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Fabrication and Characterization of a Novel Dental Filling Herbal Composite Using Biphasic Calcium Phosphate and Leaf Extracts of *Cassia occidentalis*

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Abstract

In the field of Biomedicine, allogeneic grafts that possess excellent biocompatibility and immuno-compatibility play a major role in the treatment of untreated dental bone defects. Filling these defects with bone substitute material prevents resorption of bone, preserves the alveolar ridge, and provides sufficient bone for immediate or subsequent implant placement. In this regard, the present study focused on the fabrication of a novel herbal bone graft material rich in biopolymers and phytochemicals was used as bone graft. The bone graft material was synthesized using biphasic calcium phosphate, casein, chitosan and ethanolic leaf extracts of Cassia Occidentalis. The prepared bone graft was subjected to various characterizations like FTIR, X-ray diffraction, thermos-gravimetric analysis, scanning electron microscopy to show its chemical composition, surface morphology, stability, mechanical strength to show its chemical composition, stability and porosity and GCMS analysis, anti-microbial, anti-oxidant, anti-inflammatory activity to reveal its bioactive components. Results revealed that the prepared bone graft of Cassia Occidentalis showed excellent osteogenic and can be well suggested for various biomedical applications like orthopedics, dental fillings, bone tissue engineering and in the treatment of rheumatoid arthritis. It also replaces the use of autogenous graft with high biocompatibility and osteogenesis.

Keywords: *Bone Graft, Biocompatible, Novel Methods, Health and Well-being, Bioactive Compounds, GCMS, Invitro Study, Osteogenic.*

Introduction

Dental alveolar bone defect is a challenging headway in periodontitis and therapies that aim to prevent such defects happens to be an important in treating periodontal related diseases. So, repairing the bone defects in periodontal disease is one of the challenging approaches in the field of dentistry. A dental implant selected should possess osteo-inductive and osteo-conductive properties to enhance the ossification in the defective area. In the field of biomedical applications,

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allogeneic bone grafts are prepared mainly using ceramics. Ceramics has a potential to be used as auto grafts and they help to restore the surrounding tissues, fills the space, stimulates osteogenesis and provide repair to skeletal tissues that prepares it to bear permanent stress. Ceramics used as composites make use of hydroxyapatites (HA), beta tri calcium phosphates (β-TCP), Biphasic calcium phosphates (BCP) etc. offer better osseointegration and and osteoconduction [1]. These composites made of the above ceramics mimic the natural HA of normal human bone and composition of human teeth. When these biological phosphates are implanted into human body, the Biphasic calcium phosphates which exhibited weight of 93% in crystal-like phase for HA and wt of 7 % for β-TCP for calcite bone gradually discharges calcium ions and phosphate ions into the organic medium of the body. Another characteristic feature is that the BCP is mostly brittle, ductile and tough and is better offered in treatment materials for making implants for load bearing surfaces in the body. So, to formulate a bone graft with better compatibility and strength, good bone conductive, integrative and inductive properties, very fine biodegradable nature and reformative property, the present study employs the fabrication of a novel dental implant material composing of HA , β- TCP with the incorporation of herbal extracts for repairing the bone defect [2]. Chitosan as a linking agent with good anti-microbial properties with the addition of casein to improve the strength of the material. Finally, ethanolic leaf extract of *Cassia occidentalis* was incorporated whose phytochemicals support the implant in exhibiting ossification, anti-microbial, antioxidant and anti-inflammatory property when placed *in vivo*. The properties of the component of implant (BCP-CH-CA-Co) were well analyzed by various physico-chemical properties like FTIR, XRD, SEM and TGA. The bioactive components of Co extract were revealed by GC-MS and the properties of each phytochemical was proved by performing anti-microbial, anti-oxidant and anti-inflammatory tests. Having all these properties in the BCP-CH-CA-Co implant helps in avoiding graft rejection and facilitates the implant to repair the bone defects without any inflammation and infection. The prepared dental implant (BCP-CH-CA-Co) is economically cheaper than commercial hydroxyapatite and other composite material and hence can be widely used in repairing periodontal bone defects after performing animal and human trials [3-5].

Cassia Occidentalis

Cassia occidentalis (CO) Linn, is an erect herbal plant of Caesalpiniaceae family commonly called in English as Coffee Senna and in Tamil as Ponnavarai. It is commonly grown along the sides of roads and dumped empty wet area of land. Though being commonly viable in occurrence, the medicinal potential of the plant is innumerable. The roots, leaves and seeds are the parts of the plant that have been used by traditional practitioners for various ailments [6].

Cassia occidentalis is rich in phytochemical compounds like anthraquinones, terpenoids, and carotenoids. Reports reveal that the plant is used to promote differentiation of osteoblastic cells and mineralization of the same via pathways activated by PI3K-Akt/MAPKs that caused BMP-2 expression in mouse model of MC3T3-E1 cells. Also, it is suggested that BMP-2 gene promotes anabolic activity for the anthraquinone, emodin by getting triggered in a signaling pathway namely PI3K-Akt/MAPKs-NFkB. These properties of the plant led to the development new dental implant material with leaf extracts of *Cassia occidentalis,* BCP along with casein and chitosan [7].

