM image of HAP Nanoparticle synthesized using Carica papaya leaf extract as solvent.

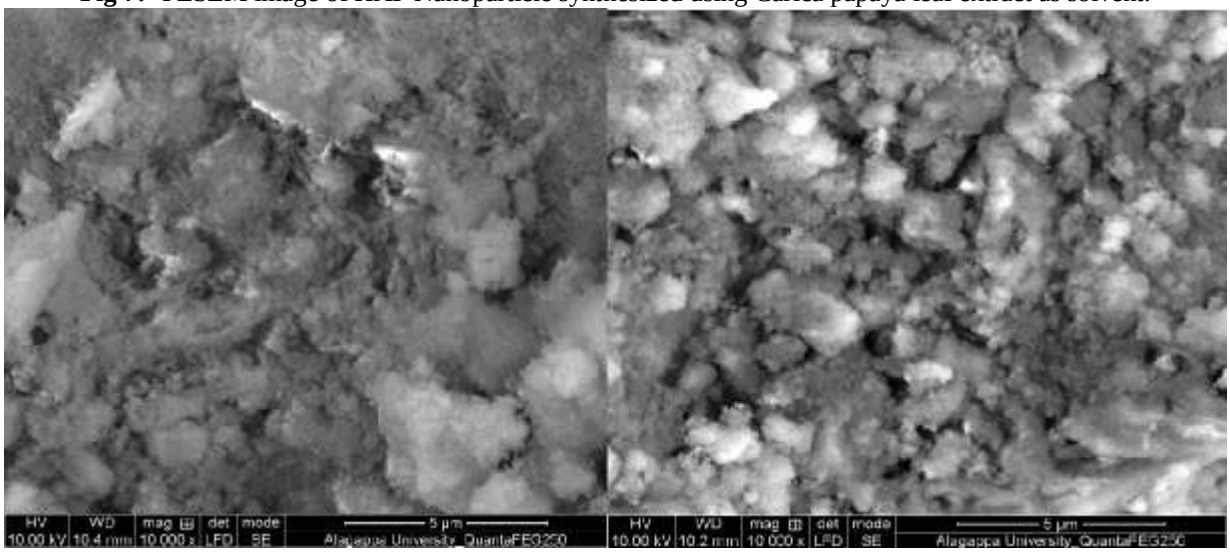

Figure 7, showed the FESEM micrograph of the sample synthesized HAP Nanoparticle (using Carica papaya leaf extract as solvent). The morphology of synthesized HAP Nanoparticle shows the agglomerated and resolved particle is represented in figure. the morphology of the particle seems to be spongy and agglomerated strcture.

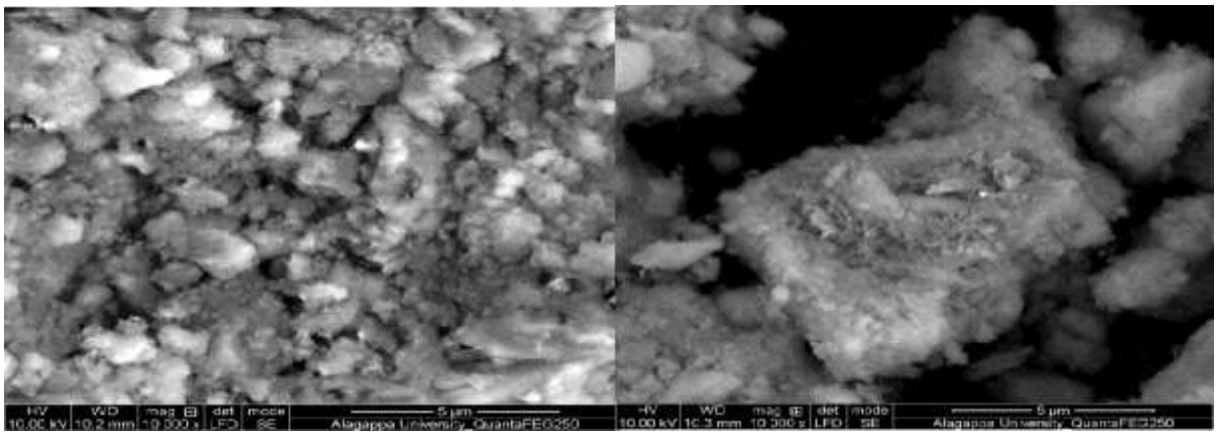

Fig 8:- FESEM image of HAP Nanoparticle synthesized using Acalypha indica leaf extract as solvent.

