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Characterization of shell, spine and Aristotle's lanterns of the invasive *Diadema setosum* in Iskenderun Bay

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A B S T R A C T

The invasive sea urchin Aristotle's lanterns, shell and spines describe as biological materials. In order to confirm this suggestion, the physical and chemical properties of Aristotle lantern, shell and spine of the invasive *Diadema setosum* distributed in the Iskenderun Bay were explained for the first time in this study. This species was prepared for the Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), energy dispersive X-ray spectroscopy (EDS) and scanning electron microscope (SEM) evaluation of Aristotle's lantern, shell and spine. The results showed that the shell, spine and Aristotle's lantern are composed of rough, microporous, multilayered, rough and fiber-like structures. According to XRD results, Aristotle's lantern, shell and spine were found to be similar to calcite. As a result, the shell, spine and Aristotle's lantern of *D. setosum* are qualified biological materials that can be used in many areas such as biosorption, biocatalyst, medicine, biomedical applications and lime welding.

1. INTRODUCTION

Diadema sp. sea urchin is a widely distributed, abundant and ecologically important tropical sea urchin genus (Muthiga & McClanahan, 2020). *Diadema setosum* (Leske, 1778) (Echinodermata: Echinoidea: Diadematidae) is one of echinoderm species distributed in the Indo-West Pacific Ocean. This invasive sea urchin species is distributed in the Red Sea (Gulf of Suez, Gulf of Aqaba, Northern and Southern Red Sea) and from the east coast of Africa to Japan and Australia (Lessios et al., 2001). *D. setosum* was first reported in 2006 in the Mediterranean Sea, the southwestern coast of Türkiye (Yokes, 2006). It has also been reported in Türkiye's Mediterranean coast, Hatay (Turan et al., 2011), Aegean Sea (Yapıcı, 2018), and Marmara Sea (Artüz & Artüz, 2019). *D. setosum* is defined by its long dark spines, five white spots around the shell, and an orange colour around the anal cone (Clark, 1925; Lessios et al., 2001). *D. setosum* is an omnivorous digger that eats free substrate and feeds on detritus. The invasive sea urchin lives in the shallow sublittoral region (0-20 m) and mostly congregates at 1-6 m depth.

Found in rocky habitats and biogenic reefs, where it hides in crevices and under ledges, especially due to intense lighting, *D. setosum* has also been reported to be distributed in sediment and seagrass meadows (Muthiga & McClanahan, 2020). The species exhibits variable reproductive patterns in altered geographic regions that are influenced by local environmental factors such as temperature, moon phases, and congener and adult densities. Because of their high densities, species can turn rocky shores into barren (Muthiga & McClanahan, 2020) and coral reefs, in particular, can erode biogenic substrates (Bronstein & Loya, 2014). Studies on echinoids are generally very limited with studies such as determination of heavy metal accumulation levels, biology, population density, distribution, chitin and chitosan production (Downs et al., 1993; Flammang et al., 1997; Al Najjar et al., 2018; Çağiltay et al., 2022; Öndes et al., 2022; Uğurlu & Duysak, 2022a, 2022b).

The aim of this study is to determine the microstructure characterization of the shell, spine and Aristotle's lanterns (teeth) of *D. setosum*. In this context, surface morphology, chemical contents and crystal structures will be determined by Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Diffraction (XRD), Energy Dispersive X-ray Spectroscopy (EDS) and Scanning Electron Microscope (SEM) analyses of sea urchin shell, spine and Aristotle structures.

2. MATERIAL AND METHOD

The sea urchin (*D. setosum*) was collected (n=5) in February 2023 from the Iskenderun station located on the northeast coast of the Iskenderun Bay (Figure 1). Sea urchins collected from the stations were transported to the laboratory in a bucket. Then the shells, spines and Aristotle's lanterns were washed abundantly with distilled water. The washed samples were left to dry separately in an oven at 70°C for one day. Dried shells, spines and Aristotle's lanterns were pulverized with the help of a mixer mill and passed through a 300 μ filter. All samples were stored in the freezer (-20°C) until analysis.

XRD Analysis

X-Ray diffraction microstructural measurements (XRD) were made in Iskenderun Technical University Central Research Laboratory. With this analysis, it was aimed to determine the crystalline structures of sea urchin shell (testa), spine (spin) and tooth (Aristotle lantern) samples. In this study, Malvern Panalytical EMPYREAN 3rd generation (UK) device was used. XRD analysis was recorded with CuKα radiation at 45 kV and 30 mA. Scanning speed was 0.033° and scanning range was between 2θ=5° and 80°.