Materials and Methods

The ethanolic extract of leaves of *Cassia occidentalis* were analyzed for the presence of bioactive compounds by GC-MS, anti-microbial property, anti-inflammatory nature, free radical scavenging property and viability concentration by *in vitro* cell culture studies. Ethanolic extract was preferred as all the bioactive compounds of *Cassia occidentalis* could be effectively extracted as per literature reports. The novel dental implant was prepared using Biphasic calcium phosphate (BCP), Casein (CA), chitosan (CH) and leaf ethanolic extract of *Cassia occidentalis* (Co) and subjecting the implant material for its composition, stability and surface morphology by various physico-chemical techniques namely FTIR analysis, XRD analysis, TGA analysis and SEM analysis.

Preparation of the Plant Extract

The dried leaves of CO were made to fine powder and extract was taken using ethanol (95%) in soxhlet apparatus and concentrated in rotary evaporator. The dried form extract was maintained at 4ºC.

Hydroxyapatite (HAP)

HAP was synthesized by the modified methodical way done by Bouyer et.al (2000) [8]. 0.5 M calcium hydroxide in distilled water was made and 0.3 M ortho phosphoric acid was supplementary added drop by drop to the solution to reach a pH of 12.5. The obtained solution was continuously stirred for half a day. The stirred mixture was centrifuged for fifteen minutes at 6000 rpm. The supernatant was collected, washed again with double distilled water and finally desiccated at 100ºC for around seven hours.

Beta Tricalcium Phosphate (β–TCP)

β-TCP was prepared by the technically modified method performed by Krithiga et. al, (2011) [9] Diammonium hydrogen phosphate (DHP) was prepared by dissolving 25.76 g of DHP in 325 ml of distilled water (double) and calcium nitrate tetrahydrate solution was prepared by dissolving 69.675 g in about 500 ml of distilled water (double). The solutions were added to make a mixture and was stirred continuously. To this known mixture, about 16.5 ml of ammonia solution was poured and again stirred for next 2 hours. The resultant solution was filtered. The obtained filtrate was washed twice with distilled water to keep away the left over calcium and phosphates. The mixture was then maximally dried in an oven at 60˚C for half a day. The obtained fragments were made to a powder and then calcined in the blast furnace at a temperature of 850º C for 12 hours and then cooled. The final creation is a white fleecy remnant called β-TCP.

Casein Glue

2 g of casein was immersed in 3ml distilled water for thirty minutes duration and it was pestle into a paste. To this, 1 g of calcium hydroxide solution in 4 ml of distilled water was joined in drops to see a glue formation.

Dental Implant Making Process

BCP was made by the addition of HA powder to β-TCP powders in a combination of 60:40 [10]. 5 g BCP was finely powdered in a mortar and casein glue, 500mg of ethanolic extract of *Cassia occidentalis* was mixed and stirred. 3 ml of 3% chitosan on further addition with the mixture was made to bring a dough like consistency. This prepared material was allowed via a 1 cm diameter tube made of glass. Now the obtained material is cylindrically shaped named with a combination of BCP-CH-CA-Co graft. The obtained cylindrical grafts were powdered and placed at RT for 3 hours. The grafts were desiccated at 55℃ in oven overnight and then it was carefully sealed in covers preferably polythene and was preserved using ethylene oxide (Fig 1)

Figure 1. Fabrication of Dental Implant

Sterilization of Dental Implant

The dental implant was subjected to standard sterilization. The dried implant was subjected to wet or dry sterilization. Since the implant contains plant extract and biodegradable proteins, a chemical method of sterilization was chosen. Ethylene oxide (ETO) was the standard method of chemical sterilization employed to sterilize medical and pharmaceutical products. Hence, the implant was sterilized by ETO sterilization and then subjected to in vitro studies [11].

Anti-microbial Activity

Sabouraud Dextrose Agar (SDA) and Mueller Hinton Agar (MHA) Preparation

6.5 g medium was added to distilled water (100ml). This was heated and boiled for one minute for complete dissolution. 15.2 g medium was added to of distilled water (400ml) separately. The mixture was purified in an autoclave machine for fifteen minutes and further cooled. Both the medium are poured onto separate sterile petri dishes of uniform depth. The test microorganisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae Escherichia coli, Candida albicans*. The plates were swabbed in eight-hour broth media to be cultured. 10 mm wells were created. 1 mg/ml CO extract was added (100 µl) to wells and made to diffuse in two hours at RT. Control consisted of injections without CO extract. The plates were placed in incubation at a temperature of 37°C for one day with bacteria load and at a temperature of 28°C for a period of 2 days for fungal load. After the prescribed time periods, the zone of inhibition was measured.

Determination of *in vitro* Pharmacological Properties of CO Extract

The *in vitro* total antioxidant activity of CO extract was assessed by phospho-molybdenum technique [12]. The in vitro anti-inflammatory activity was assessed by protein denaturation assay during Bovine serum albumin [13] and HRBC membrane stabilization studies [14].

Characterization of the Dental Implant

The prepared material was characterized using FT IR spectroscopy on a Bruker Alpha II device, the functional groups were identified. To create the pellet for FTIR analysis, 2 mg of sample and 200 mg of (potassium bromide) KBr were compressed under hydraulic pressure. Averaging 100 scans, the spectra was recorded between 400 and 4000 cm^{-1} at a resolution of 2 cm^{-1}. A Bruker D8 Advance X Ray diffractometer operating at 40 kV and 30 mA was utilised to

record X-ray diffraction (XRD) patterns using a Cu K anode (= 0.1542 nm). The diffraction patterns were recorded at 25°C and at an angular range of 20 to 70°C using a step size of 0.05° per step and a dwell time of 12 sec per increment. The International Centre for Diffraction Data's stoichiometric HAP (JCPDS, card number 01 072 1243) was compared to the resulting pattern. Samples were fully dried before being ready for SEM analysis. Samples were sputter coated with platinum for 30 seconds after being placed on stubs using carbon tape. JEOL FE SEM IT800 was used to take SEM pictures. Software in the same instrument was used to record EDS at 20 KV. The samples were prepared and subjected to a 10°C/min heating rate in a nitrogen atmosphere using a Seiko SSC 5200 H thermo gravimetric analyzer. This study documented the implant's primary weight loss as a function of temperature.

