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Research Article/Araştırma Makalesi

Extraction and Physicochemical Characterization of Chitin from *Galeodes araneoides* (Pallas, 1772) (Arachnida: Solifugae)

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Abstract: Chitin is a biomaterial which has a high potential of use in many areas of technology. It is found as a structural material in the outer skeletons of arthropods, the cell walls of mushrooms, and the shells of marine invertebrates. Chitin is the most abundant biopolymer in nature after cellulose and contains nitrogen in its structure. In recent years, apart from traditional chitin sources, insects, arachnids, coral/crustacean eggs and even bat guano have been reported as alternative chitin sources. In this study, chitin was first extracted from external skeleton of a solifugids species, *Galeodes araneoides* (Pallas, 1772) and the isolated chitin was characterized by SEM, FTIR, XRD, and TGA. The obtained chitin has been found to have high thermal stability, nanofiber and nanoporous surface, and alpha form and it is suggested that it can be an alternative chitin source.

Keywords: Biomaterial, nanofibre, solifugid, arthropods, biopolymer.

Galeodes araneoides (Pallas, 1772) (Arachnida: Solifugae) Türünden Kitin Ekstraksiyonu ve Fizikokimyasal Karakterizasyonu

Öz: Teknolojinin birçok alanında yüksek bir kullanım alanına sahip olan kitin önemli bir biyomateryaldir. Mantarların hücre duvarında, eklembacaklıların ekzoiskelet iskeletlerinde ve kabuklarının temel yapısında bulunmaktadır. Kitin, selülozdan sonra doğada en fazla bulunan ve yapısında azot bulunduran biyopolimerdir. Son yıllarda geleneksel kitin kaynakları dışında böcekler, araknidler, mercan/kabuklu yumurtaları ve hatta yarasa guanosunun alternatif kitin kaynakları olduğu bildirilmiştir. Bu çalışmada bir böğü türü olarak bilinen *Galeodes araneoides* (Pallas, 1772) türünün ekzoiskeletinden ilk kez kitin elde edilmiştir. FTIR, SEM, TGA ve XRD değerlerine bakılarak bu kitinin karakterizasyonu yapılmıştır. Elde edilen kitinin alfa formda olduğu, termal kararlılığının yüksek, yüzey morfolojisinin nanofiber ve nanoporlardan oluştuğu görülmüş ve alternatif bir kitin kaynağı olabileceği önerilmiştir.

Anahtar kelimeler: Biyomateryal, nanofibril, eklembacaklılar, biyopolimer.

1. Introduction

Chitin is abundant in the basic structure of the exoskeletons of arthropods, the cell walls of fungi, and the shells of marine invertebrates. Chitin is also found in natural structures such as bat guano, insect and crustacean eggs, and etc. (Kaya et al., 2014a). Although chitin is a biopolymer generally found in the bodies of invertebrates, a recent study also recorded its presence in vertebrates (Tang et al., 2015). These studies show that more than 70% of all living things in the world have chitin in their structure.

It is estimated that chitin, which is an aminopolysaccharide and widely found in nature, is produced as much as annual cellulose for commercial purposes. Chitin and chitosan, one of its most important derivatives, are widely used in the industrial field because it is a natural resource, it is biodegradable, and does not cause environmental pollution, compatible with both plant and animal tissues and has no toxic effects, is a biologically functional compound, and its molecular structure is modifiable (Aranaz et al., 2009; Dutta et al., 2004). With the increase in studies on chitin and its derivatives and the emergence of new application areas in this field, there is a greater need for chitin and its derivatives and the orientation towards new chitin sources is increasing.

The order Solifugae, which belongs to the class Arachnida (Arachnida) of the arthropods, is one of the largest orders of arachnids in terms of the number of species. They are represented by 140 genera and 1075 species belonging to 12 families in the world and 15 genera and 44 species belonging to 6 families in our country. Compared to neighboring countries, our country is among the countries with the richest taxon diversity in terms of solifugids (Erdek, 2019; Punzo, 1998). As in other arthropods, solifugids have an exoskeleton made of chitin that covers the outer part of their bodies (Fig. 1).

