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Research Article

Sium sisarum L. var. *lancifolium* (M. Bieb.) Thell -a traditional spice from eastern Anatolia: chemical composition and biological activities

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Abstract: Traditionally consumed food and spices are significant sources in the daily life diet and constitute a large portion of the cuisine in Eastern Anatolia in Turkey. However, limited data available necessitate further analysis of their chemical composition and health attributing properties. This study aims to present phytochemical composition and biological activities of *Sium sisarum* var. *lancifolium*, a commonly consumed spice and food species in the region. Analytical studies to date have revealed the presence of high levels of phenolics (chlorogenic acid and isoquercetin) and volatiles (α-terpinene, camphene, cyclohexene, carene and p-cymene), which exhibits significant potential of digestive enzyme suppressive and antioxidant abilities. Data collected in this study suggest the use of *Sium sisarum* plant to obtain nutraceuticals and/or biotherapeutic agents that are able to regulate oxidative stress and enzyme activities.

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1. INTRODUCTION

The use of plant-based materials as food and medicine has been formed of the experiences, beliefs and practices of different cultures and preferred due to their effective health enhancing properties and minimized side effects across the world (Firenzuoli & Gori, 2007; Robinson & Zhang, 2011). Ethnobotanical studies reveal significant knowledge of plant-based preparations which possess promising candidates of nutraceuticals. Several ethnobotanical studies that have been conducted in the region (Kaval *et al*., 2014; Mükemre *et al*., 2015; Dalar and Mükemre, 2020) reveal extensive use of several locally used plant samples including *Sium sisarum* var. *lancifolium* (Apiaceae). It is a perennial plant species that can reach 100 cm long (Figure 1), which mericarps becoming arcuate at maturity (Davis, 1965-1985). *Sium* taxa are very popular in food and medicine culture worldwide. For instance, skin care products formulations and preparations for treating asthma and allergy (Ashraf, 1999) have been commonly used in Siberia. In Europe, their roots are commonly consumed as raw or added to soups due to its

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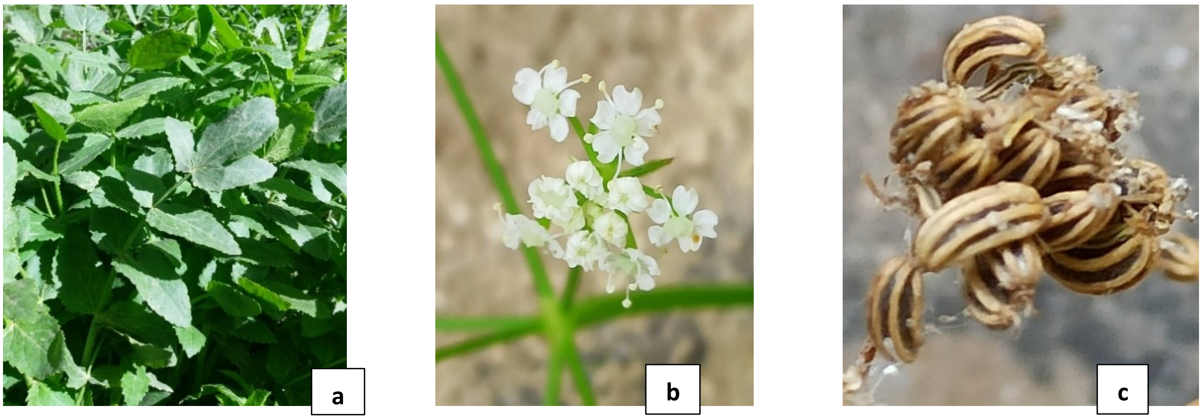
sweet taste. Also their leaves are used as a vegetable (Harvey, 1984). In Eastern Anatolia the above-ground young shoots of the *Sium sisarum* var. *lancifolium* plant are added to cheese. Fresh shoots are boiled, then fried together with egg, and cooked. The young stem part of the plant is peeled and eaten raw due to its pleasant smell and its leaves are used as a spice and medicine (Kaval *et al*., 2014; Mükemre *et al*., 2015; Dalar & Mükemre, 2020). Although *Sium sisarum* var. *lancifolium* plant is used extensively in food and in the treatment of diabetes in Hakkari and Van regions in Eastern Anatolia, Turkey by the local population, there is limited data in regard to its chemical composition and potential biological activities. Therefore, this study aims to determine the phytochemical composition, antioxidant, and enzyme inhibition effects of the ethanol-based extract and traditional preparation (infusion) obtained from *Sium sisarum* var. *lancifolium* plant.