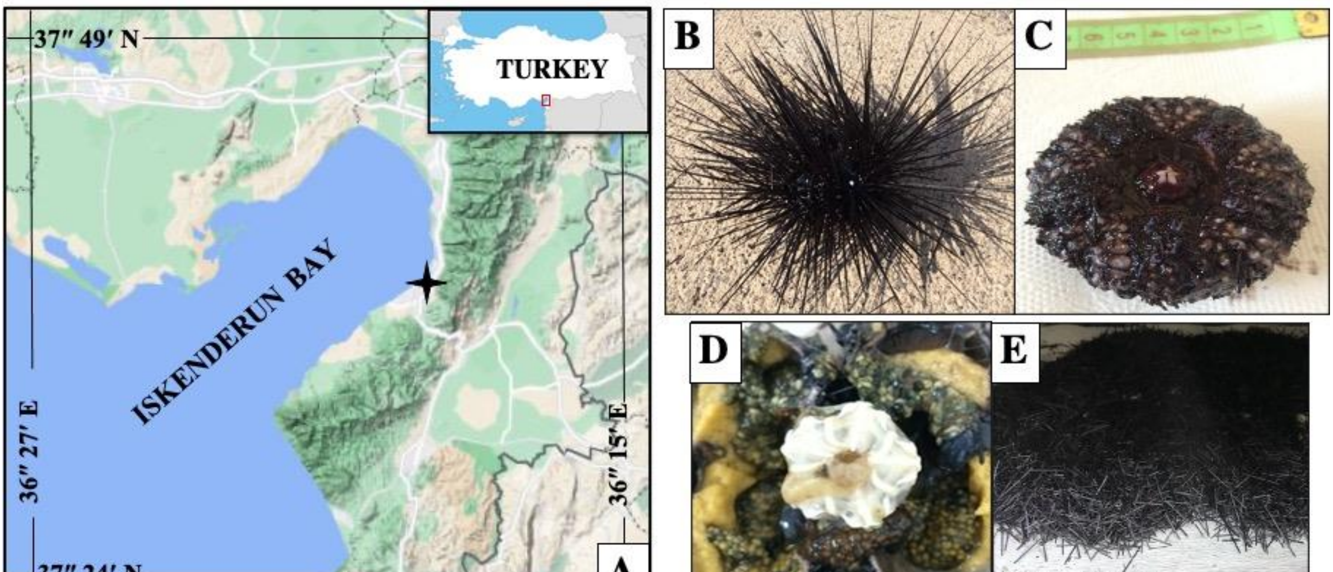

Figure 1. A) Study area (Anonymous, 2023; GM, 2023), B) *Diadema setosum*, C) Shell D) Aristotle's lantern and E) Spine

FT-IR Analysis

Fourier Transform Infrared (FT-IR) Spectroscopy measurements were made in Iskenderun Technical University Central Research Laboratory. It was determined by FT-IR spectrophotometer (Jasco/FT/IR-6700) device at 400-4000 cm^{-1} wavelength using ATR (Attenuated Total Reflectance) technique for shell, spine and Aristotle's lanterns analysis.

SEM-EDS Analysis

The obtained powder samples were coated with gold-palladium (Au-Pd) using POLARON SC7620 spray coating device before being displayed in the Central Research Laboratory of Iskenderun Technical University. Different sizes of images were taken under SEM (Scanning Electron Microscopy) (JEOL JSM-638OLA) using samples at 10-15 kV. In addition, to determine the chemical contents of these samples, energy dispersive X-ray spectroscopy (EDS) connected to the SEM device was used.

3. RESULTS AND DISCUSSION

The surface morphology, chemical components and crystal structures of the hard structures (shell, spines and teeth) of the sea urchin *D. setosum* taken from the Iskenderun Bay were investigated. In this context, XRD, FTIR, SEM and EDS analyses of *D. setosum* shell, spines and Aristotle's lanterns are presented in Figures 2-5.

XRD

Crystal structures of sea urchin shell, spine and Aristotle's lanterns raw powders were determined by XRD analysis (Figure 2). In Figure 2, the peaks obtained from the XRD patterns of 3 different powders of sea urchin represent the calcite phases. The most severe peaks seen in the XRD pattern are calcite of $CaCO_3$ at 2θ = 23.08°, 29.41°, 31.43°, 36.02°, 39.41°, 43.19°, 47.55° and 48.53°. It has been found to be related to its structure (Downs et al., 1993).

