# Persian Gulf Corals: A New Hydroxyapatite Bioceramics in Medicine

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## ABSTRACT

Background: Marine Organism such as coral is known as unique natural hydroxyapatite (HA) resource for preparation of bioceramic. Ceramic based biomaterials comprise calcium phosphate ceramics, bioactive glasses and glass ceramics. They are characterized by their bioactivity and unique bone bonding properties, which are usually related to their surface chemistry. The use of bio-structures and bioceramics derived from the marine environment for their application as biomaterials is very recent.

Objectives: The purpose of this research was to apply Persian Gulf calcined corals for production of hydroxyapatite bioceramic.

Materials and methods: Marine corals (Goniopora sp. and Stylophora sp.) were crushed in a mill and cleaned with NaClO, solution of KOH and then dried at 100°C before use. Calcined coral samples were produced by addition of phosphoric acid, di-ammonium hydrogen phosphate, and finally calcination program at 800-1200 °C for 4 hours in air in Muffle furnace were carried out for the effective synthesis of Hydroxyapatite bioceramics (HA) respectively. At the end of the experiments, the microstructures of the crystals were monitored by X-ray diffraction (XRD), scanning electron microscopy (SEM) and the composition was investigated using infrared spectroscopy (FTIR).

Results and Discussions: The FTIR of hydroxyapatite shows absorption bands at 562, 597, 962, 1032 and 1095 $cm^{-1}$ corresponding to the $PO_4^{3-}$ ions of the hydroxyapatite. The XRD pattern of converted hydroxyapatite demonstrated crystallinity. The XRD pattern of the samples obtained by thermal transformation at 800-1200 °C show the diffraction pattern belonging to Hydroxyapatite, highest intensity peak at 32, 37, 53° corresponding to crystalline and other highest peak intensity is 28° crystals suitable for $Ca_{10}(PO_4)_6(OH)_2$. SEM images show the formation of HA microstructures in the thermal process can be attributed to the tendency of particles to crystallize and agglomerate at high temperatures. The particles had irregular shapes, including small spheres, agglomerated together in some parts.

Conclusions: Present study has demonstrated that under a controlled condition exchange, strong and porous coralline hydroxyapatite bioceramic from Persian Gulf coral can be produced at relatively low pressure. The hydroxyapatite obtained retains the structure and strength of coral.

**Key Words:** Marine coral, Goniopora sp., Stylophora sp., Persian Gulf, Hydroxyapatite, Bioceramic, Calcined.

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## INTRODUCTION

Marine organisms such as eggshells, sea urchins, sponge, coral, nacre, mussel and land snails in addition to being a great help to have a healthy environment can be applied as natural resources in medicine, in pharmaceutical production, and biological compounds. Biological natural

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compounds from these marine organisms have been used to synthesize bioceramics due to their chemical composition and their unique architectural structure (1-5). Ceramic based biomaterials comprise calcium phosphate ceramics, bioactive glasses and glass ceramics. They are characterized by their bioactivity and unique bone bonding properties, which are usually related to their surface chemistry. The use of bio-structures and bioceramics derived from the marine environment for their application as biomaterials is very recent. For instance, several authors have proposed in the last years, the use of different marine species like coral skeletons, sea urchins and sponges as three dimensional biomatrices [6-9].

Coral consists mostly of calcium carbonate in the form of aragonite crystals ($CaCO_3$) which can easily be converted to calcium phosphate bioceramics. It has been reported that, coral shows cell ingrowth and bone formation similar to the host bone tissue.

Hydroxyapatite, $Ca_{10}(PO4)_6(OH)_2$ or (HA) is a calcium phosphate material found in mammals hard tissues, teeth and bones. Because of the biological implications, the interface between metallic and organic components with the hydroxyapatite crystals has been a subject of great research interest (10).

Coral reefs are also interesting marine organism to study the phosphate-carbonate exchange and the preferential growth directed by chemical and morphological composition. In this way, HA can be suitably prepared from natural carbonate-phosphate compounds of coral for applications in biomedicine and in technology.