2. MATERIAL and METHODS

2.1. Plant Materials

Leaf samples of *Sium sisarum* var. *lancifolium* were collected from marshy and damp habitats in the villages of Narlı, Çukurca, Hakkari on August 20th, 2020 (Global Positioning System (GPS) coordinates 38S 374905 4125792; 810 m) and transferred to the laboratory (Figure 1). Taxonomical identification of the samples was done at Van Pharmaceutical Herbarium (VPH), Faculty of Pharmacy, Van Yuzuncu Yil University, Turkey and the voucher specimens were stored at VPH (Herbarium code: VPH-510, Collector Code: MM-687). Plant materials were dried in the dark, subsequently ground into a fine powder, and stored at -20°C for a maximum of 4 weeks until they were analyzed.

Figure 1. General view of *Sium sisarum* var. *lancifolium* (a: Leaf, b: Flower, c: Fruit (mericarp).



2.2. Chemicals

All chemicals were obtained from Sigma-Aldrich, Inc. (St Louis, MO, USA)

2.3. Preparation of extracts

2.3.1. Ethanol-based extract

The ethanol-based lyophilized extracts were prepared as described previously by Dalar and Konczak (2013).

2.3.2. Herbal infusion extract

The herbal infusion was prepared from the powder according to Baytop (1999).

2.4. Antioxidant Capacity

2.4.1. Folin-Ciocalteu Reducing (FCR) capacity

FCR capacity was measured using the Folin-Ciocalteu assay as described according to Dalar *et al* (2012).

2.4.2. *Ferric reducing antioxidant power* (FRAP)

The total reducing capacities of the extracts were determined as previously described by Dalar *et al*. (2012).

2.4.3. *Oxygen radical absorbance capacity* (ORAC)

The ORAC assay was conducted as previously described by Dalar *et al*. (2012).

2.4.4. 2,2-*Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity*

The DPPH assay was done according to Konczak *et al*. (2003).

2.4.5. *The metal chelating activity*

The metal chelating activities of the extracts were determined as described by Dinis (1994).

2.5. Enzyme Inhibitory Activities

2.5.1. *α-Glucosidase inhibitory activity*

The inhibitory activity of *α*-glucosidase was determined according to Dalar and Konczak (2013).

2.5.2. *α-Amylase inhibitory activity*

α-Amylase inhibitory activity was done using the Caraway–Somogyi iodine/potassium iodide (IKI) method as previously described by Dalar and Konczak (2013).

2.5.3. *Pancreatic lipase inhibitory activity*

Lipase inhibitory activity was assayed as previously described by Dalar and Konczak (2013).

2.6. Identification and Quantification hf Phenolic Compounds

Identification and quantification of phenolic compounds were conducted as described previously by Dalar and Konczak (2013).

2.7. Identification and Quantification hf Volatile und Fatty Acid (FA) Compounds

Identification and quantification of volatile and fatty acid compounds were done as described previously by Uzun, Dalar, and Konczak, (2017).

2.8. Data Analysis

The mean values were calculated based on at least three determinations (n = 3). One-way ANOVA followed using the Bonferroni *post-hoc* test was done to measure differences between the samples at $p < 0.05$ using Graphpad Prism 5 (Graphpad Software, San Diego, CA, USA).

3. RESULTS and DISCUSSION

3.1. Extraction Yields

The infusion preparation gave a higher yield than that of the ethanol extract. However, the ethanol extract exhibited better antioxidant and digestive enzyme inhibitory activities which indicate its effective extraction ability of biologically active compounds from plant matrix (Tables 1, 2, and 3). This finding was also confirmed by chromatographic analyses. The higher yield of water solvent can be explained by other hydrophylic chemical compounds such as sugars and proteins present in the extract.