Statistical Analysis

The results were analyzed by one-way ANOVA and significance among groups were analyzed by Tukey's multi comparison test. $p<0.05$ was well-thought-out as significant.

Results and Discussion

Gas Chromatography-Mass Spectrometer (GC-MS)

GCMS reveals presence of flavonoids, anthraquinone, terpenoids, carotenoids and saponins. High concentration of polyphenols and emodin imparts the antimicrobial, anti-inflammatory, antioxidant property for CO extract (Fig 2). The content of flavonoids and anthraquinones attribute to the ossification property of the dental implant [15].

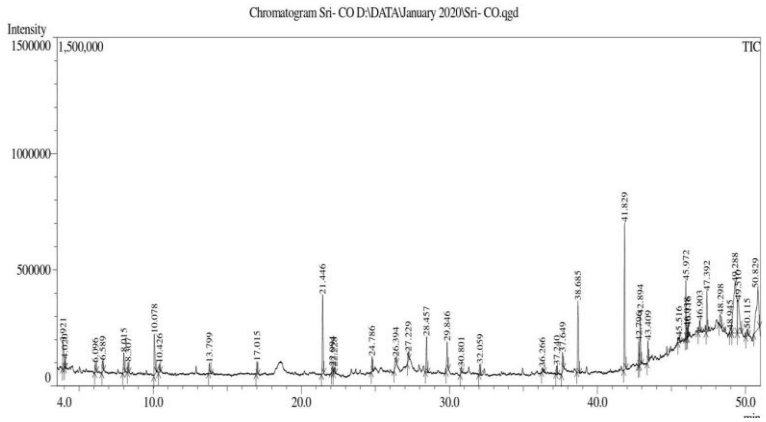

Figure 2. GC-MS Analysis of Cassia Occidentalis

Determination of Total Anti-oxidant Activity

Table 1 and Fig. 3 shows the antioxidant activity of Co extract. Plant-derived antioxidants like tannins, flavonoids, phenolic acids, anthocyanins, lignans, quinones, catechins, etc., are rich in reducing capabilities and they significantly prevent the occurrence of degenerative diseases. This reducing capability of the bioactive compound act as donors of hydrogen or scavengers of hydroxy and superoxide radicals. The CO extract revealed the presence of flavonoids, anthraquinone, terpenoids, carotenoids and saponins. High concentration of polyphenols and emodins in the extract could be the possible reason for Co to exhibit the antioxidant property.

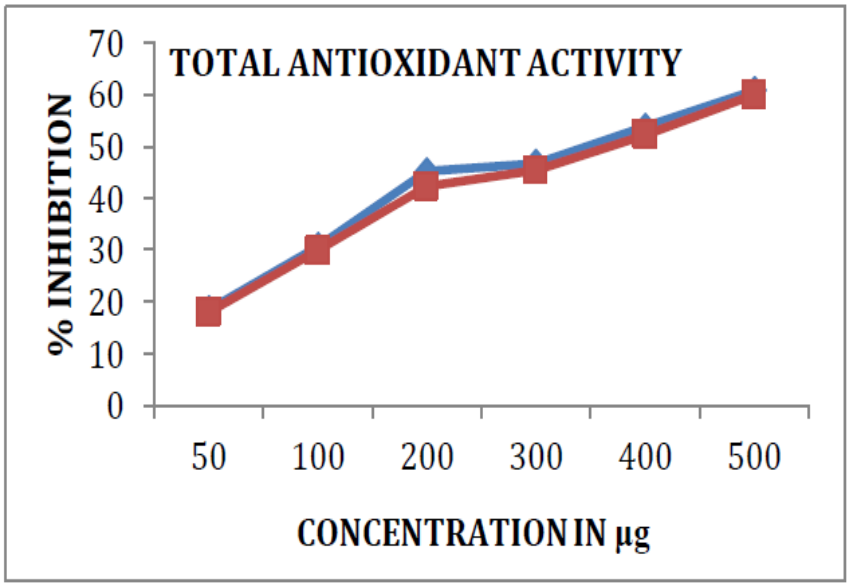

Figure 3. Determination of Total Antioxidant Capacity

Table 1.Total Antioxidant Capacity

Conc (µg/ml)	**Ascorbic acid % Inhibition**	**Cassia occidentalis % Inhibition**
50	18.47±1.08	17.98±1.19
100	30.64±1.62	29.87±1.77
200	45.12±1.94	42.25±2.33
300	46.73±1.37	45.33±1.77
400	53.75±1.82	52.12±0.81
500	60.72±1.09	59.94±0.03
IC $_{50}$ (µg/ml)	284	321