The aim of this study is to isolate chitin for the first time in the world from *Galeodes araneoides* (Pallas, 1772), a solifugid species with a wide distribution in our country, and to characterize the obtained chitin using FTIR, TGA, XRD, and SEM analyses.

2. Method

2.1. Sample collection and identification

The specimens of *Galeodes araneoides* (Pallas, 1772) species used in this study were collected from Niğde province and its surroundings with the help of Dr. Melek ERDEK, who is an expert on these species in our country. Six specimens of *Galeodes araneoides* (Pallas, 1772) were subjected to the following steps in the laboratory to obtain the chitin.

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2.2. Chitin extraction process

Six *Galeodes araneoides* (Pallas, 1772) samples were washed, dried, and milled. Then the milled materials were processed with solutions (100 ml of 2 M HCl) to eliminate the inorganic content at 60-65 °C for 2 h. The solutions were then filtrated and the raw extracts were treated several times with pure water. After that, the isolated materials were placed in 50 mL of 1.0 M NaOH solutions to eliminate proteins. This stage lasted 16 h at a temperature of 130-135 °C. The solutions were re-filtered and re-washed with purified water. The extracts were treated in a mixture of methanol, chloroform, and water (2:1:4 ratio) for one hour at ambient temperature. This stage caused discoloration and lipids elimination. Lastly, the process was completed by drying the washed chitin materials in an oven at 60 °C for 24 h (Fig. 2).

Figure 1. General habitus of *Galeodes araneoides* (Pallas, 1772)

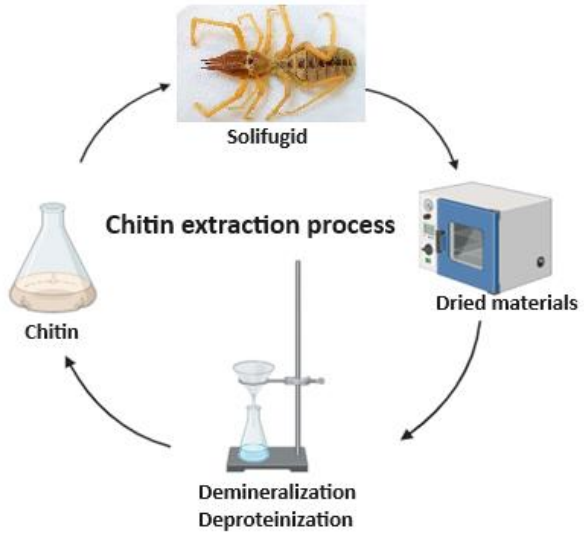

Figure 2. Scheme of chitin extraction process from *Galeodes araneoides* (Pallas, 1772)

2.3. FTIR analysis

The FTIR spectrum of the chitin sample extracted from *Galeodes araneoides* (Pallas, 1772) solifugid species were analyzed with a Bruker Vertex 70 FTIR spectrometer with a frequency range of 625–4000 cm^{-1}.

2.4. SEM analysis

The surface images of *Galeodes araneoides* (Pallas, 1772) chitin was comparatively analysed by scanning electron microscopy (Carl Zeiss, Evo LS 10). The surfaces were coated with Au by Sputter Coating System (SCS) before SEM analysis.

2.5. TGA analysis

The samples of chitin were analyzed by TGA machine (STA PT1600) with a temperature change rate of 10 °C /min from 25 to 650 °C.

2.6. XRD analysis

The samples of chitin were examined by Rigaku D max 2000 at 2θ in the range of 5-45°C. The value of crystalline index (CrI) was calculated according to formula: CrI110 = [(I110 − Iam)/I110] × 100. I110 = the maximum intensity at 2 θ = 20 °C. Iam = the intensity of amorphous diffraction at 2 θ = 16 °C (Sajomsang & Gonil 2010).

3. Results and Discussion

Chitin was obtained from *Galeodes araneoides* (Pallas, 1772) and physicochemical characterization of the obtained chitin was performed using FTIR, TGA, XRD, and SEM. In this study, chitin was obtained from solifugids of this species for the first time and was characterized.