3.2. Phytochemical Composition

The major contributor of biological activities of the extracts were composed of phenolics, volatiles, and fatty acids (Table 1 and 2 and Figure 2, 3, and 4). Based on GC-MS analysis five volatiles and three fatty acids were found in the ethanol extract. No volatiles were detected in the infusion preparation and fatty acids were only at trace levels. Major volatiles were α-terpinene and p-cymene and fatty acids were dominated by linolenic acid (Table 1 and Figure

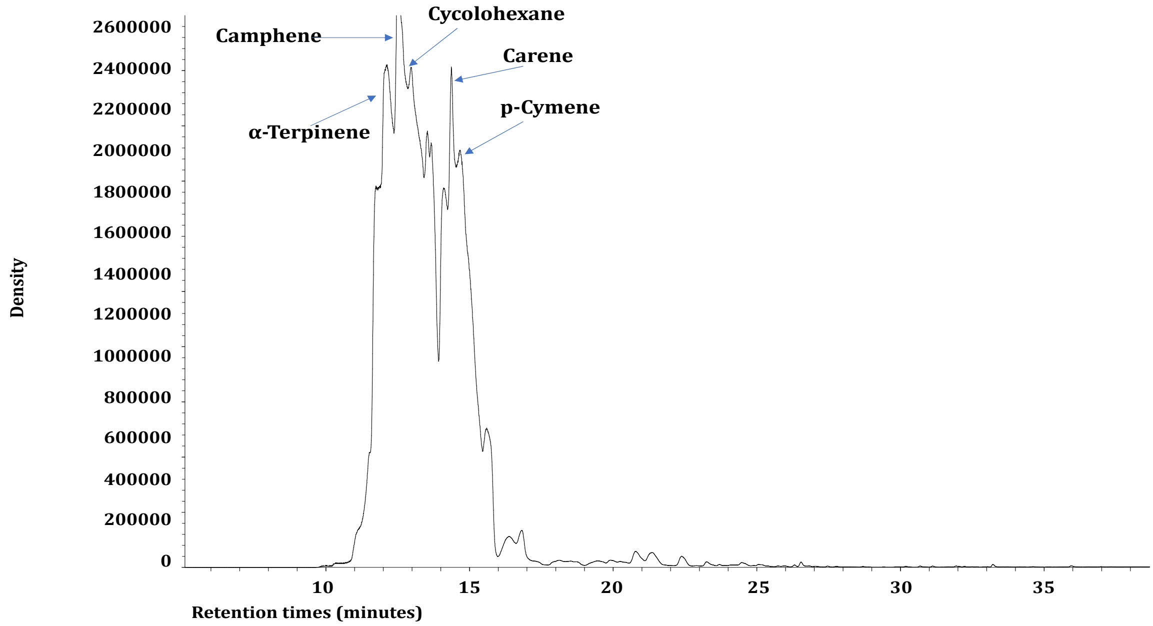
2 and 3), which is in agreement with previous chromatographic reports of *Sium sisarum* (Ozturk *et al*., 2017).

Table 1. Gas chromatography mass spectrometry (GC-MS) profiles of *Sium sisarum* var. *lancifolium* extracts.

	Retention time	Compound	Infusion	Ethanol
Volatile component (Relative concentration; %)	12.1	*α*-Terpinene	ND	22±1
	12.5	Camphene	ND	17±1
	12.9	Cyclohexane	ND	15±1
	14.1	Carene	ND	6±0.4
	14.5	*p*-Cymene	ND	23±1
Fatty acids component (Relative concentration; %)	36.7	Palmitic acid	T	14±1
	40.8	Linoleic acid	T	9±0
	44.8	Linolenic acid	T	35±2

Means with different letters in the same column were significantly different at $p < 0.05$; all data were determined as a result of at least three independent experiments. T: Trace level; concentration ≤2 % ND; Not Detected.

Figure 2. Volatile component (GC-MS) profiles of *Sium sisarum* var. *lancifolium* extracts.