Values are expressed as mean+ Stdev

Table 2. The Antimicrobial Activity of Co Extract Against Various Pathogens

S.NO	**Selected Pathogens**	**Zone of Inhibition in mm**	**Positive Control**
1	*Psudomonas aeruginosa*	30mm	Linezolide 30 mm
2	*Candida albicans*	13mm	Flucoconazole 26 mm
3	*Klebsiella sp*	16mm	Cephalosporin 19 mm
4	*Staphylococus aureus*	NO ZONE FORMED	Piperacillin/tazobactam 28 mm

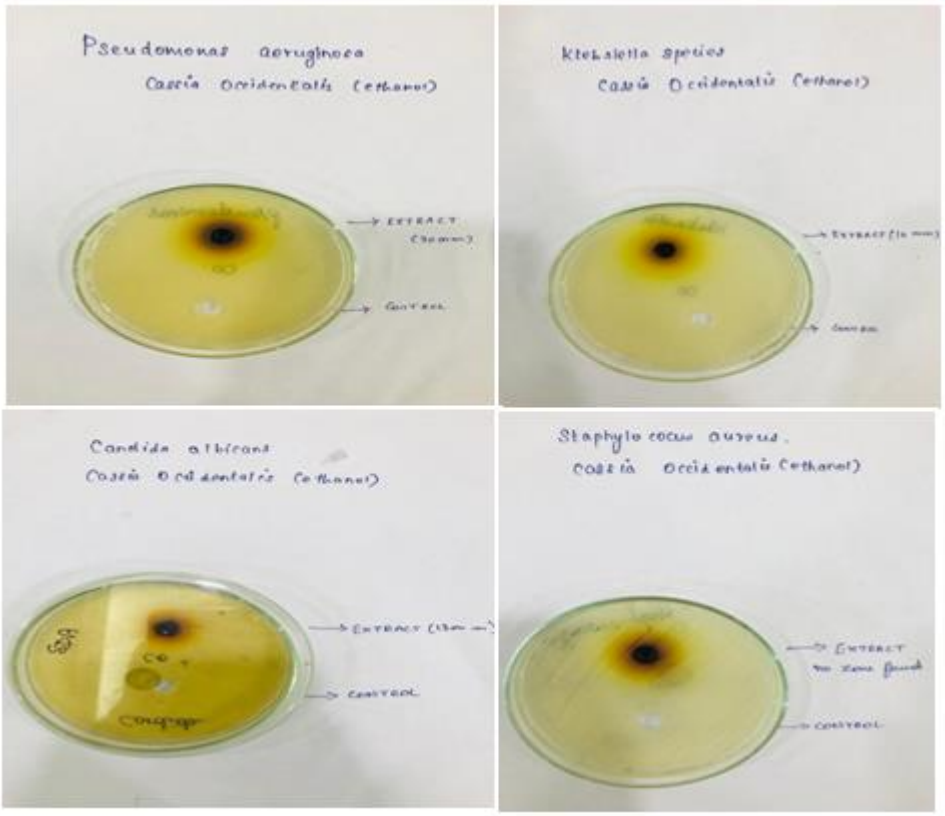

Figure 4. The Antimicrobial Activity of Co Extract Against Various Pathogens

Anti-microbial Assay

Plant derived phytochemicals are generally have lower toxicity and can act as effective therapeutic agents to stop the growth of microbes like fungi or any bacteria or virus [16]. Reports reveal many plant extract materials that contribute much for their anti-microbial effects [17]. The present research work evaluated the antimicrobial effect of leaf ethanolic extract for Co against certain Gram positive as well as negative bacteria, and few fungi that are generally made to consider as major pathogenic organisms for human diseases. This pharmacological susceptibility of the plant extract against the organisms was tested by well diffusion technique. The reports reveal that leaf extract of Co acted effectively on isolated human pathogenic species mainly *Pseudomonas aeruginosa, Klebsiella species and Candida albicans* as shown in table 2 and figure 4 and among all these the extract was highly resistant towards *Pseudomonas aeruginosa*. The antimicrobial property supports the implant in preventing any infection and facilitates bone repair when placed *in vivo* (Table 2 and Fig 4).

Fourier Transform Infrared Spectroscopy (FTIR)

Fig 5 depict that the band from 605 cm^{-1} is due to be allotted to the anti-symmetrical winding motion of ν4 groups of phosphates in the HAP. Band at 962 cm^{-1} representing ν1 phosphate bands of HAP and the band at 1044 cm^{-1} and 1087 cm^{-1} represent ν3 phosphate groups of HAP. The –OH vibrations of HAP were represented by a band at 3435 cm^{-1}. Highlighted peaks at 966 cm^{-1} and 943 cm^{-1} represent ν***1*** phosphate bands for β-TCP. Further the groups at 1117 cm^{-1} and 1040 cm^{-1} represent ν3 phosphate bands for β-TCP. ν4 phosphate band of β-TCP was observed as a peak at 603 cm^{-1}

