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Araştırma Makalesi / Research Article

Haşhaş (*Papaver Somniferum L.*) Çiçeğinin Uçucu Yağ İçeriğinin Belirlenmesi ve Antimikrobiyal Özelliklerinin Araştırılması**Meltem Dilek¹, Alparslan Gültepe², Nuray Öztaşan³**¹Afyon Kocatepe Üniversitesi, Mühendislik Fakültesi, Kimya Mühendisliği Bölümü, Afyonkarahisar.²Afyon Kocatepe Üniversitesi, Fen Bilimleri Enstitüsü, Organik Kimya Anabilim Dalı, Afyonkarahisar.³Afyonkarahisar Sağlık Bilimleri Üniversitesi, Tıp Fakültesi, Temel Tıp Bilimleri Bölümü, Afyonkarahisar.

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Anahtar kelimelerHaşhaş; *Papaver Somniferum L.*; Uçucu yağ; Antimikrobiyal aktivite**Öz**

Afyon Alkaloidleri Fabrikası İşletme Müdürlüğüne ait ekim alanlarından toplanan *Papaver Somniferum L.* çiçeklerinin uçucu yağı hidrodestilasyon yöntemi kullanılarak elde edildi. Elde edilen uçucu yağın bileşenleri GC ve GC-MS metodları ile tespit edildi. Haşhaş çiçeği uçucu yağının ana bileşenlerinin n-nonadekan (%8,96), heneikosan (%10,83), n-pentakosan (%7,91), n-heptakosan (%5,19), 1-heptakosanol (%4,09), palmitik asit (%7,26) ve 1-nonadekanol (%16,31) olduğu belirlendi ve antimikrobiyal aktiviteleri incelendi. Haşhaş çiçeği uçucu yağının tridekanoik, miristik, palmitik, steraik, oleik, linoleik, linolenik, eikasadienoik, eicasatrienoik doymuş ve doymamış yağ asitlerinden oluştuğu tespit edildi. Uçucu yağın ana bileşenleri tespit edildikten sonra antimikrobiyal aktiviteleri disk difüzyon testi ve bouillon mikrodilüsyon testi ile belirlendi. Haşhaş çiçeği uçucu yağının hem Gram (+) hemde Gram (-) bakteriler üzerinde antimikrobiyal etkiye sahip olduğu tespit edildi.

Determination of Essential Oil Composition and Investigation of, Antimicrobial Properties of Poppy (*Papaver Somniferum L.*) Flower**Keywords**Poppy; *Papaver Somniferum L.*; Essential oil; Antimicrobial activity**Abstract**

The essential oil of *Papaver Somniferum L.* gathered from crop fields of Opium Alkaloids Plant is obtained by hydrodistillation method. The component of essential oil was determined by GC and GC-MS methods. It was determined that the components of the essential oil are n-nonadecane (8,96%), heneicosane (10,83%), n-pentacosane (7,91%), n-heptacosane (5,19%), 1-heptacosanol (4,09%), palmitic acid (7,26%), 1-nonadecanol (16,31%) and antimicrobial activities were studied. It was determined that essential oil consist of tridecanoic, myristic, palmitic, stearic, oleic, linoleic, linolenic, eicasadienoic, eicasatrienoic saturated and unsaturated oil. After determining the components of the essential oil; the antimicrobial activities were studied disc diffusion and bouillon micro dilution tests. It was determined that the essential oil was an impact on both Gram (+) and Gram (-) bacteria.