3.1. FTIR spectroscopic analysis

In many studies, FTIR spectroscopy analysis of chitin has revealed characteristic peaks for α-chitin. These bands are: 3263 (N-H stretching), 1620 (Amide I), and 1552 cm−1 (Amide II) (Fig. 3). In this research, the chitin extracted from the *Galeodes araneoides* (Pallas, 1772) was analyzed by FTIR, two peaks at around 1650 and 1620 cm^{-1} were monitored and shown to be consistent with former studies (Fig. 3). These peaks reveal that the chitin isolated from *Galeodes araneoides* (Pallas, 1772) is in the α-form.

3.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis is widely used to learn the temperature resistance of the obtained material (Dutta et al., 2004). In the present study, TGA analysis was performed to reveal the degradation of chitin obtained from FTIR spectrum of chitin isolated from *Galeodes araneoides* (Pallas, 1772). The results obtained show that chitin experiences mass loss in two steps under the influence of temperature. In the first step, the mass losses around 100°C are due to the water that is removed from the structure. The significant mass losses observed in the second step indicate that the chitin started to degrade (Fig. 4). As a result of the literature review, mass loss of chitin isolated from different organisms was also observed in two different steps as in the present study (Jang et al., 2004; Juarez-de la Rosa et al., 2011; Sajomsang & Gonil, 2010). The maximum decomposition temperature of chitin (DTGmax) recorded for *Galeodes araneoides* (Pallas, 1772) was 334°C. This value is found lower than other arachnids DTGmax (Kaya et al., 2014a; Seyyar & Demir, 2017). These

results indicate that chitin was successfully produced from *Galeodes araneoides* (Pallas, 1772) in the present study.

3.3. X-Ray diffraction analysis (XRD)

XRD analysis results of chitin isolated from *Galeodes araneoides* (Pallas, 1772) are shown in Fig. 5. In the literature, XRD analysis of chitin and its derivatives showed two sharp peaks around 9°C and 19°C (Jang et al., 2004; Liu et al., 2012; Sajomsang & Gonil, 2010). The one around 19°C is more intense than the one around 9°C. These are the characteristic XRD peaks used in understanding of chitin and its derivatives.

XRD analysis of chitin showed two sharp peaks at 9.75°C and 19.51°C and three weak peaks at 12.93°C, 23.24°C, and 26.81°C. These peaks are very similar to the chitin peaks described in the literature (Jang et al., 2004). The crystalline index value (CrI) of chitin of *Galeodes araneoides* (Pallas, 1772) calculated as %51.14. According to the literature, the CrI values of the chitin of different arachnids were examined as 89.17% in opilionid (*Phalangium opilio*), and spiders 58.9% (*Hogna radiata*) and 78.6% (*Geolycosa vultuosa*), respectively (Kaya et al., 2014b; Seyyar & Demir, 2017). This value is lower than other arachnids. This result emphasizes that CrI value is very variable depending on species.

3.4. Scanning electron microscopy (SEM) analysis and imaging

The surface morphology of chitin of *Galeodes araneoides* (Pallas, 1772) was visualized by SEM (Fig. 6). It is seen that the surface of the chitin consists of many nanofibres and pores.