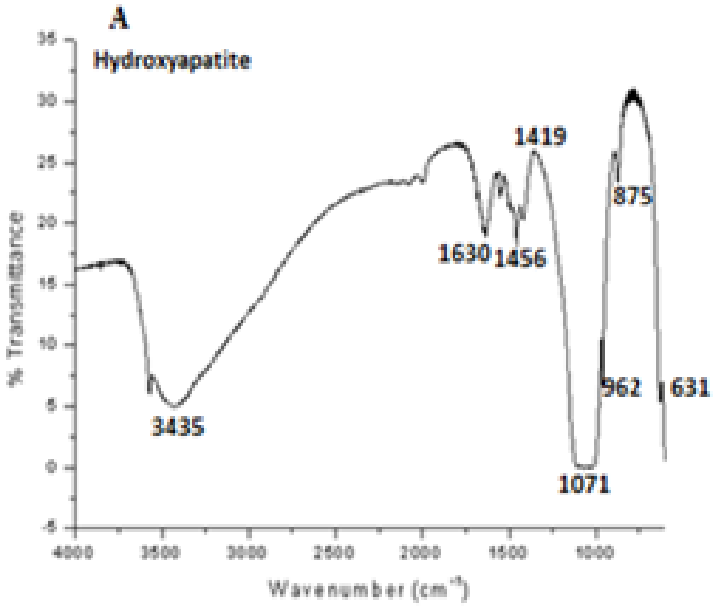

Figure 5. FTIR OF HAP

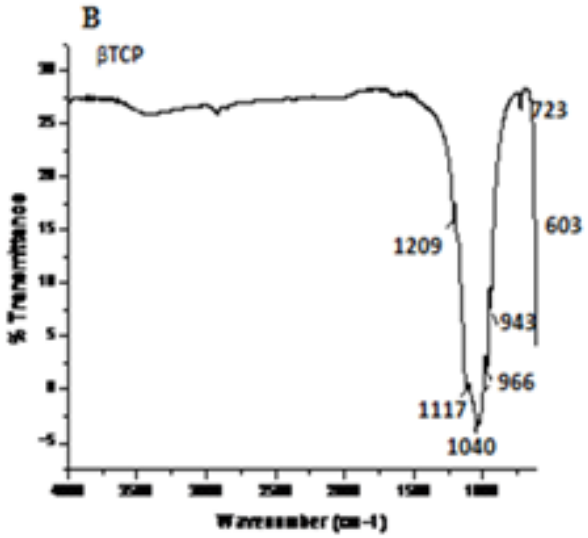

Figure 6. FTIR of β-TCP

The width of band from 603cm^{-1} is attributed to the oppositely symmetric bonding position of groups of phosphates in the structure HAP as shown in Figure 5. ν1 for phosphate depicted that β-TCP on 875.96cm^{-1} whereas the –OH band of hydroxyl moiety was visibly present on 3400-3700 cm^{-1}. FTIR studies showed that the prepared dental implant was showing absorption bands for amide I and II on 1489cm^{-1} and 1643cm^{-1} respectively. The group of hydroxyl moiety of chitosan was visibly present on broad band 1046.31cm^{-1}. Fig 6, 7. The FTIR spectrum of casein depicted the highlighted peak at 3415cm^{-1} for (OH) and 2930cm^{-1} for (C-H) and 1589cm^{-1} for (Hydrogen bond N-H) [15].

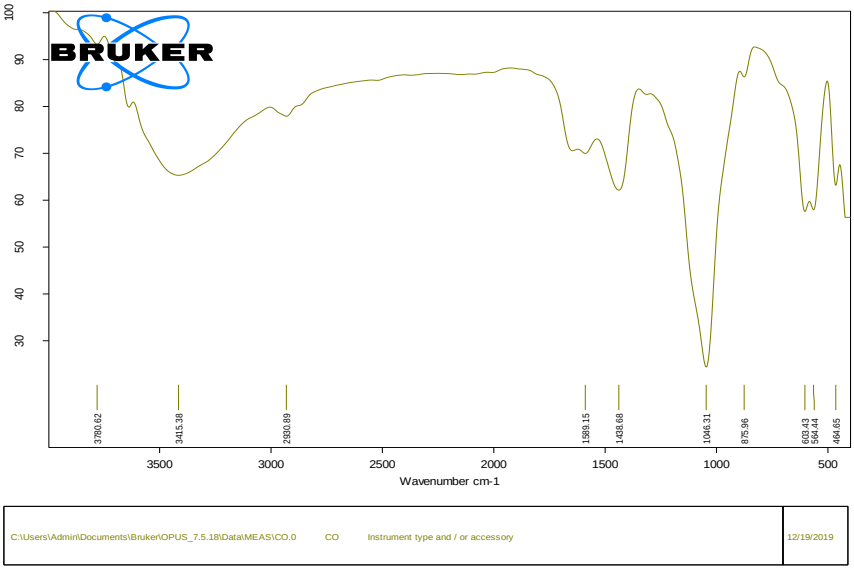

Figure 7. FTIR of BCP-CH-CA-Co implant

X-Ray Diffraction Studies (XRD)

The peaks that reflected at 19.54 deg, 21.26 deg, 26.10 deg and 28.03 deg depicted the echo from planes namely 111, 202, 002, and 210, thus attributing to the occurrence of HAP crystals.