As can be seen in Figure 2, it has been determined that the majority of the structure belongs to the calcite phase. It is important that components such as shell and spines of *D. setosum*, which is an invasive species in Turkish waters, contain calcite in terms of providing an economic benefit for this species. Because calcite is one of the main ingredients used in plastic, rubber, paint, sticky, pigments, toothpaste, cosmetics, paper, feed, pharmaceutical and food industries (Yener, 2015).

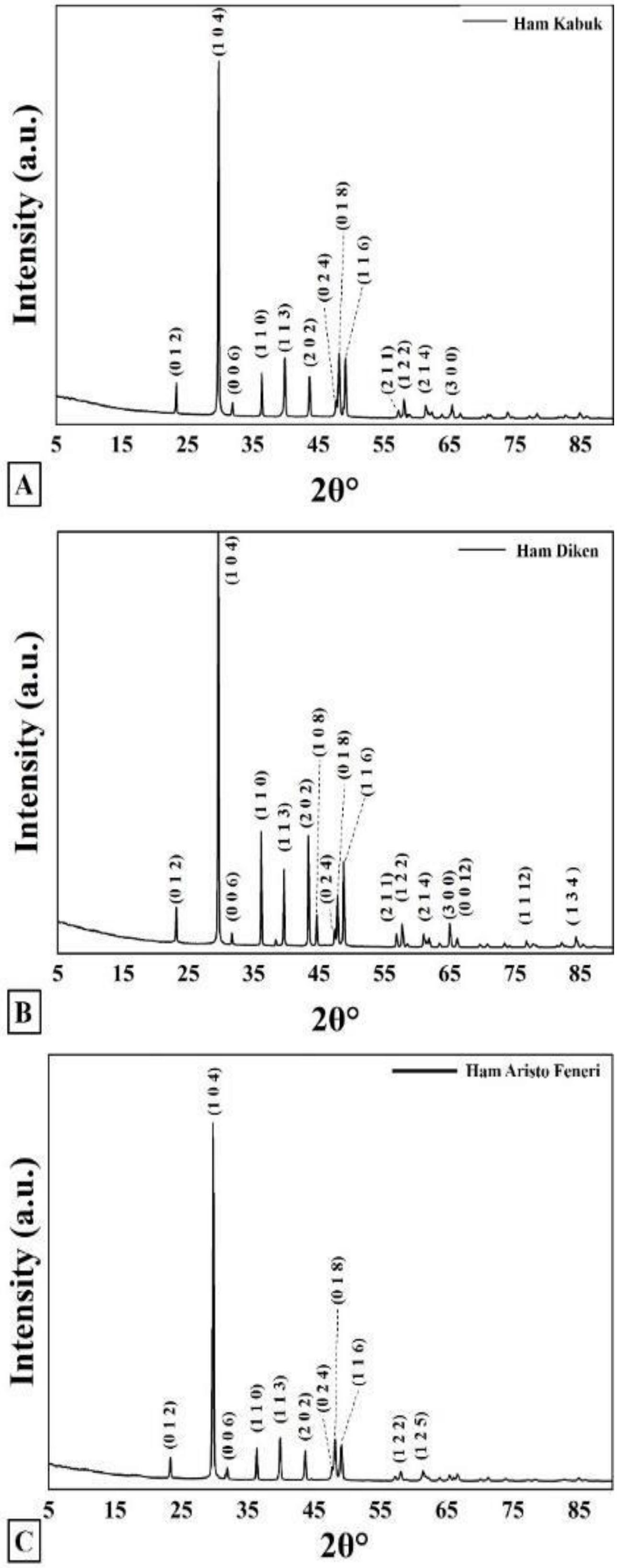

Figure 2. X-ray diffraction of sea urchins (*D. setosum*) shell (A), spines (B) and Aristotle's lanterns (C) samples.

FT-IR

Infrared characterization of the samples was performed to examine their spectral properties indicative of chemical bonding in sea urchin shells, spines and Aristotle's lanterns. Infrared spectra *D. setosum* shells, spines and Aristotle's lanterns powder is shown in Figure 3.