1. Introduction

The use of quality perfumery and cosmetics has increased worldwide. Parallel to this development, the significance of aromatic plants has increased globally as well. Turkey a country marked by a rich flora is also rich in aromatic plants. However, several plants have not been investigated for the properties of their essential oils despite the fact that they are known to bear aromatic qualities (Öztürk 1990). Essential oils are fragrant and generally colourless natural products obtained from several plant parts such as leaves, fruits, bark and roots that turn into liquid at about 24-25 °C. Due to their pleasant odour, they are also called essences or etheric oils (Ceylan 1983). It is known that most medical plants and their essential oils with economic significance are used in pharmacological and perfume industries (Kırbağ and Bağcı 2000). Essential oils can be obtained with several methods including steam distillation, hydro-distillation, high pressure solvent extraction, supercritical CO₂ extraction, ultrasonic extraction and solvent-free microwave extraction. However, the properties of essential oils vary based on the method used (Okoh et al. 2010). Essential oils generally indicate antibiotic and antiseptic properties against bacteria, fungi and yeasts. In recent years, numerous studies have been conducted on antimicrobial activities of essential oils (Sarıkurkcu et al. 2010, Eryiğit et al. 2013, Meng et al. 2016, Ebrahimabadi et al. 2010). Opium poppy plants have been grown in several parts of the world since ancient times. The poppy, which is the genetic origin of several culture plants, has a special place in Turkey. It is known that poppy plant has been cultivated in Anatolia since the Hittite era. Thus, Anatolia is significant as the native land and the cultural source of the poppy plant. Poppy is both an important medicinal plant and essential oil source. Alkaloids such as morphine, which are obtained from poppy capsules, are used as sedatives and, pain killers and as raw material in several drugs. Poppy seeds contain 40-55% oil and are widely used as cooking oil in the areas where they are cultivated. Since poppy belongs to the semi-siccative oils group, it is

extensively used in painting, soap industry and other industrial branches (İncekara 1964). The seeds are roasted and can be eaten as snacks and can also be used for pastry ornaments in its natural form. Moreover, it can be roasted, crushed, and used as a supplementary material in pastries. During the first developmental stages, the leaves of the poppy plant can be used in salads. Its stems can be used as fuel. There are several studies on the alkaloids in capsules and the oil in seeds of the poppy, a very significant industrial plant due to its capsules and seeds; however there is no previous study on obtaining essential oil from poppy flowers and the characterization of this essential oil. It is difficult to determine the antimicrobial activities of essential oils due to their water solubility, volatility and complex structures. The structure of the essential oil, microorganisms, growth medium and selected methodology are important parameters in determination of antimicrobial activities (Cowan 1999, Janssen et al. 1987, Hammer et al. 1999, Dorman and Deans 2000, Delespaul et al. 2000, Pattnaik et al. 1997). The present study aimed to obtain essential oil from the *Papaver somniferum* L. flowers, widely cultivated in Turkey, with the hydrodistillation method and to determine the gas chromatography (GC), Gas Chromatography-Mass Spectrometer (GC-MS) and components of the obtained essential oil. Furthermore, in order to investigate the biological activity of the essential oil obtained from flowers, antimicrobial properties were determined with the disc diffusion and bouillon microdilution methods and antifungal properties were determined with the agar diffusion plate method.

2. Materyals and Methots

2.1. Materials

The white poppy flowers used in the study were obtained from the cultivation sites in the Afyon Alkaloids Plant Directorate in Afyonkarahisar province Bolvadin district in June (Figure 1). Standard material, media, and solvents were obtained from Fluka, Merck, Sigma and Aldrich

corporations. Culture media used for biological activity tests were obtained from Merck.



Figure 1. Afyon Alkaloids Plant cultivation site

2.2. Isolation of the essential oil with hydro-distillation

The collected white poppy flowers were spread as a thin layer on a clean and dry environment without sunlight and dried for one week until the time of the experiment (Figure 2). Clevenger system was used to obtain essential oil from the dried poppy flowers. In this process, 250 g material was placed in a 2000 mL flask, and 1000 mL distilled water was added to initiate hydrolysis for 3.5 hours. At the end of this process, the oil phase was separated from the water phase. The water phase was extracted 3 times with 30 mL CH₂Cl₂ (dichloromethane) in order to recover the essential oil in the water phase. The CH₂Cl₂ solvent was removed with a rotary evaporator. The oil phases were combined and dried on anhydrous Na₂SO₄. The resulting oil phases were combined and placed in amber bottles, and nitrogen gas was allowed to pass through the bottles and the lids were tightened. The oil samples were stored at 4 ± 1°C in an incubator until the analysis was performed (Gültepe 2013).



Figure 2. Dried poppy flowers

2.3. Determination of density and refractive index

The density was determined using a 1 µL capillary tube. The capillary tube was weighed empty and then weighed after it was filled with water. Finally, after the oil sample was weighed, the density was calculated with the formula given below (Williams 1984).

$$d = \frac{c - a}{b - a}$$

a= Weight of the empty capillary tube (g)

b= Weight of the capillary tube filled with water (g)

c= Weight of the capillary tube filled with oil (g)

The refractive index of the essential oil was determined with an Abbe Refractometer.