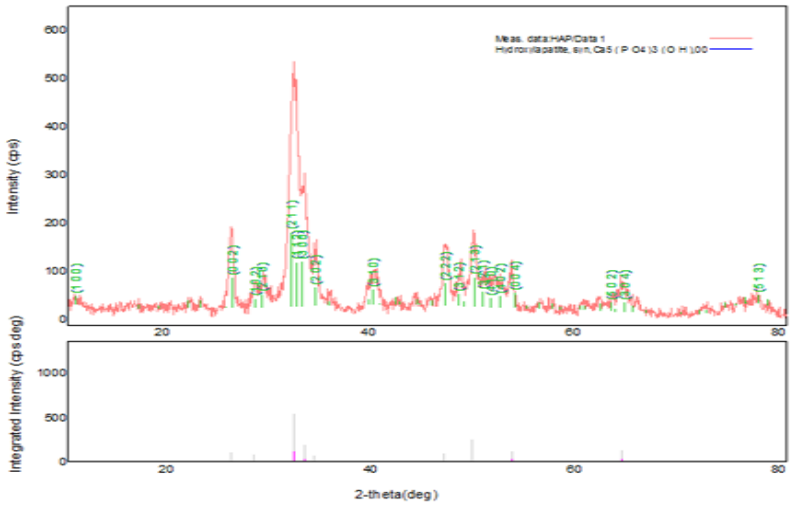

Figure 8. XRD Pattern of HAP

The Fig 8 depicts the XRD image of HAP structure and Fig 9 depicted the XRD image of prepared dental implant BCP-CH-CA-Co. It confirms the presence of Hydroxyapatite and its crystalline nature of the material gradually declines with a higher increase in addition of chitosan. The 2θ values at 24.61º, 26.54º and 29.77º indicates the reflection respectively indicating presence of HAP in the implant. The peak at 31.67º, 33.08º, 48.20º and 51.33º depicted the occurrence of material in crystalline planes namely 222, 112,130 &315 crystal planes respectively depicting the occurrence of the two materials β-TCP and chitosan. 2θ values at 30.48º, 33.08º and 38.57º corresponds to the crystal planes 331, 400 and 511 which reveal the presence of casein in the implant [18].

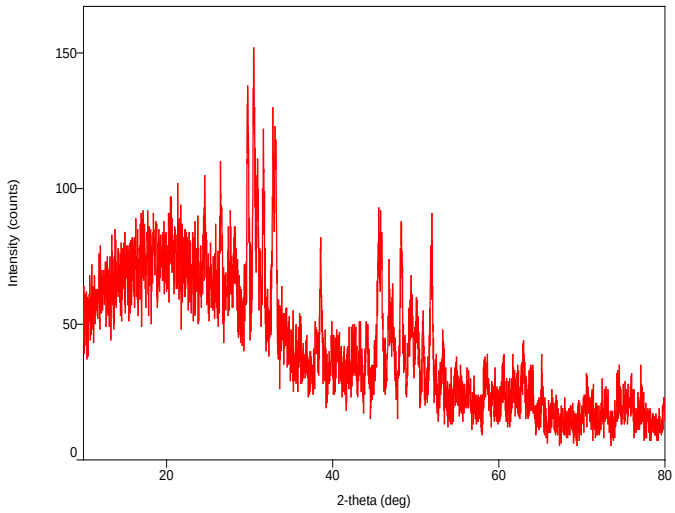

Figure 9. XRD Pattern of BCP-CH-CA-Co

Thermo Gravimeric Analysis (TGA)

The prepared dental implant was exposed to TGA analytical techniques and the results depict as in figure 10. A descent in gradient loss were detected in between 30.06 ˚C and 241.76 ˚C. This was attributed to dehydrative action on the implant. The second descent in gradient loss were detected in between 248.18˚C and 585.11˚C and this was attributed to the putrefactive effect of chitosan and casein. A final residue of 11.646 mg was achieved due to the presence of inorganic content namely calcium and phosphorous of dental implant which imparts thermal stability to the prepared implant [6].