Figure 8, shows the FESEM micrograph of sample synthesized HAP Nanoparticle (using Acalypha indica leaf extract as solvent). The morphology of synthesized HAP Nanoparticle shows the agglomerated and resolved particle is represented in figure. the morphology of the particle seems to be spongy and agglomerated strcture.

Antibacterial Activity:

E. coli and Bacillus subtilis were the most common bacteria found in bone infection such as osteomyelitis. Antibacterial activity of the prepared samples against E. coli and Bacillus subtilis were shown in Fig 9, 10. The diameters of inhibition zone of synthesized samples against and are E. coli-25mm and Bacillus-26mm. HAP (using Acalypha indica, Carica papaya leaf extract) Nanoparticle showed significant inhibition zone against both the tested bacteria. However, the HAP (using Acalypha indica, Carica papaya leaf extract) showed inhibition zone against E.coli with Bacillus subtilis. The observed antimicrobial activity may be due to the presence of biomolecules such as flavonoids, terpenoids, and protein compounds in the samples, which inhibit the enzymes required for bacterial growth and replication. The green synthesis of HAP Nanoparticle using leaf extract with excellent antibacterial properties. The difference in the antibacterial activity depends on the particles size, nature of the particles, and types of bacteria used for the antibacterial test.

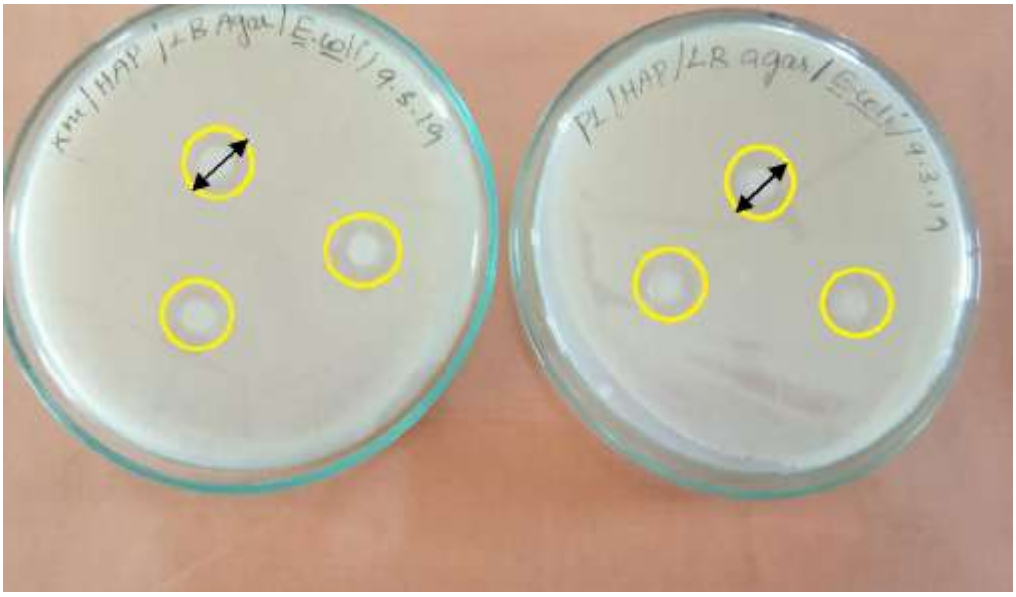

Fig 9:- Antibacterial activity of the prepared samples of HAP (Acalypha indica, Carica papaya leaf extract) using both nanoparticle against E. coli.