With regard to phenolic composition of the extracts, molecular data showed that two major compounds (isoquercetin and chlorogenic acid) dominated the composition (Table 2 and Figure 4). Chlorogenic acid dominated infusion preparation, while isoquercetin dominated ethanol extract (Table 2). These compounds are among biologically active compounds and several scientific reports have revealed their strong biological activities such as radical scavenging and reducing oxidative stress, enzyme inhibitory activities, anti-inflammatory, antidiabetic, antiobesitic, neuroprotection, cancer, cardiovascular disorders, allergic reactions, and antidepressants in both *in vitro* and *in vivo* studies (Dalar *et al*., 2014; Oboh *et al*., 2015; Cruz-Zuniga *et al*., 2016; Gonçalves & Romano, 2017). Though the levels of volatile and fatty acids compounds were low in the extract, they might also be among secondary contributor of the biological activities detected with the present study due to their high biological activities reported previously such as reducing the risk of hypertension, arteriosclerosis, cancer, and allergic diseases and lowering serum cholesterol, triglycerides, and LDL cholesterol levels (Lee *et al*., 2002; Agoramoorthy *et al*., 2007; El Tahir *et al*., 2003; Lahlou *et al*., 2003).

Table 2. High performance liquid chromatograpy mass spectrometry (HPLC-MS/MS) profiles of *Sium sisarum* var. *lancifolium* extracts.

Phenolic compound	MS/MS		Concentration (µg/mg extract)	
	-/[M-1]$^-$	Fragment ions (m/z) (+/)	Infusion	Ethanol
Chlorogenic acid	-/353	-/191	19±1b	23±1a
Isoquercetin	-/463	-/301	6±1b	34±2a

Means with different letters in the same column were significantly different at $p < 0.05$; all data were determined as a result of at least three independent experiments.

Figure 3. Fatty acids component (GC-MS) profiles of *Sium sisarum* var. *lancifolium* extracts.

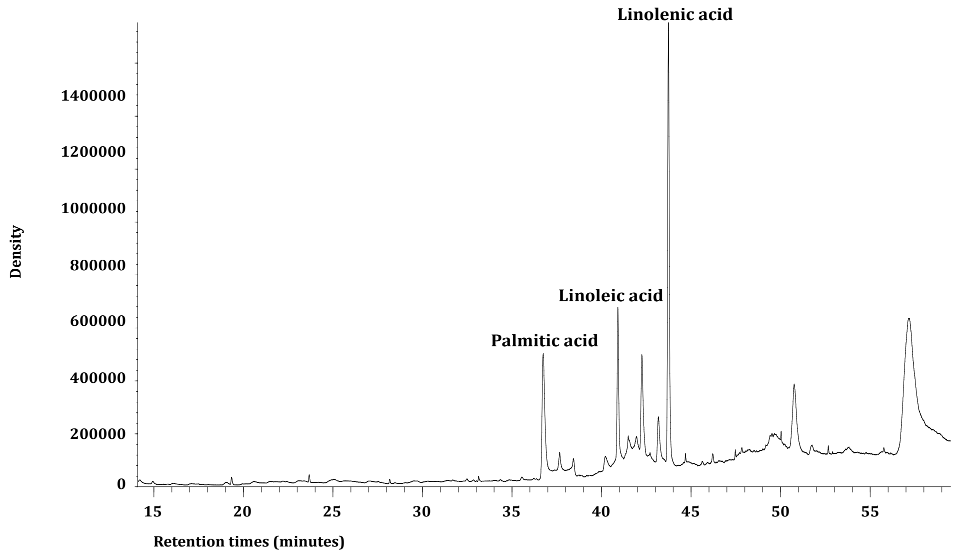
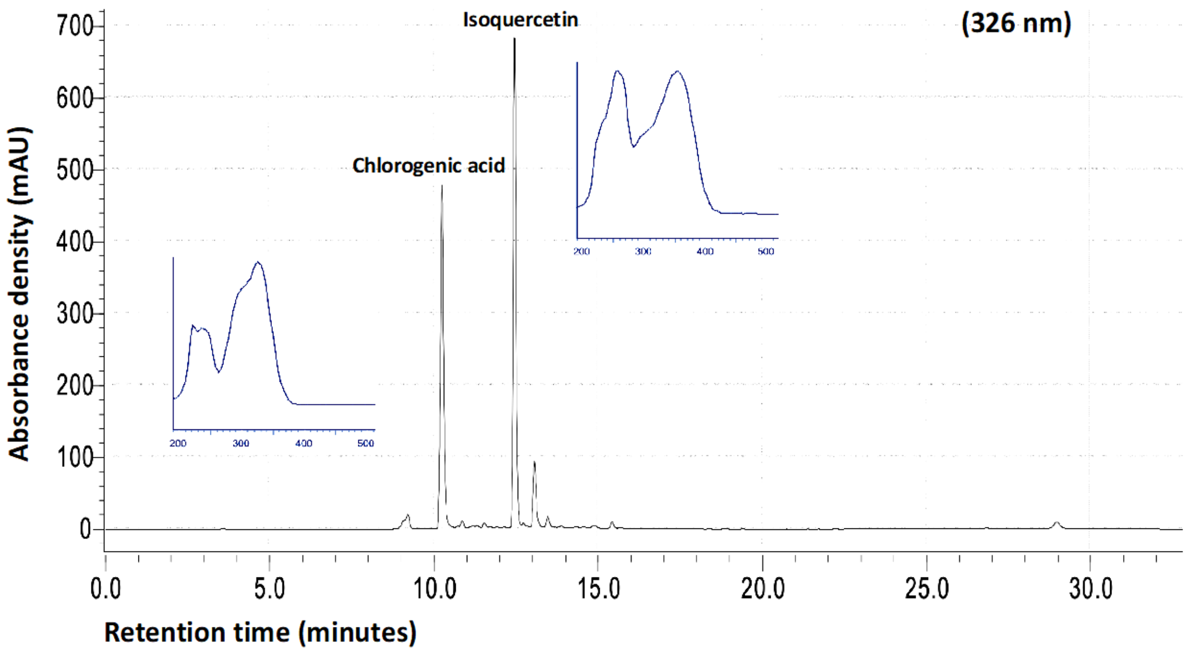


