International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 02, 2020
ISSN: 1475-7192

Synthesis and Characterization of Natural Hydroxyapatite (Hap) from Black Sumatraand Gallus Domesticus Bone for Orthopedic Applications

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Abstract--*Biomaterial substances are widely used in the biological system. Different physical, chemical and natural methods are used to biomaterials are synthesized. In This present work focused on Biomaterials was selected from different two rare species of chicken (Black Sumatra and Gallus Domesticus) bone. Thermal calcinations method used to the natural Hydroxyapatite (HAP) was isolated from the Black Sumatra and Gallus Domesticus chicken bones. The isolated Hydroxyapatite (HAP) has been characterized using X-Ray Diffraction analysis (XRD), Fourier Transformed Infrared (FTIR), Scanning Electron microscope (SEM).After characterization this material is applicable for Bone cement, Bone implantation, Bone stabilization, Bone injury.*

Key words-- *Black Sumatra and Gallus Domesticus, Scanning Electron microscope (SEM), Fourier Transformed Infrared (FTIR).*

I. INTRODUCTION

Natural synthesized biomaterials are widely used in medicinal field [1]. Different types of physical chemical methods are used to the materials are synthesized [2, 3]. Nowadays natural biocompatible techniques are applied particularly in orthopedic application areas [4,5]. Basically natural human bone contains 70% inorganic materials, mineral 20% and organic material 10% water [6]. This organic material contains Hydroxyapatite (HAP) and Calcium Phosphate in many forms [7]. Here mainly Hydroxyapatite (HAP) in vital role in bio compatible, drug delivery, orthopedics, dentistry, bone tissue engineering.HAP was mainly present in the bones [8, 9].

HAP was synthesis from thermal calcinations method and hydrothermal method [10]. In our research work is carry on natural Hydroxyapatite (HAP) was isolated from the very rare and different chicken species [11]. One is *Black Sumatra* this chicken country of origin was Indonesia and another was in *Gallus Domesticus* this chicken found in India[12].These two chicken species bones contain so many biological and Therapeutic applications in the growth of modern research era[13]. Our research work is mainly focused on these chicken bones waste was further used and recycles [14]. It is applied in sustainable development for cheap and low cost [15].

The present study focus on *Black Sumatra* and *Gallus Domesticus* chicken bones are collected and natural Hydroxyapatite (HAP) was isolated. Further the bones are characterization studied and applied in orthopedic.

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DOI: 10.37200/IJPR/V24I2/PR200441
Received: 20 Dec 2019 | Revised: 05 Jan 2020 | Accepted: 18 Jan 2020

II. MATERIAL AND METHODS

Bone sample collection

Black Sumatra and Gallus Domesticus these two rare species of chicken are obtained from Thootukudi district.

Flesh removal

First *Black Sumatra* and *Gallus Domesticus* chicken bone flesh and meat will remove. (NaOH-0.5N-48 hours) Solution used to the bone and flesh are removed. NaOH remove oil content from chicken bone without any corrosion. *Black Sumatra and Gallus Domesticus* need 48 hour to remove flesh because of muscle hardness.

Buried method

It is an effective method used to chicken bones is removed within six to seven days. In this technique the bone contain organic materials and bone marrows cartilage of the bone corner then microorganisms everything decomposed easily.

Extraction of HPA

Flesh removal method used to the chicken bones contain moistures are washed and bones are cut into small pieces. Muffle furnace used the (150g+150g chicken bone) at different temperature 200 ◦C to 1000 ◦C heated within 20 hours. Every 100° C cooling needed. And constant heating rate is 10°C/min in the muffle furnace. And record the changes. Normally chicken bones are colour changes in 200° C in black colour. *But Black Sumatra* and *Gallus Domesticus* chicken bones are heated 1000° C.This bones are look like tube and brittle nature material and its pure white. No colour changes are variations in these bone material. That indicates the complete calcination of biomaterials due to the annealing process.

Grinding Process

The ball milling and mortar and pestle used to the chicken bone final products got fine powder and eventually perform the characterization process such as X-ray Powder Diffraction (XRD), Fourier transform infrared (FTIR),Scanning Electron Microscope (SEM) for both *Black Sumatra and Gallus Domesticus* bone samples.

III. CHARACTERIZATION STUDY

X-ray Powder Diffraction (XRD)

The *Black Sumatra and Gallus Domesticus* bone samples were characterized by X-ray powder diffraction (XRD) [Philips X 'Pert MPD] at 40 kV and 40 mA. The angular range was set at 5 ° to 75 ° at steps of 0.05 ° and counting time was 3 seconds per step.