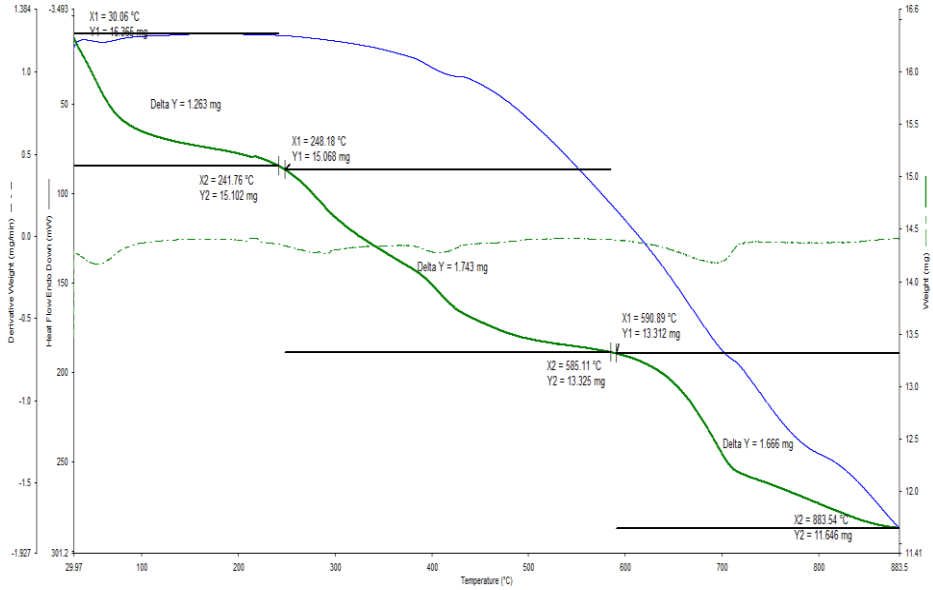

Figure 10. TGA of the Dental Implant BCP-CH-CA-Co

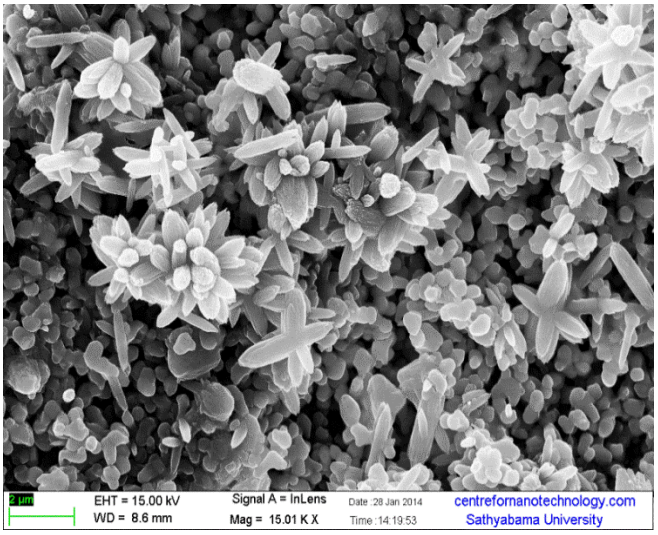

Figure 11. SEM Image of HAP which Appears as Rod Shaped Crystals and Arranged as Flower Like Patterns.

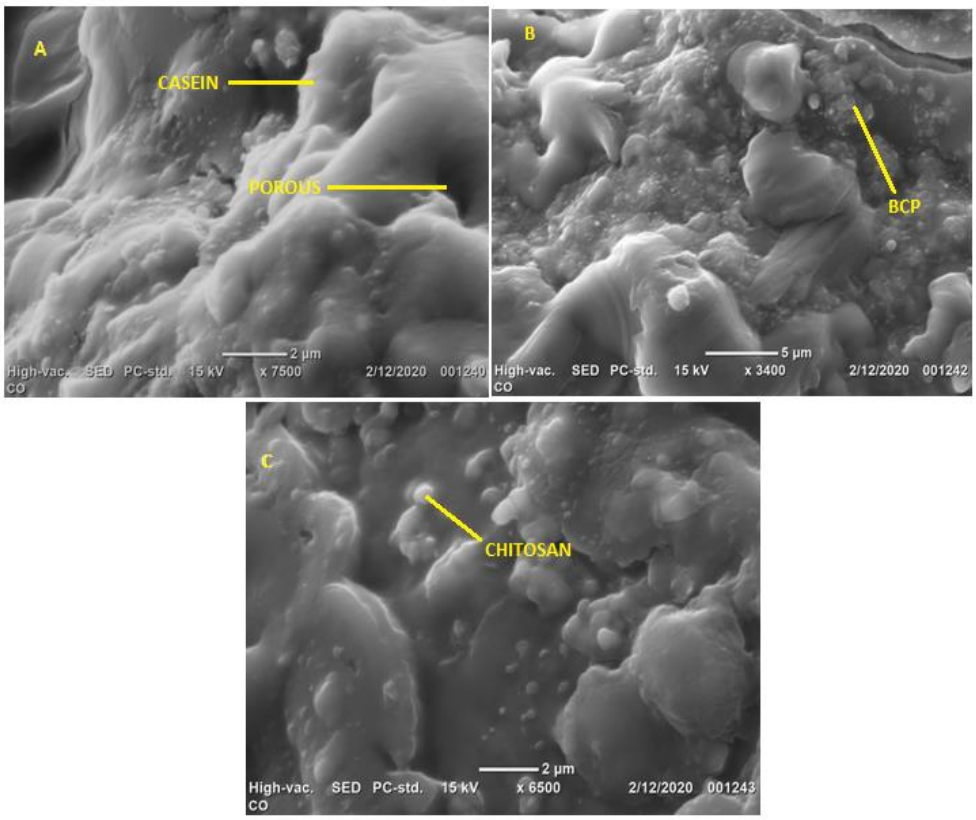

Figure 12. (A) SEM Image of the Implant Showing Porosity (B) SEM Image Showing BCP(C) SEM Image Showing Chitosan Impregnated with Casein

SEM Analysis

Figure 12 shows the SEM image of the implant BCP-CH-CA-Co in which figure (A) clearly shows the presence of casein and the porous nature of the implant. The porosity of the implant facilitates the diffusion of the Co extract from the implant into the bone defect area (implant site) [17]. Figure (B) shows the BCP impregnated in the casein-chitosan matrix which stays in the implant site and acts as an osteo-inductive and osteo-conductive material. Figure (C) clearly shows the chitosan and case in matrix in which BCP is impregnated along with the extract.

Thus, incorporation of a herbal or a nano composite with a dental implant has proved its

efficacy in a better way. Previous work with biological incorporation of silver and iron nanoparticles with clove extracts proved a better antibacterial activity with good characterizations [19-21].

Periodontitis is an inflammatory condition that affects the periodontal tissues and has multiple causes. Loss of support from the alveolar bone is one of the many telltale indications of periodontal disease, and it typically indicates the anatomical consequences of the disease's apical advancement. Periodontitis causes bone loss, which can happen alone or in a variety of combination forms. It is clinically difficult to identify these osseous flaws after surgically exposing the bone, and it is crucial for a clinician to comprehend these defects and classify them accurately in order to develop more effective therapy strategies. Therefore It is important that a clinician have a thorough understanding of all these periodontal osseous abnormalities that are linked to periodontal disease, and so that a proper dental implant material could be designed towards it [22].