In Figure 3, the FT-IR spectrum of the sea urchin shell, spine and Aristotle's lanterns was determined between 4000-400 cm^{-1} wavelengths. The absorption band of the CO_3 group at 874.56–1403.92 cm^{-1}. Accordingly, it can be characterized as O-H stretching of the peak around 3270 cm^{-1}, C=O stretching of the peak around 1400 cm^{-1}, O-H bending of the peak around 1370 cm^{-1}, C-C stretching of the peaks around 1000 and 710 cm^{-1}. An important absorption peak of carbonate was observed at 1403.9 cm^{-1}, 1412.8 cm^{-1} and 1421.1 cm^{-1} in sea urchin shells, spine and Aristotle's lanterns, respectively. Amide I functional groups represented by smaller absorption bands in sea urchin shells, spine and Aristotle's lantern powders are 1645.95, 1644.81 and 1642.76, respectively.

The band representing Amide I functional groups has been reported between 1639 cm^{-1} and 1646 cm^{-1} (Yang et al., 2005; Li et al., 2005). The FT-IR results also showed the absorption peak of calcite at 874.56 cm^{-1}, 874.43 cm^{-1} and 874.12 cm^{-1} of CO_3^{-2} in the sea urchin shells, spine and Aristotle's lantern, respectively (Figure 3). Jung et al. (2000) reported that they observed the absorption peaks of calcite at 875 cm^{-1} in their study. These results supported that the sea urchin shell, spine and Aristotle's lantern were calcite.

SEM-EDS

The SEM and EDS analysis results of the shell (Figure 4A), spine (Figure 4B) and Aristotle's lanterns (Figure 4C) of the pulverized sea urchin *D. setosum* are given in Figure 4 and Figure 5, respectively. The surface morphologies of shell, spine and Aristotle's lantern samples of sea urchin are shown in Figure 4. Although the surface of the sea urchin shell (Figure 4A), viewed at 5 μ and 1 μ magnification, appears straight at first glance, it was determined that its structure was rough and consisted of micro-pore and fibre-like structures. As a result of the SEM analysis of the sea urchin spines (Figure 4B), it was determined that there were vertical and transverse overlapping structures, and at the same time, each layer had a surface area as if it were made up of very dense fibres. Finally, in the SEM image of the tooth structure of the sea urchin, which is called Aristotle's lantern (Figure 4C), it was understood that it was gathered from vertical columns of dense fibrous and regionally different thicknesses. In addition, it was observed that the surface structure was multi-layered and rough.