FTIR analysis

Fourier transform infrared **(**Bruker, Alpha T, Germany) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum. Dried powder of *Black Sumatra and Gallus Domesticus* bone samples were subjected to FT-IR instrumental analysis. The dried powder of bone sample (150g) was mixed with 100 mg of KBr to prepare translucent sample pellets. The pellet discs were evaluated by KBr pellet mode of FTIR spectroscopy.

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SEM

The samples were analyzed by Scanning Electron Microscope (SEM, model JSM-7000F, JEOL Ltd.). SEM was linked to an EDS/INCA 350 (Energy dispersive X-ray analyzer, Oxford Instruments Ltd.). Samples were not coated with a conductive layer. Analysis was done at low accelerating voltage.

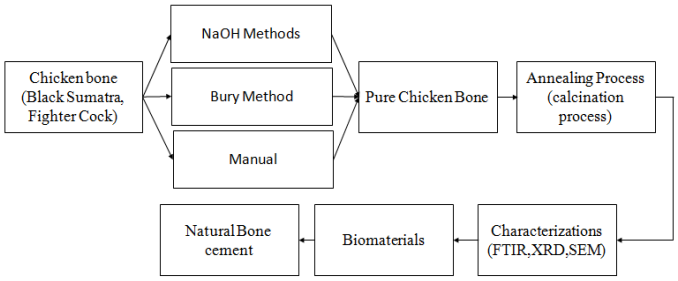

Fig 1: Block diagram of methodology

IV. RESULTS

Bone sample collection

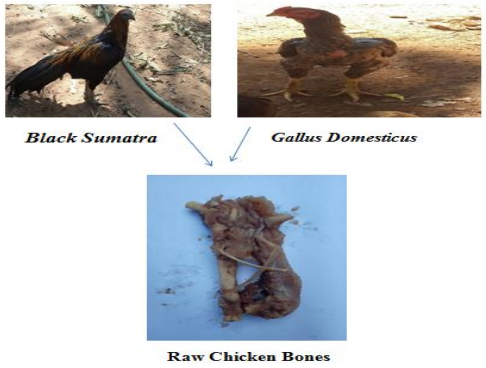

Fig 2: Chicken bones collected from *Black Sumatra* and *Gallus Domesticus*

Flesh removal

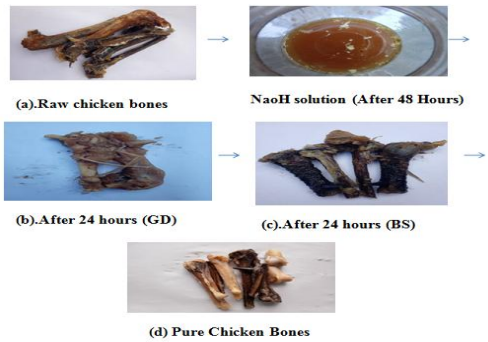

Fig 3: Pure Bone without the Flesh

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Extraction of Hydroxyapatite (HAP)

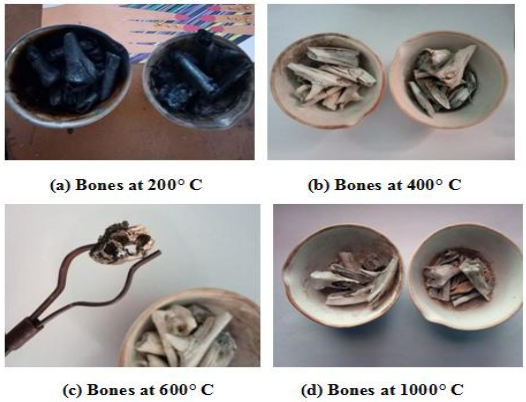


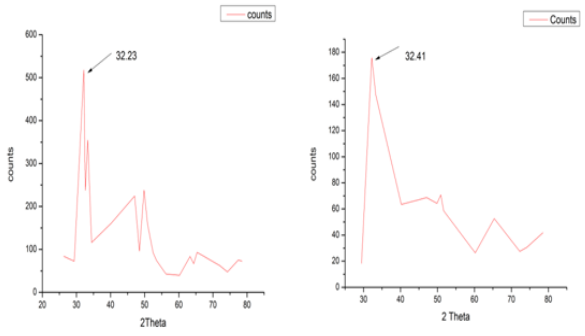
Fig 4: Calcined in a muffle furnace at different temperatures (200, 400, 600, 1000° C)

Grinding Process

Fig 5: *Black Sumatra* and *Gallus Domesticus* Bone powder

V. CHARACTERIZATION STUDY

X-ray Powder Diffraction (XRD)



(a) Black Sumatra (b) Gallus Domesticus

Fig 6: The XRD pattern of *Black Sumatra* and *Gallus Domesticus* Bone broad reflection seen at 2 theta =32.23 and 32.41 shows natural Hydroxyapatite (HAP) was present in the chicken bone sample.