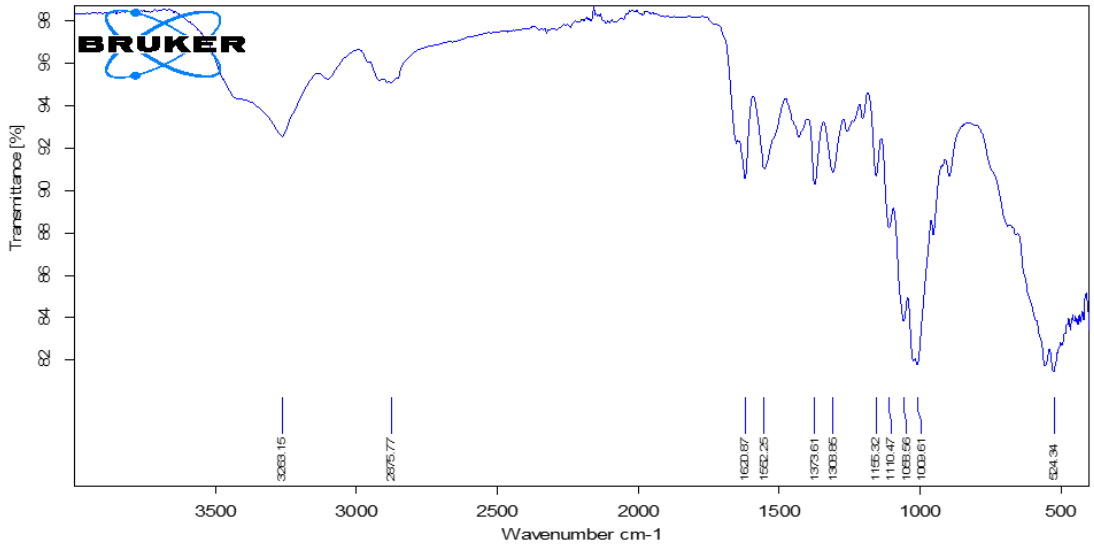

Figure 3. FTIR spectrum of chitin isolated from *Galeodes araneoides* (Pallas, 1772)

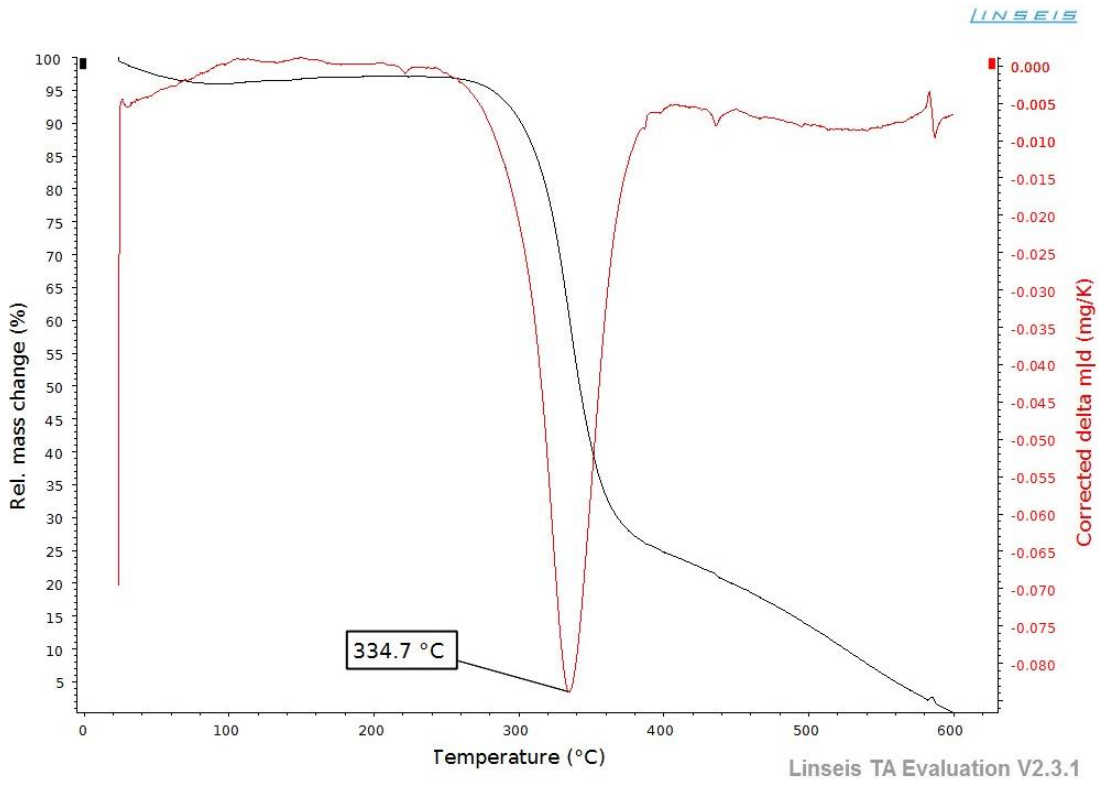

Figure 4. TGA analysis of chitin isolated from *Galeodes araneoides* (Pallas, 1772)