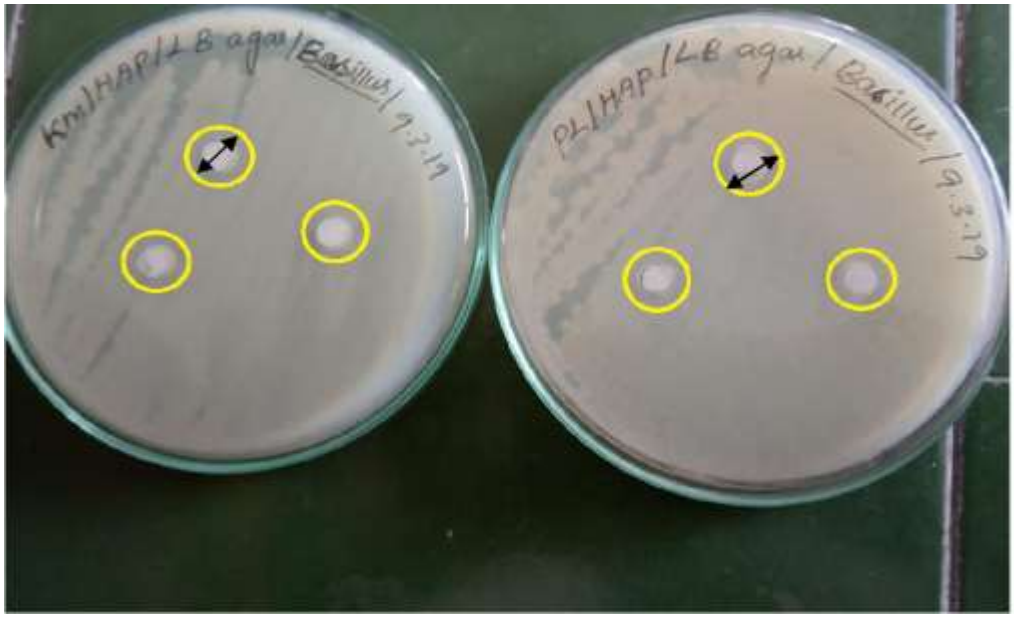

Fig 10:- Antibacterial activity of the prepared samples of HAP (Acalypha indica, Carica papaya leaf extract) using both Nanoparticle against Bacillus subtilis.

Anti- Diabetic Activity

Inhibitory assay of α-amylase activity of HAP (using Acalypha indica & Carica papaya leaf extract) Nanoparticle

The inhibitory action of HAP (using Acalypha indica & Carica papaya leaf extract) Nanoparticles were increased as the concentration increases in both the HAP Nanoparticle and the standard Acarbose and both the charts and table were shown below:

α-amylase inhibition assay

Standard: Acarbose

Blank: Phosphate buffer (0.2 M, pH: 6.9)

Concentration (µg)	Acarbose (std)	Inhibition (%)
10	15.67	3.2
50	36.89	15.49
100	68.71	35.89
250	80.6	42.1
500	89.92	49.09

Table 3:- α-amylase Inhibitory activity of HAP (using Acalypha indica leaf extract) Nanoparticle.

Fig 11:- α-amylase Inhibitory activity of HAP (using Acalypha indica leaf extract) Nanoparticle.

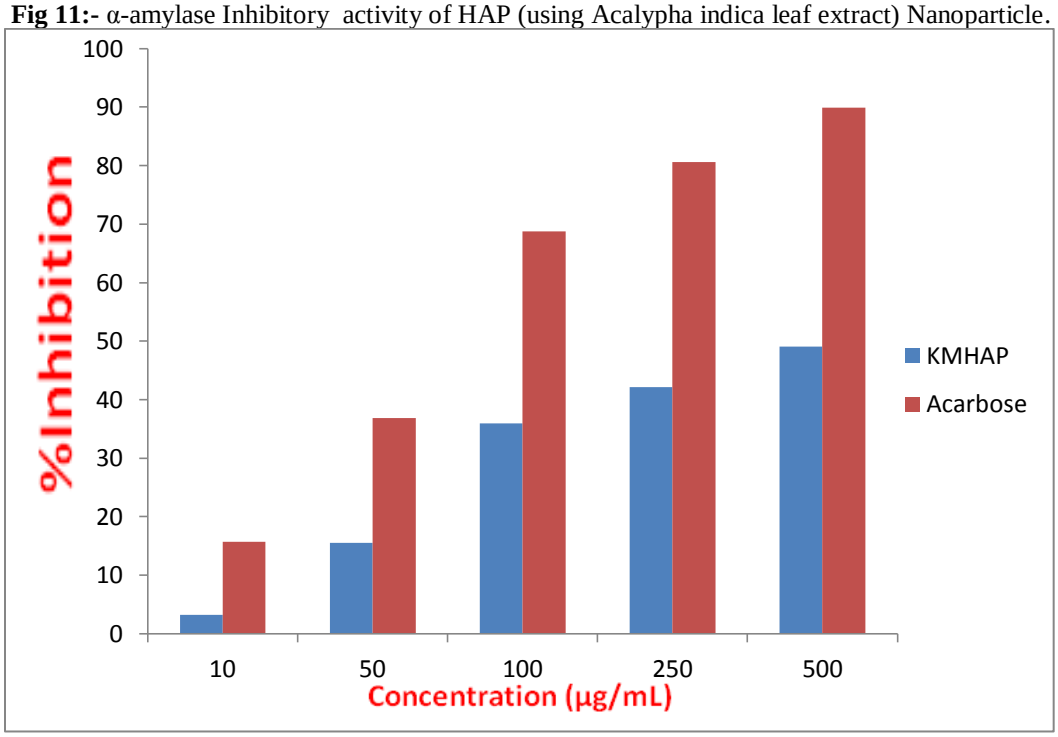

Table 4:- α-amylase Inhibitory activity of HAP (using *Carica papaya* leaf extract) Nanoparticle