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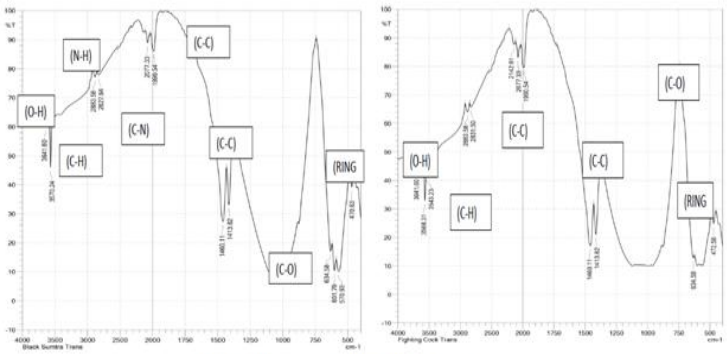
FTIR analysis



(a) Black Sumatra (b) Gallus Domesticus

Fig 7: FTIR spectrum of *Black Sumatra* and *Gallus Domesticus*Bone

Table 1: Characteristic peaks in FTIR spectrum of *Black Sumatra* chicken Bone

Peak values Frequency, cm--1	**Bond**	**Functional groups**
3570	C-H	Aliphatic
3641	O-H	Oxygen
2883	N-H	Nitrogen
2077	C-N	Carbonyl
1460	C-C	Carbonyl
1000	C-O	Carbonyl
470	Ring	Mono

Table 2: Characteristic peaks in FTIR spectrum of *Gallus Domesticus* chicken Bone

Peak values Frequency, cm--1	**Bond**	**Functional groups**
3568	O-H	Oxygen
2883	C-H	Aliphatic
2142	C-C	Carbonyl
2077	C-N	Carbonyl
1460	C-C	Carbonyl
750	C-O	Carbonyl
472	Ring	Mono

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Scanning Electron Microscope (SEM) analysis

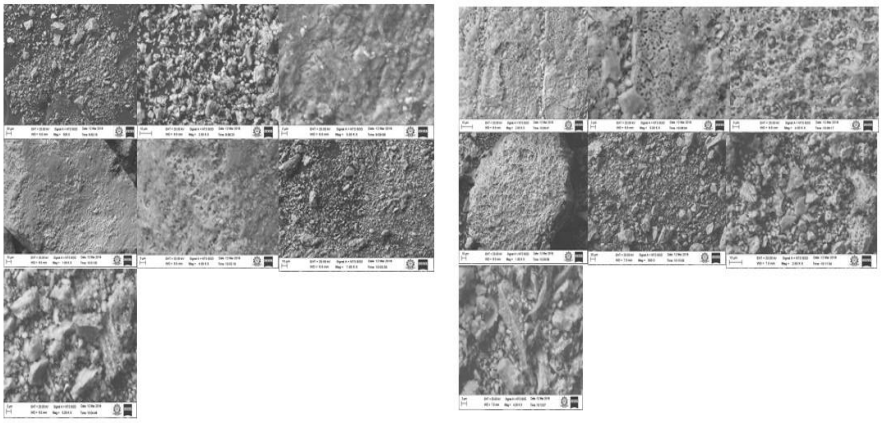

Fig 8: Microstructure (20 µm, 10µm, 3µm, 2 µm) of *Black Sumatra* and *Gallus Domesticus* bone sample in SEM analysis.

VI. CONCLUSION

Black Sumatra and *Gallus Domesticus* chicken bone samples are collected and natural Hydroxyapatite (HAP) was isolated Thermal calcination method. Buried technique used to unwanted flesh waste is washed in the samples. Muffle furnace used to HAP was isolated different temperature. Then grinding method ball milling used to final product got fine powder. The characterization analysis of *Black Sumatra* and *Gallus Domesticus* chicken bones are comparative analysis in XRD, FTIR and SEM. The XRD broad reflection seen at (2Theta= 32.41) shows natural Hydroxyapatite (HAP) was present in *Gallus Domesticus.* FTIR analysis also shows that *Gallus Domesticus* peaks value frequency (2142cm-1-C-C-Carboxyl groups) are mostly present in the bone sample.