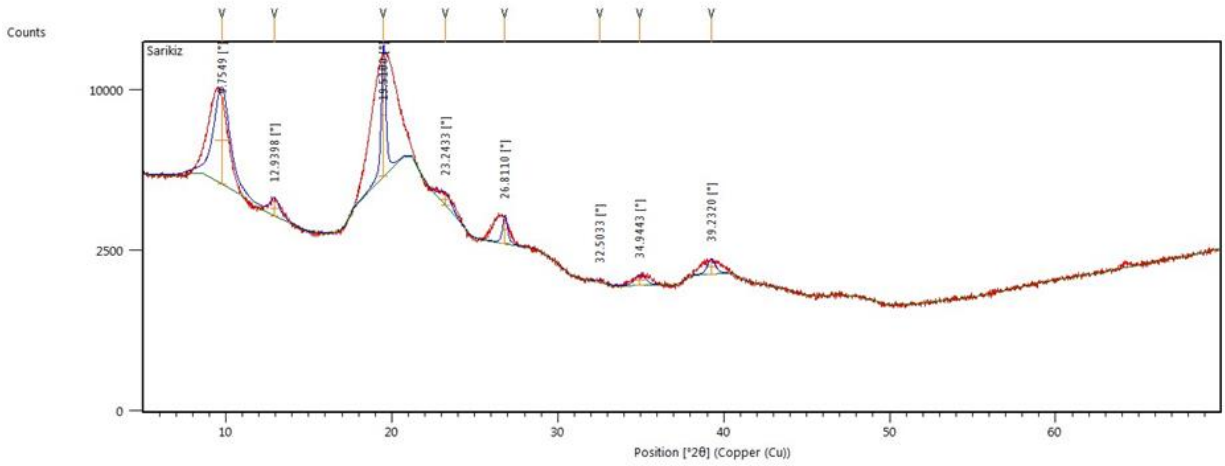

Figure 5. XRD analysis of chitin isolated from *Galeodes araneoides* (Pallas, 1772)