**Objectives:**

In continuation of our research program concerning the development of marine natural compounds, we report the conversion of calcium carbonate-phosphate present in a Persian Gulf corals Goniopora djiboutiensis Vaughan and Stylophora pistillata Esper, into hydroxyapatite bioceramics, by calcination at 800-1200 $^{oC}$. At the end of the experiments, the microstructures of the crystals were monitored by X-ray diffraction (XRD), scanning electron microscopy (SEM) and the composition was investigated using infrared spectroscopy (FTIR).

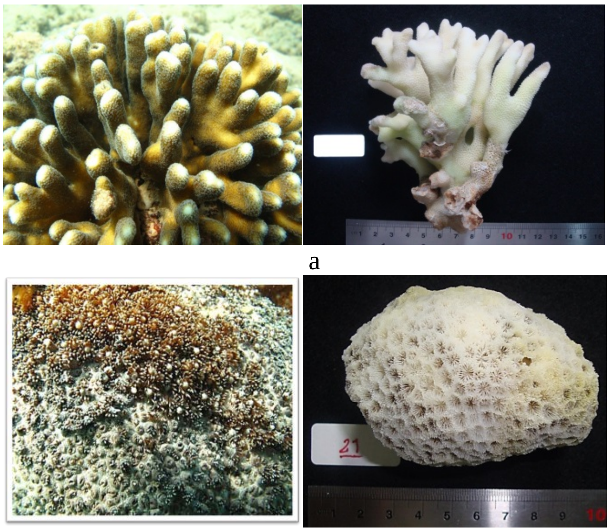
## MATERIALS AND METHODS

All starting materials were purchased from Merck and Aldrich Companies. The IR spectra were recorded on a Perkin-Elmer RXI infrared spectrometer. The X-Ray Diffraction was recorded on ID3003 SIEFRET model. The SEM pictures were taken with a Zeiss Supra 55VP SEM with RAITH E-beam Lithography System & EBSD for the secondary electron imaging (SEI) the energy was kept at 20 kV, working distance and magnification were varied to obtain best possible pictures. The samples were emulsified by dispersing of hydroxyapatite bioceramic solution in ultrasonic bath for 4h and were dropped on aluminum foil and dried on air.

3.1. Preparation of marine coral hydroxyapatite

3.1.1. Preparation of coral powder

In this study marine corals were collected from the Larak island of Persian Gulf, Iran (North coast of Persian Gulf, 26˚53΄14.92″N, 56˚23΄43.11″E), in October 2013 and were washed several times using tap water. The coral samples were crushed in a mill and cleaned with 2% (v/v) NaClO. For removing the organic compounds, coral powder was maintain in 5% solution of KOH for 18 hours and then dried at 100˚C for 1 hour before use. The dried corals were ground to give fine powder. Identification of corals, Goniopora djiboutiensis Vaughan 1907 and Stylophora pistillata Esper 1797 as scientific names, was carried out kindly by Khoramshahr marine science and Technology University. The pictures of Persian Gulf corals were shown in figure 1.


a

b

**Figure 1.** The pictures of Persian Gulf corals: a) Goniopora sp. and b) Stylophora sp.

3.1.2. Addition of phosphoric acid

100 ml of 1 N $H_3PO_4$ was added, drop by drop, to 3 g of coral powder suspended in 50 ml of distilled water at room temperature with magnetic stirrer for 10 hour and finally filtered.

3.3.2. Preparation of calcined coral/$H_3PO_4$

Calcined coral and $H_3PO_4$ was obtained heating the sample in an oven at 800 ºC for 2 hours, 1100 ºC for 1 hour and 1200 ºC for 1 hour subsequently. Then it was washed with water and dried in an oven at 100 ºC for 1 hour.

3.3.3. Addition of $(NH_4)_2HPO_4$

3 g of Di-ammonium hydrogen phosphate was added to 3 g of calcined coral/ $H_3PO_4$ powder suspended in 50 ml of distilled water at room temperature with magnetic stirrer for 10 hour, filtered and finally calcined in an oven at 800 ºC for 1 hour. The samples were washed twice with distilled water and separated by centrifuge. The samples were then dried in an oven at 80 ˚C for 24 hours for further analysis.