Concentration (µg)	Acarbose (std)	Inhibition (%)
10	15.67	6.23
50	36.89	18.52
100	68.71	37.71
250	80.6	44.45
500	89.92	56.89

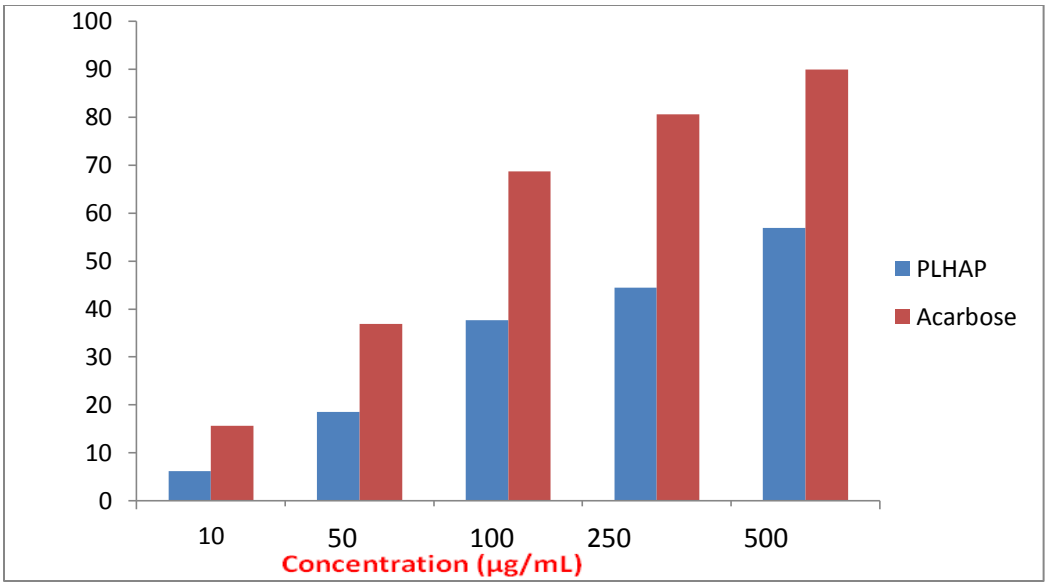

Fig 12:- α-amylase Inhibitory activity of HAP (using Carica papaya leaf extract) Nanoparticle.

Conclusion:-

1. The synthesis and calcinations of hydroxyapatite Nanoparticle using Carica papaya and Acalypha indica leaf extracts as a solvent by Sol-gel process was done. The precursor's calcium hydroxide and orthophosphoric acid was investigated using UV, XRD, FTIR and SEM.
2. UV investigates the revealed the confirmation of HAP Nanoparicle exhibit absorption at 255nm.
3. XRD investigation revealed that the crystallite size calculated using the Debye-Scherrrer's equation lies between the Nano particle sizes.
4. FTIR studies enumerated the functional group present in the HAP Nanoparticle synthesised by Sol-Gel method using leaf extract as a solvent.
5. SEM revealed the formation of Nanoparticle which stimulates the surface morphology, particle size and shape.
6. Antimicrobial studies showed good zone of exhibition in both the samples of the HAP Nanoparticles were prepared by green synthesis using Carica papaya and Acalypha indica leaf extracts as a solvent.
7. The obtained HAP Nanoparticles exhibited excellent antibacterial activity against E. coli and Bacillus subtilis.
8. The HAP Nanoparticles were prepared by green synthesis (using Carica papaya and Acalypha indica leaf extracts) as a solvent also exhibit antidiabetic activity.
9. Hence, green synthesis of the HAP Nanoparticles using Sol-Gel method derived

10. biopolymer possess excellent bioactivity and biocompatibility along with
11. strong antimicrobial activity that make them as potential materials for tissue engineering, orthopaedic and dental applications.

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