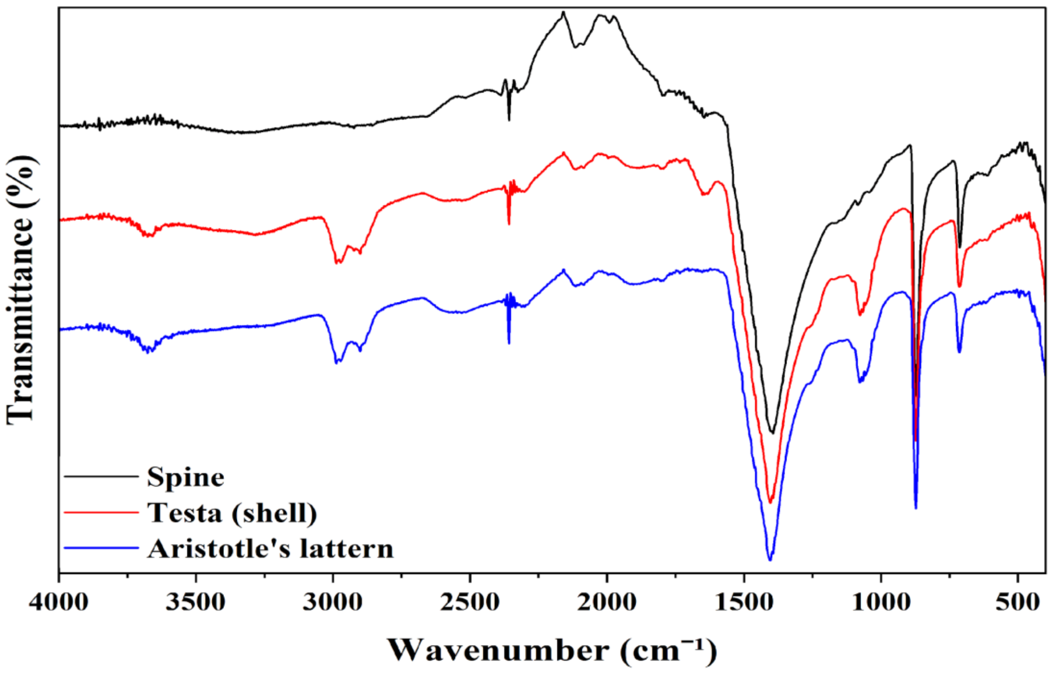

Figure 3. FT-IR spectrum of *D. setosum* shell, spine and Aristotle's lantern

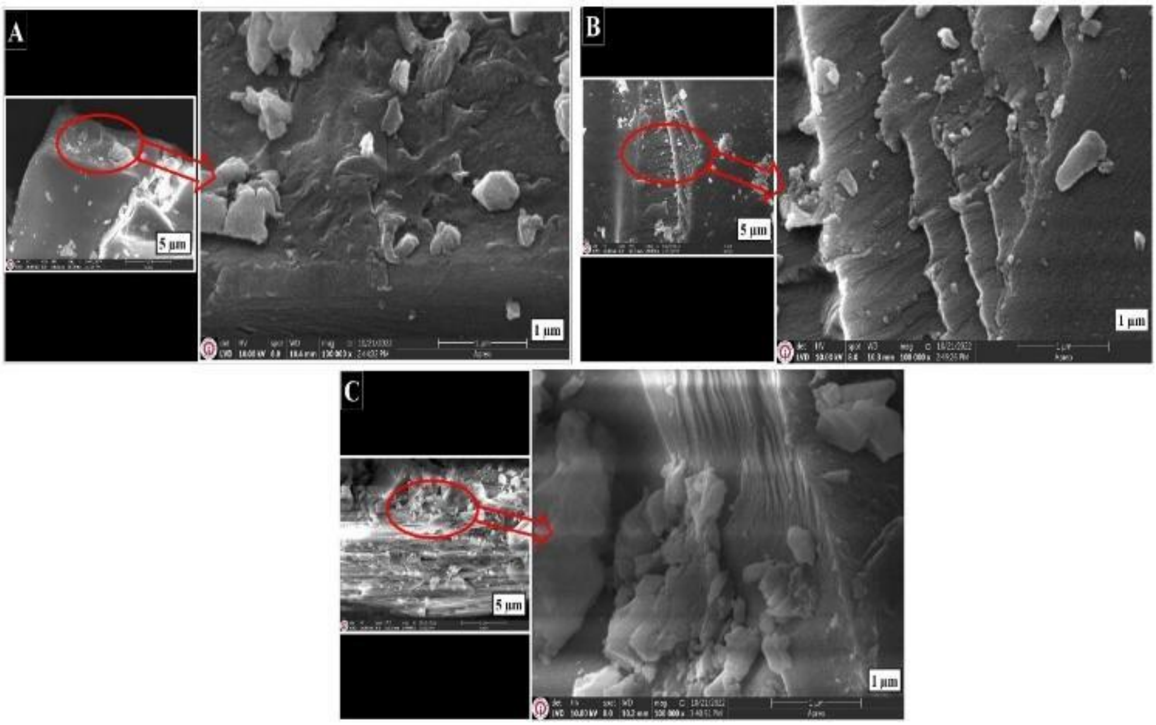

Figure 4. Structure of the *D. setosum* **(A)** Raw shell, **(B)** Raw spine **(C)** Raw Aristotle's lantern.