3.2. Characterization

The raw materials and products were characterized before and after hydrothermal conversion. Characterization of powders was studied by FTIR, SEM and XRD.

Fourier-Transform Infra-Red (FTIR) analysis was conducted using Bruker Vector 33 FT-IR spectrometer. KBr was used for preparing of pellet shaped samples and tests were performed according to ASTM 1252. SEM micrographs were prepared by using Cam Scan MV2300 SEM (with 15 kV accelerating voltage). Phase analysis of the products were carried out by X-ray powder diffraction using Bruker d8 X-ray Diffractometer employing CuKα radiation (λ=0.15418 nm). The diffractometer was operated at 40 kV, 0.02 step size from 20 – 70° with step time 0.5 s. The powder samples were loaded into a holder and flattened before analysis. The phase quantification was done using Xpowder 12 software on full profile quantitative analysis of components using 'Dirac' patterns and convolution and PIR scale factor.

## RESULTS AND DISCUSSIONS

Two samples of corals, Goniopora sp. and Stylophora sp. were taken for FT-IR experiment, as shown in figure 2. As indicated in figure 2, the FTIR spectrum of pure coral shows sharp intense peaks at 1480 and 853 cm$^{-1}$ which may be attributed to the presence of the $CO_3^{2-}$ group. The absorption peaks at 2527, 1786, and 711 cm$^{-1}$ can be explained owing to the C-O stretching vibrations. The FTIR of hydroxyapatite shows absorption bands at 563, 564, 968, 1043, 1057 and 1157 cm$^{-1}$ corresponding to the $PO_4^{3-}$ ions of the hydroxyapatite. The band around 3421, 3437, 3642 cm$^{-1}$ and peaks around 726 cm$^{-1}$ is due to the OH group. However, the appearance of peaks at 1470 and 1426 cm$^{-1}$ indicate the presence of trace contamination of residual carbonate in the hydroxyapatite. Studies have already shown similar results using FTIR analysis to confirm the absorbance bands assigned to $CO_3^{2-}$ group. (11-12)