The SEM analysis shows that the microstructure of *Gallus Domesticus contain (*20 µm-width7.0mm) high dense due to presence of organic substances natural Hydroxyapatite (HAP) associated with chicken bone sample numerous pores and few small inter granular or intra granular pores agglutinating and present in the spherical shape in the *Gallus Domesticus* bone. The characterization analysis of *Black Sumatra* and *Gallus Domesticus* these two chicken compared *Gallus Domesticus contain* more natural Hydroxyapatite (HAP) and further analysis for bone cement and orthopedic application.

Future work

Next step on words in this research work will carry on orthopedic nanocomposites biomaterial synthesis and applied in medicinal and therapeutic fields.

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Acknowledgement

This research work was supported by Manonmaniam Sundaranar University-Department of physics, (Tirunelveli), Kalasalingam Academy of Research And Education, (Srivilliputhur), As a part of this work will completed supported the biotech lab Xcellogen and Nanodot Pvt Ltd-(Nagercoil).

REFERENCE

1. Balamurugan A, Michel M, Faure J, Benhayoune H, Wortham L, Sockalingum G et al. (2006) Synthesis and structural analysis of sol gel derived stoichiometric monophasic hydroxyapatite. CeramSilikaty;50 : pp27-31.
2. Elkayra A, Elshazly Y, Assaad M (2009).Properties of hydroxyapatite from bovine teeth. Bone Tissue Regene Insights;2 :pp31-36.
3. Dominic Ravichandran and A. Hariharasubramanian, (2012)"CHICKENBONE AS A BIORESOURCE FOR THE BIOCERAMIC" journals of biomaterial related elements. DOI:10.1080/10426507.2011.650806.p917-935.
4. A. Harisubramanian and N. Senthilkumar (2012)"A Biocompatible And Load Bearing Composite Of Multi Walled Carbon Nanotubes Chitosan And Natural Hydroxyapatite Derived From The Chicken Bones Wasted In The Slaughter Houses" International journals of Pharmacy and pharmaceutical science. pp 345-348.
5. Hiteshkumar, D. Tailor, and Wasim S. Khan(2010)" The Use Of Growth Factors And Mesenchymal Stem Cells In Orthopaedics "Journal of Orthopaedics. pp 7-12.
6. IdrisAbdulrahman and Sulaiman Mohammad (2012)"From Garbage To Biomaterials: An Overview On Egg Shell Based Hydroxyapatite" Journals of biomaterials.pp 223-228.
7. Jaafari K, Ruiz T, Elmaleh S, Coma J, Benkhouja K. (2004) Simulation of a fixed bed adsorber packed with protonated cross-linked chitosan gel beads to remove nitrate from contaminated water. ChemEngJ ;99 :pp153-160.
8. JeyachandranVenkatesan and Se Kwon Kim (2010) "Effect Of Temperature On Isolation And Characterization Of Hydroxyapatite From Tuna (ThunnusObesus) Bone" Journals of Bio-medical Engineering. pp4471-44.78.
9. Kulkarni PV, Keshavayya J, Baek SH, Kim B, Suh KD.(2010) Chitosansodium alginate biodegradable interpenetrating polymer network (IPN) beads for delivery ofofloxacin hydrochloride. Int J Pharm PharmSci ;2: pp77-82.
10. Liu YL, Chen WH, Chang YH.(2009). Preparation and properties of chitosan/carbon nanotube nanocomposites using poly(styrene sulfonic acid)-modified CNTs. CarbohydrPolym ;p76
11. Lombardi M, Palmero P, Haberko K, Pyda W. Gelcast (2010)Components having controlled porosity features obtained from a natural hydroxyapatite powder. Ceram Materials ;62 : pp342-348.
12. Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN. (2007).Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. Biomaterials;22 : pp165-173.
13. Ooi CY, Hamdi M, Ramesh S.(2007) Properties of hydroxyapatite produced annealing of bovine bone. Ceram Int;33 :11711177. pp 342-356.
14. Sambarkar PP, Patwekar SL, DudhgaonkarBM.(2012) Polymer nanocomposites: An overview. Int J Pharm Pharm Sci;4 :pp60-65.
15. Tang C, Chen N, Zhang Q, Wang K, Fu Q, Zhang X. (2009) Preparation and properties of chitosan nanocomposites with nanofillers of different dimensions. PolymDegrad Stab;94 : pp124-131.

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