The most important factor contributing to the eventual loss of teeth due to periodontal attachment loss is most likely bone resorption. On radiographs, the condition is identified by signs of "bone loss" surrounding the tooth. The cemento-enamel junction (CEJ) of that particular tooth is typically located between 0.4 and 1.97 mm apically from the crest of the alveolar bone. Periodontitis causes osseous defects, or bone loss, which can occur alone or in many combination forms. Since the osseous procedures are predicated on this diagnosis, it is clinically difficult to identify these osseous anomalies on postoperative bone exposure. During implant making, metals are usually selected based on a factor involved with its characteristics and properties. Its machining, surface-finishing, and biomechanical traits include these properties. In the dental implant industry, metals like Co-Cr, stainless steel, and gold are no longer used. The dental metals that are currently accessible are zirconium and titanium. However, some dental implant components, including as the abutment, abutment screws, and different attachments, are composed of stainless steel, gold alloys, and co-cr alloys [23, 24].

Titanium is the preferred material for intraosseous applications because it possesses common qualities such high passivity and chemical dosage resistance. It can also heal itself if it is damaged. Its 116 GPa modulus of elasticity is consistent with that of titanium oxide and bone. Therefore, during the implant-making process, the manufacture of a composite film using basic materials to create scaffolds and insert herbal supplements increases the strength and osteogenic property of the implant. A better matrix material for cell adhesion in the tissue engineering process was found after numerous described investigations were conducted on the variously manufactured herbal implant materials [25, 26].

A novel implanting material was developed by Chin with an incorporation of nanoparticles in a magnetic fibrin and was characterized by physicochemical characteristics using Saos 2 cells, the cell viability, adhesion, and alkaline phosphatase assay. This material was very cost effective and possesses good osteogenic property and could be a better product for bone tissue engineering [27, 28].

Another bone substitute with the bark extracts *Terminalia arjuna* were were collected and added with BCP, casein gel, and cast into cylindrical bone grafts The grafts were submerged in SBF for 21 days before being subjected to standard methods of analysis. These prepared grafts were subjected to in vitro cell studies to analyze for its ossification property. Another bone graft implant was designed using BCP, casein that is biocompatible along with fresh extracts of *Myristica fragans*. The developed graft was observed for in vitro bioactive nature and also subjected to in vitro cell analysis. The outcomes showed that apatite deposition

occurred on the graft upon immersion in SBF and that MG-63, NIH-3 T3, and Saos 2 cell lines all significantly increased ALP activity. According to this study, adding plant extract to the transplant improves its ability to promote bone growth [29].

Another report investigated the efficacy of *Cassia occidentalis* extract along with the incorporation of biphasic calcium phosphate (BCP) that acts as an osteoinductive material. FTIR, TGA, XRD, SEM, and EDX were used to physically and chemically analyse the produced bone implants. The mechanical and biological characteristics of the implants were examined in more detail. According to the findings, bone implants made with *Cassia occidentalis* extract had greater compression strength and were able to stimulate SaOS-2 cell proliferation and alkaline phosphatase activity. This implant proved to be a promising tool for bone tissue engineering, and hence it demands further in vivo evaluation [30]. Hence, the present study used the same extract of *Cassia occidentalis* along with the incorporation of Biphasic calcium phosphate (BCP), Casein (CA), chitosan (CH) and leaf ethanolic extract of *Cassia occidentalis* (Co)

In present days, plant extracts were used in the green synthesis processes had received more and more attention for the development of metal nanoparticles. The term "green synthesis" refers to a variety of synthetic methods that produce nanomaterials using largely benign chemicals. Examples of such methods include the use of nontoxic solvents like water, biological extracts, and microwave assisted synthesis. A study synthesized an implant material using silver and copper nanoparticles along with leaf extract of *Cassia occidentalis* and the material was analyzed by antibacterial, antioxidant activity and characterized by many spectroscopic techniques like UV-Vis, SEM- EDX, TEM, and XRD measurements and toxicity assays. CuNPs were more effective against S. typhi, while the biocapped AgNPs shown better antibacterial activity against E. coli. Compared to AgNPs, CuNPs had greater scavenging activity. The reduction of 4-NP and 2-NP can be accomplished more efficiently by the CuNPs than the AgNPs [31].

Another report suggested that antibacterial preparation of iron oxide nanoparticles are capable of producing crystalline γ-Fe2O3 nanoparticles. This is prepared in a combination of *Achillea millefolium, Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna,* and *Tamarix gallica.* Incorporation of iron oxide nanoparticles along with the herbal composites act as effective antibacterial agents to kill the bacteria and inhibit the growth of a bacteria and fungus, mainly in the formation of a biofilm [32].

Conclusion

The present study showed that the novel dental implant (BCP-CH-CA-Co) is more biocompatible and promotes bone growth compared to a commercial implant. The various characterizations, namely FTIR, XRD, TGA and SEM, revealed the composites present in the material possess a stable effect and porous in nature. Moreover, bioactive compounds of *Cassia occidentalis* which was revealed by GC-MS analysis, in the implant provides various properties to the implant such as ossification, anti-microbial, antioxidant and anti-inflammatory due to the presence of phytoconstituents chemicals namely alkaloids, flavonoids, triterpenoids, phenols, steroids and saponins. This novel combination enhances the osteoinductive and osteoconductive property of the implant with no side effects and thereby, the implant can be used in repairing periodontal bone defects and also it can be used in other biomedical applications.

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Conflicts of Interest

The authors declare no conflict of interest.

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