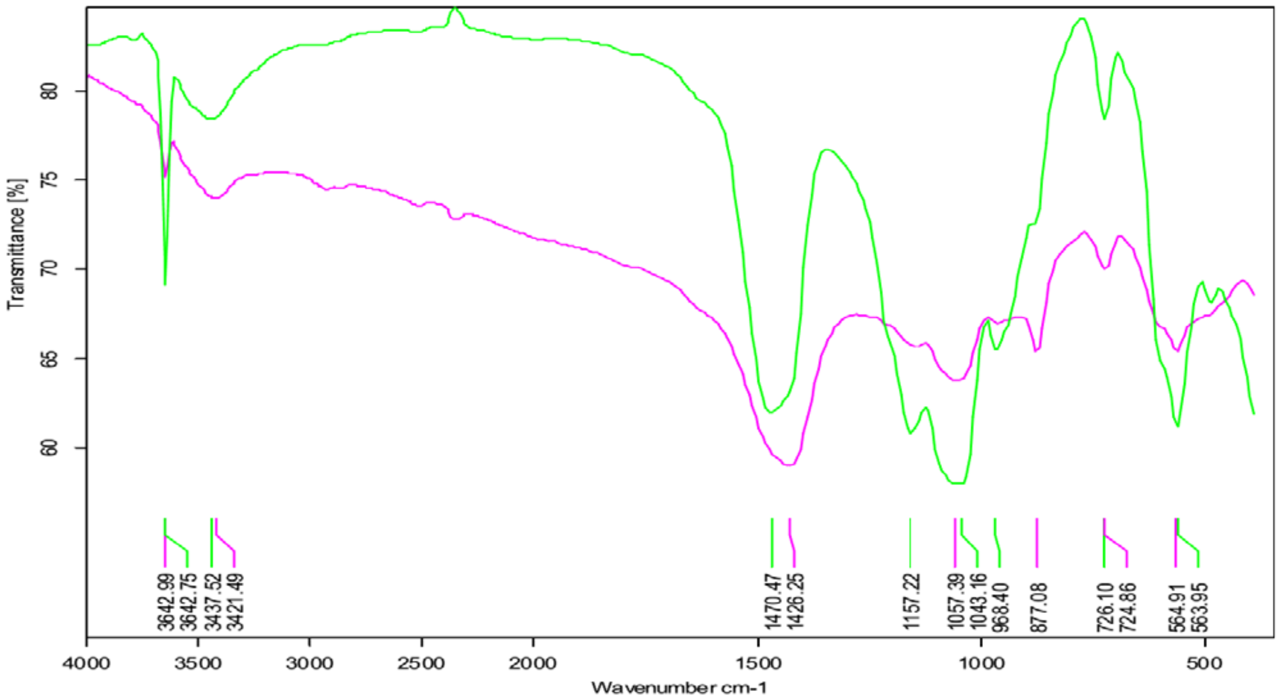
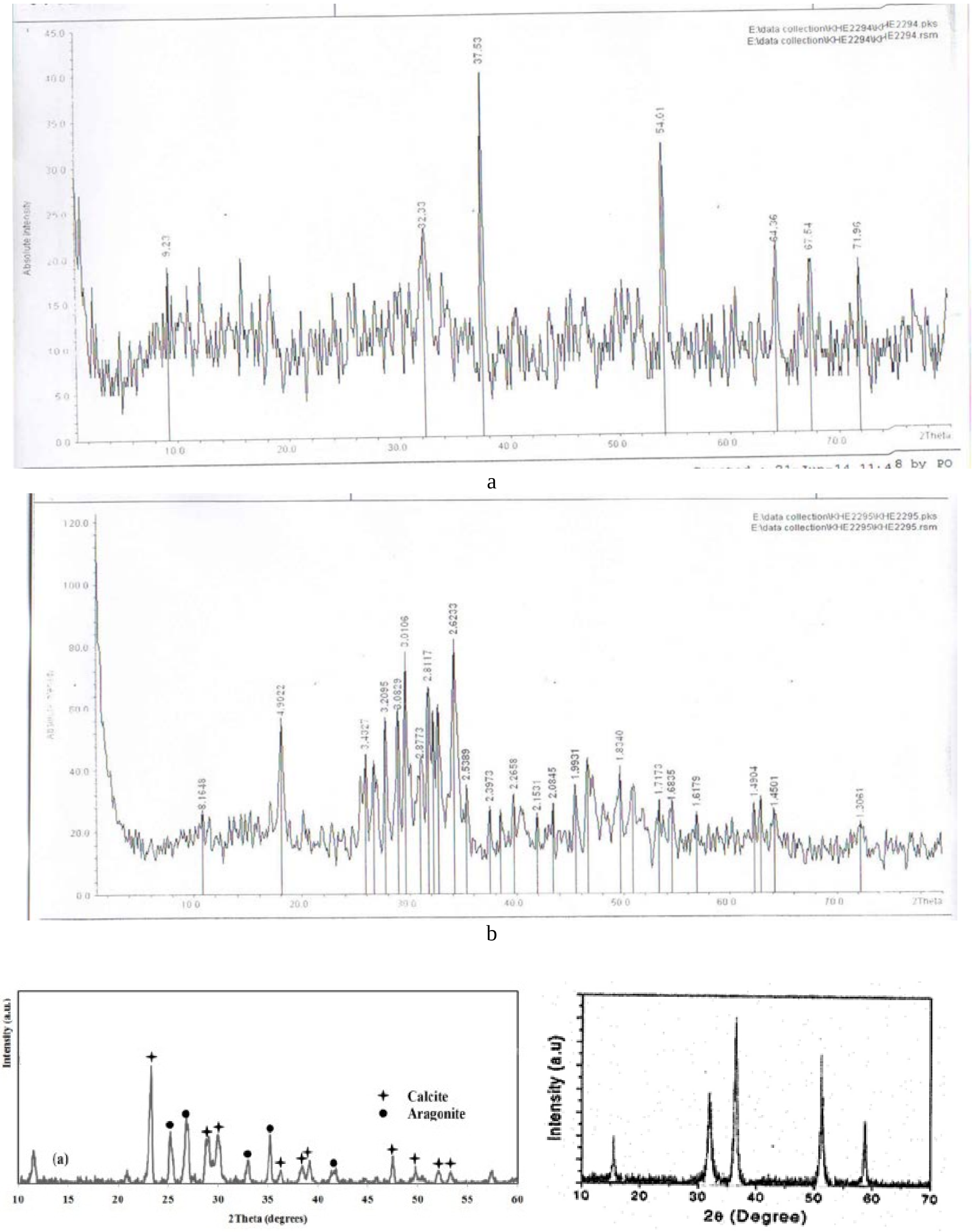