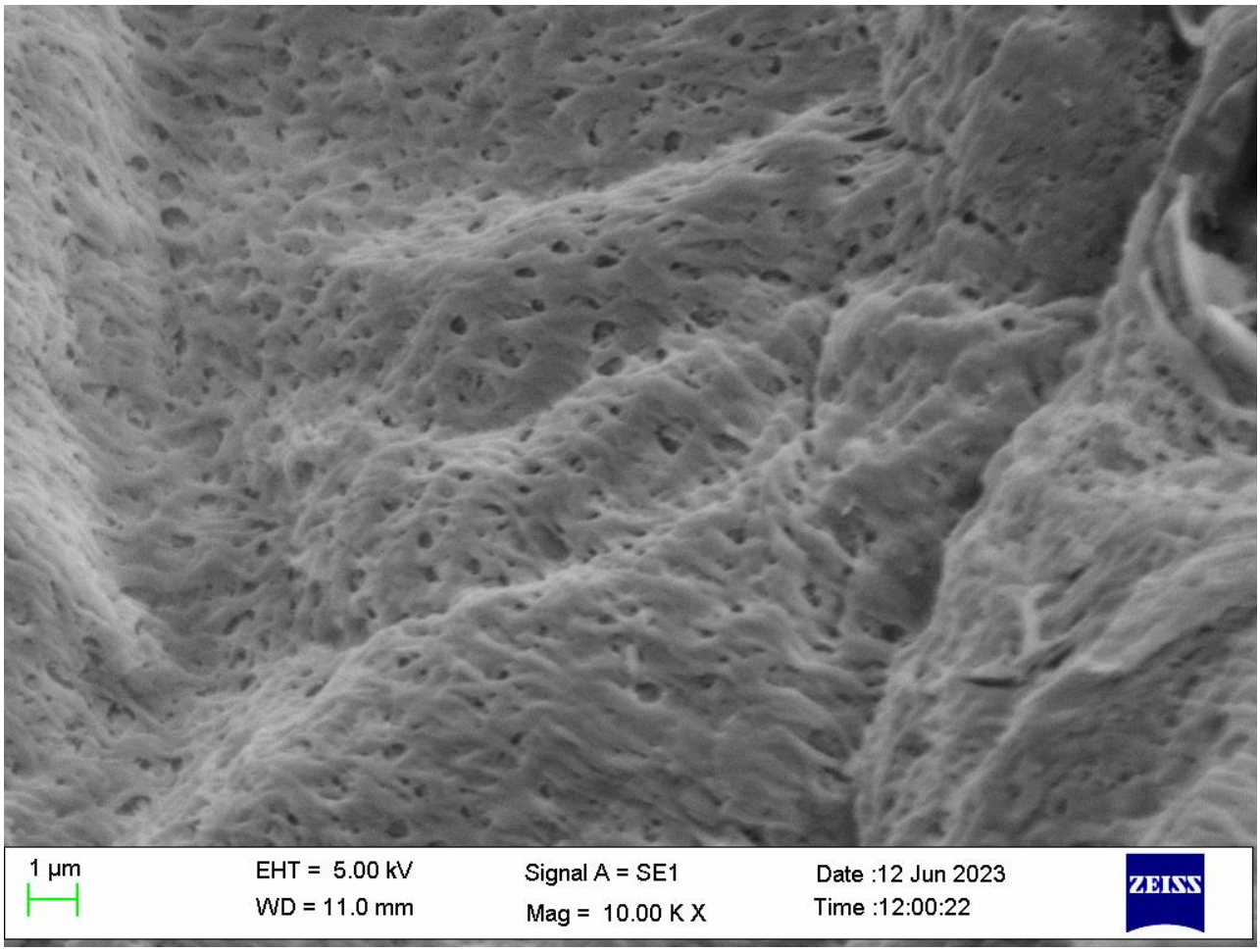

Figure 6. SEM surface morphology of chitin isolated from *Galeodes araneoides* (Pallas, 1772)

Looking at the studies in the literature, it is known that the surface of chitin varies according to the living group. During this study, it was observed that nanofibers and pores were not observed in the morphology of chitin obtained from fungi and the chitin surface was flat (Yen & Mau, 2006, 2007). It is revealed in other studies that the surface morphology of chitin obtained from some living groups is only nanofiber, while in some other living groups, the chitin surface contains both nanofibers and nanopores (Kaya et al., 2013). In very few groups of organisms, the chitin surface containing nanofibers, nanopores, and micropores together was also observed (Kaya et al., 2014b).

So far, chitin studies have only been carried out on a few species of spiders, harvestmen, and scorpion from arachnid groups in Türkiye. Among these, the chitosan surface morphology of *Mesobuthus gibbosus* (Brullé, 1832), a scorpion species, was reported to consist of dense nanofibers and pores when examined by SEM (Kaya et al., 2015, 2016). In addition, chitin was isolated from three different spider species (*Geolycosa vultuosa*, *Hogna radiata* and *Aculepeira ceropegia*) in our country and it was revealed

that they have different surface morphologies (Demir & Seyyar, 2017; Kaya et al., 2014b). In addition, in a study on opilionid species *Phalangium opilio* Linnaeus, 1758, another arachnid group, it was observed that they have a surface morphology consisting of nanofibers and nanopores (Seyyar & Demir, 2017).

4. Conclusions

The classic source of chitin isolation is the shells of crabs, shrimps, and mollusks that form waste from the processing of marine food products. In recent years, people have sought unique sources of chitin beyond these traditional sources. As a result, many fungal, insect and arachnid are now being discovered as well. In our study, we isolated chitin for the first time from solifugid which may provide a unique alternative source of chitin. Then, we investigated its characterization and some physicochemical properties obtained from *Galeodes araneoides* (Pallas, 1772) by FTIR, XRD, TGA, and SEM analyses. The findings obtained from the FTIR spectrum clearly show that the chitin obtained from this solifugid species is in alpha form. Images obtained by scanning electron microscopy show that the surface morphology of the chitin consists of nanofibers and nanopores. Thermogravimetric analysis revealed that the thermal stability of the obtained chitin was lower than that of many known insects and crustaceans. This isolated chitin has wide applications in many different industries. Therefore, we propose that solifugids can also be used as an alternative source to extract chitin on a large scale without relying on conventional sources.

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Conflict of interest: The authors declare that there is no conflict of interest.

Author Contributions: Conception – D.D., O.S.; Design – D.D., O.S.; Materials – D.D., O.S., F.S.; Data Collection and Processing – D.D., O.S., F.S.; Analysis Interpretation – D.D., O.S., F.S.; Literature Review – D.D., O.S., F.S.; Writing – D.D., O.S., F.S.; Critical Review – O.S.

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