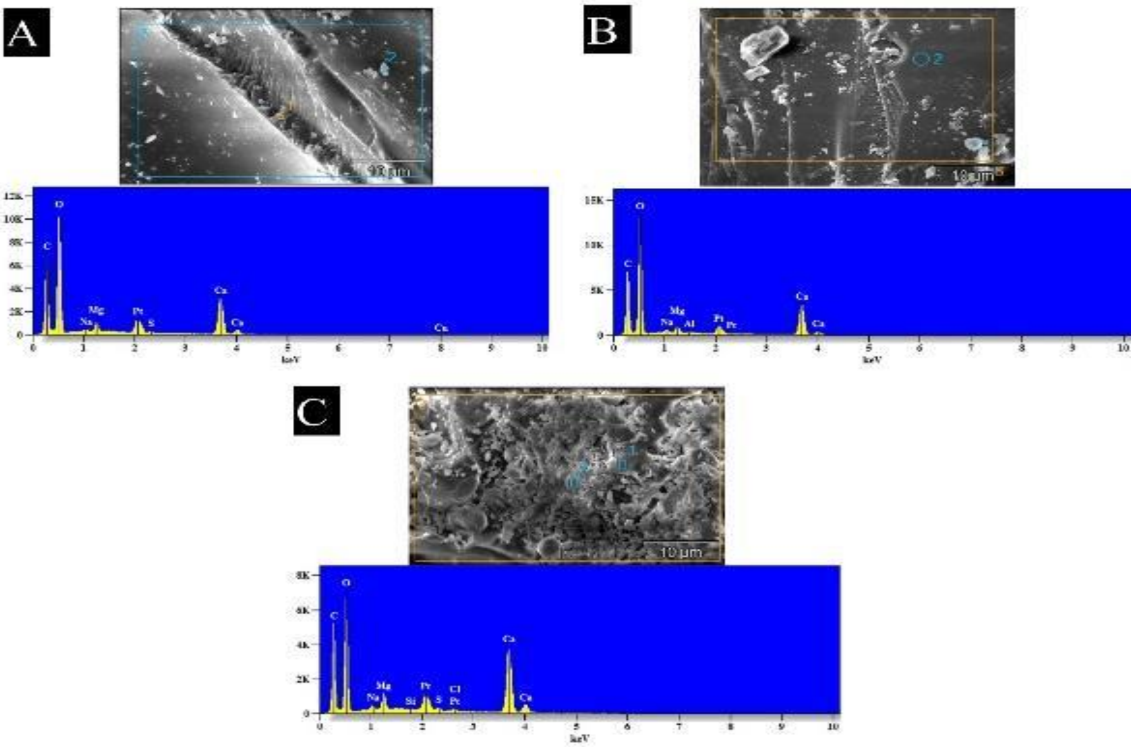

Figure 5. *Diadema setosum*: **A)** Raw shell **B)** Raw spine **C)** Raw Aristotle's lantern

The microstructure of the shell, spines and Aristotle's lantern of *D. setosum* has not been studied, except for investigations on the heavy metals, biomaterials or distribution (Flammang et al., 1997; Al Najjar et al., 2018; Öndes et al., 2022; Uğurlu & Duysak, 2022a, 2022b). The present study contributes new data on the morphology and the crystalline characterization of calcium carbonate structures in a shell, spines and Aristotle's lantern of *D. setosum*.

Table 1. EDS results of sea urchin shell, spines and Aristotle's lantern (Wt: weight and at: atom)

Elements	Shell		Spine		Aristotle's lantern	
	Wt %	*At %*	*Wt %*	*At %*	*Wt %*	*At %*
C	13.19	23.04	11.92	19.66	11.27	20.92
O	45.22	59.27	51.87	64.24	39.24	54.67
Mg	1.79	1.54	1.48	1.20	2.17	1.99
Cl	-	-	0.16	0.09	0.58	0.37
Ca	26.79	14.02	27.44	13.57	35.78	19.90
Pt	11.87	1.28	6.48	0.66	9.69	1.11

In the element analysis of the outer shell of the sea urchin, its spine and the Aristotle's lantern with the EDS device, oxygen (O), calcium (Ca), carbon (C) and platinum (Pt) elements were determined, from largest to smallest, respectively, according to their weight percent ratio (Figure 5). The results of EDS analyses are shown in Figure 5 indicating that shell, spine and Aristotle's lantern powders own similar elements such as Ca, C, Mg (magnesium) and O.

It is thought that the platinum element detected as a result of the EDS analysis of sea urchin shell, spines and Aristotle's lantern is due to the coating of the samples with gold platinum before imaging (Table 1). Similar results were reported by Saharudin et al. (2018), Jones et al. (2011), Suteu et al. (2012) and Kamba et al. (2013) where the elements of raw powders detected through EDS were Ca, C, Mg and O.

4. CONCLUSION

The surface morphology, chemical contents and crystal structures of the shell, spine and Aristotle's lantern materials of the invasive *D. setosum* sea urchin distributed in the Iskenderun Bay were revealed for the first time in this study. SEM, FT-IR and XRD analyzes have been made to bring the hard structures of this species, consisting of calcium carbonate, into the economy, which reach dense populations in the bay and are not consumed as human food, in various fields. The shell, spine and Aristotle's lantern of *D. setosum* is qualified biological materials that can be used in many areas such as biosorption, biocatalyst, pharmaceutical, biomedical applications and lime source. The use of hard structures of this invasive species, which is distributed along the Turkish coasts, will ecologically reduce the pressure on the endemic species and thus provide economic support.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest

The author declares that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

The data that support the findings of this study are available from the author upon reasonable request.

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