**Figure 2.** FTIR spectrum of hydroxyapatite bio-ceramics from Goniopora sp. (green) Stylophora sp. (red)

The observed XRD pattern of the coral powder is shown in Figure 3a. The coral used exhibits the presence of both aragonite and calcite phases. The stable phase of the calcium carbonate at atmospheric pressure is called „calcite‟ with R3c structure, while the stable high pressure phase is called „aragonite‟ with Pmcn structure [13]. The products after thermal process were identified by powder X-ray diffraction to be single phase of hydroxyapatite. The XRD pattern of converted hydroxyapatite demonstrated crystallinity. The XRD pattern of the samples obtained by thermal transformation at 800-1200 ◦C show the diffraction pattern belonging to Hydroxyapatite, highest intensity peak at 32, 37, 53◦ corresponding to crystalline and other highest peak intensity is 28◦ crystals suitable for $Ca_{10}(PO_4)_6(OH)_2$.



a

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**Figure 3.** X-ray diffraction spectrum of hydroxyapatite bio-ceramics from a) Goniopora sp. b) Stylophora sp. and standard

Scanning electron microscopy (SEM) analysis: The morphology of the hydroxyapatite (HA) bioceramics prepared from Persian Gulf corals was studied using SEM. SEM images of samples are given in Figure 4. From the Figure 4a synthesized HA powders are almost regular and round in shape; with a little agglomerated structure whereas sintered HA powders are agglomerated almost irregular in shape. It can be proposed that calcination treatment of the raw corals has a significant effect on sintering temperatures. The formation of these microstructures of HA in the thermal process can be attributed to the tendency of particles to crystallize and agglomerate at high temperatures. The particles had irregular shapes, including small spheres, agglomerated together in some parts. It can be concluded that higher temperature decreases the probability of grain growth and the densification can be improved. Moreover, the differences of the morphology of the particles of the powders, likely occurring due to the different species of corals, are apparent (14). In particular, according to Fig. 4a, the particles formed thin cubicles with a size of ca. 2 µm. In Figure 4b, the material surface indicates different shapes of microstructures with the range sizes of ca. 2-5 µm.