Figure 4. HPLC-MS/MS profiles of *Sium sisarum* var. *lancifolium* extracts.



3.3. Antioxidant Capacities

Free radicals and reactive oxygen species are produced through the normal process of metabolism and external sources. Imbalance between antioxidant defense system and free radicals result in oxidative stress related metabolic and neurological diseases. As synthetic antioxidants might improve defense system capacity despite their toxic and mutagenic effects, there is a need to research and develop natural source-derived bioactive substances or standardized extracts with tolarable side effects (Pham-Huy & He, 2008; Akata *et al*. 2019)

To reveal the comprehensive antioxidant potential of plant extracts which contain complex and various phytochemicals, complementary methods including single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms were applied in the study. The results gave a common pattern of positive control ≥ ethanol extract > infusion preparation. Among all tests applied, a significant higher result was obtained in ORAC assay which directly measures the level of inhibition of antioxidant formation of the peroxyl radical compared to positive control (Butylated hydroxyanisole) (Table 3). Data obtained revealed superior antioxidant capacities than those of plant species that belong to Apiaceae (Dalar *et al*. 2014) and also those of previous studies that discussed *Sium sisarum* (Samancığlu *et al*., 2016). Also there is a positive correlation between phenolic content and antioxidant activity in the present and earlier studies (Cruz-Zuniga *et al*., 2016), which indicates that they can be among major contributors of the activity.

Table 3. Total phenolic contents and antioxidant capacities of *Sium sisarum* var. *lancifolium* extracts.

		Extraction yield (%)	FCR[1]	FRAP [2]	ORAC [3]	DPPH [4]	Metal chelation[4]
Sium sisarum	Infusion	15.174	32±2b	950±14c	3716±278c	217±11c	131±9c
	Ethanol	25.886	66±2a	2165±60b	6281±96a	103±8b	78±5b
Positive control	Ascorbic acid	-	-	4984±43a	-	-	-
	Butylated hydroxyanisole	-	-	-	5912±42b	-	-
	Trolox	-	-	-	-	54±4a	-
	Ethylenediaminetetraacetic acid	-	-	-	-	-	28±3a

Means with different letters in the same column were significantly different at $p<0.05$; all data were determined as a result of at least three independent experiments. [1]Folin–Ciocalteu values; mg gallic acid equivalent/g extract. [2]Ferric reducing antioxidant power; μ mol Fe^{2+}/g extract. [3]Oxygen radical absorbance capacity; μ mol trolox equivalent/g extract. [4]DPPH radical scavenging activity; IC_{50} (μg extract /ml), [4]Metal chelation activity; IC_{50} (μg extract /ml).