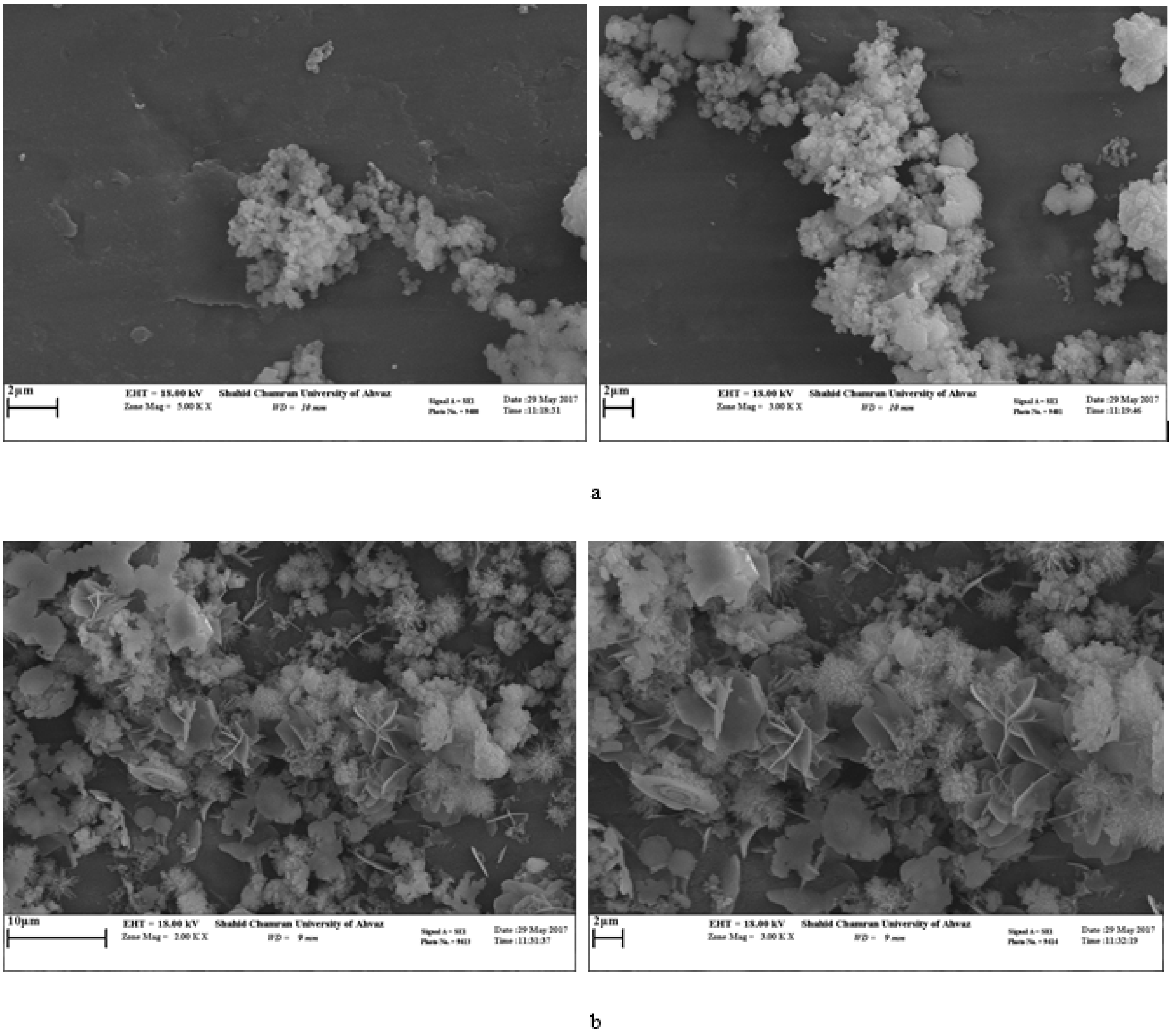

**Figure 4.** SEM of hydroxyapatite bio-ceramics from a) Goniopora sp. b) Stylophora sp.

## CONCLUSION

The samples of Persian Gulf corals Goniopora sp., Stylophora sp. were collected. Most of the marine structures are made up of pure calcium carbonate (calcite or argonite) with the addition of very small amount of an organic matrix, bioceramics are synthesized. Calcined coral samples were produced by addition of phosphoric acid, di-ammonium hydrogen phosphate, and finally calcination program at 800-1200 °C for 4 hours in air in Muffle furnace were carried out for the effective synthesis of Hydroxyapatite bioceramics (HA) respectively. FTIR analysis of samples was performed to identification of the calcium carbonate and phosphate contents for Hydroxyapatite bioceramics (HA). Structural characterization and elucidation were done using X-ray diffraction analysis, Scanning electron microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR) analysis.

## ACKNOWLEDGMENTS

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