3.4. Enzyme Inhibition Activities

Phytochemical compounds present in spices have multiple effects, not only antioxidant but also enzyme inhibitory activities through binding to enzymes that cause hypertension, metabolic disorders, inflammation, and various neurodegenerative diseases (Mai *et al*., 2007; Zengin, 2016). Alternative plant-orginated enzyme inhibitors have been searched for a long time due to unwanted effects of synthetic inhibitors (Sakulnarmrat & Konczak, 2012). The results of our study as summarized in Table 2 show pronounced digestive enzyme inhibitory activities and display a similar pattern to antioxidant findings.

Isoquercetin, one of the most dominant compounds identified in the phenolic composition of the extracts has been reported for its high antihyperglycemic activity *in vivo* (Jayachandran *et al*., 2018). It has also been reported that chlorogenic acid can suppress the activity of α-glucosidase enzyme in very low dose applications effectively (Exteberria *et al*., 2012). Low alpha-amylase and high alpha-glucosidase results (Table 4) suggest *Sium sisarum* as a potential candidate of nutraceuticals that can be utilized in the management of diabetes due to its potential to minimize digestive system problems such as diarrhea and gastric gas (Weiss *et al*., 2013).

Table 4. Enzyme inhibitory activities of *Sium sisarum* var. *lancifolium* extracts.

		Enzyme inhibition activity ($IC_{50;}$ µg/ml))		
		Alfa-Amylase	Alfa-Glucosidase	Pancreatic lipase
Sium sisarum	Infusion	2018±38c	517±52c	505±20c
	Ethanol	1013±41b	187±23b	95±3b
Positive control	Acarbose	34±3a	75±6a	-
	Orlistat	-	-	8±1a

Means with different letters in the same column were significantly different at $p<0.05$; all data were determined as a result of at least three independent experiments. * The equivalent of commercial standards calculated based on a standard curve and against control.

Various experimental studies showed that herbal materials rich in phenolic compounds can effectively inhibit the activity of pancreatic lipase enzyme *in vitro* and *in vivo* which is linked to the formation of obesity and other related diseases (Cho *et al*., 2010; Dalar *et al*., 2014; Zhang *et al*., 2015). The extracts had pronounced levels of antilipase activity which is consistent with those of Zhang *et al*. (2011), who reported a positive correlation between the levels of phenolic compounds and enzyme inhibitory activities of plant extracts. Zhang *et al*. (2011) reported that isoquercetin had an antidiabetic effect in diabetic mice and a regulatory role in sugar level and lipids. Various studies showed that the chlorogenic acid rich extracts inhibited lipase activity effectively (Zhang *et al*., 2015; Dalar *et al*., 2014), which explains the strong antilipase ability of *Sium sisarum*. Metabolic diseases such as diabetes and obesity are closely related to excessive amounts of reactive oxygen radicals produced or accumulated in the body. Plant materials rich in phenolic compounds are powerful antioxidants and have important functions in preventing or controlling metabolic diseases such as diabetes and obesity because of their free radical scavenging activities (Styskal *et al*., 2012). Therefore, it is important to prevent or control metabolic diseases such as diabetes and obesity, along with the inhibition of related enzymes, as well as the elimination of reactive oxygen radicals.

4. CONCLUSION

The present study reports phytochemical composition and biological activities of a traditional spice- *Sium sisarum* var. *lancifolium*-commonly used by local people of Eastern Anatolia, Turkey. Its major chemical compouds are composed of phenolics (isoquercetin and chlorogenic acid), volatiles (α-terpinene, camphene, cyclohexene, karen and p-cymene), and fatty acids (palmitic, linoleic and linolenic acid). Phytochemical rich ethanol extract and infusion preperation showed its high antioxidant and enzyme inhibitory (alpha-glucosidase and pancreatic lipase) activities, but not alpha-amylase. These findings suggest the use of *Sium sisarum* extracts as potential sources of antioxidant and digestive enzyme inhibitors that can be used in the daily diet.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Muzaffer Mukemre: Investigation, Methodology, Project administration, Visualization. **Abdullah Dalar**: Investigation, Methodology, Project administration, Visualization. **Sengal Bagci Taylan:** Investigation, Methodology. **Metin Ertas**: Investigation, Methodology.

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