2.4. Gas Chromatography - Mass Spectrophotometer (GC-MS)

The major components and relative percentages of poppy seed oil were determined using a Shimadzu QP 5050A GC-MS instrument. The operating conditions of the GC-MS device are presented in Table 1.

Table 1. The operating conditions of Gas Chromatography - Mass Spectrophotometer (GC-MS)

Instrument	Shimadzu QP 5050A GC-MS
Sensor	FID
Column	Agilent Innowax (60 m, length, 0.25 mm diameter, 0.25 µm thickness)
Injection temperature	250 °C
İyonlaştırma Modu	EI

Electron Energy	70 eV
Mass range	45-475 m/z
Temperature program	60 °C 10 min //4 °C/min 220 °C //1 °C/min 240 °C // 30 min
Carrier gas and flow rate	Helium, 1 mL/min
Library	Wiley

2.5. Determination of Poppy Flower Essential Oil Fatty Acids

Initially, the extracted essential oil sample was converted to fatty acid methyl esters according to the International Union of Pure and Applied Chemistry (IUPAC) method 2.301 (IUPAC 2.301) to detect the fatty acids in the composition of the poppy seed essential oil obtained with the Clevenger apparatus. The conversion of fatty acids to methyl esters increases the volatility of the components as well as enhances the detector sensitivity during GC analysis, facilitates separation, and reduces tailing in the peaks.

2.5.1. Preparation of methyl esters with the BF_3 methanol method

1.5 mL 0.5 M methanolic NaOH (sodium hydroxide) solution was added to 25 mg essential oil extracted from poppy flowers. Nitrogen gas was sent into the solution and the flask was closed and mixed. It was heated in a 95 °C water bath. Then 2 mL 20% BF_3 - methanol complex was added, nitrogen gas was released into the tube, and the tube was closed. It was kept in the water bath at 95°C for 30 minutes. 1 mL hexane was added to the solution and the mixture was heated for 1 minute. Then the test tube was cooled to room temperature and the aqueous phase was saturated with NaCl (sodium chloride) to separate the organic and aqueous phases. 1 mL supernatant n-hexane phase was analyzed in a gas chromatography device (IUPAC 2.301).

2.6. Gas chromatography (GC)

After the poppy seed essential oil methyl esters were prepared, fatty acid content was determined

with GC under the operating conditions specified in Table 2.

Table 2. The operating conditions of Gas Chromatography

Instrument	HP Agilent 7890A GC SYSTEM
Sensor	FID
Column	HP5 – MS (60 m, length, 0,32 mm diameter, 1 µm thickness)
Injection temperature	250 °C
Detector temperature	250 °C
Temperature program	60 °C, 3 °C /min, 240 °C
Carrier gas and flow rate	H ₂ 40 mL/min, air 400 mL/min, N ₂ 50 mL/min

2.7. Determination of the antimicrobial effect of poppy flower essential oil

The standard bacterial strains such as *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (NRRL-B 767), *Salmonella typhimurium* (NRRLB-4420), *Bacillus subtilis* (NRS-744), *Proteus vulgaris* (ATCC-13315), *Micrococcus luteus* (ATCC-9341), *Listeria monocytogenes* (ATCC 7644), and *Pseudomonas aeruginosa* (ATCC-7664), *Bacillus cereus* (ATCC-11778) were used to determine the antimicrobial effects of the poppy flower essential oil. Standard bacteria strains were obtained from Anadolu University, Faculty of Sciences, Department of Biology. Fungus isolation was performed on air, black pepper and thyme. Malt agar was used in the identification and isolation of the species. Point seeding was conducted in petri dishes containing adequate agar for the species using a needle. The cultures were incubated for 14 days and the resulting colonies were examined macroscopically and microscopically, and identified at the genus level. Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), and Malt Agar media were used for determination of antimicrobial activities. The weighed materials were mixed with distilled water in erlenmeyer flasks and the media were prepared. The prepared media were sterilized at 121°C for 15 minutes in the autoclave, and then placed in sterile petri dishes to determine the antimicrobial activities of the microorganism strains.

The effects of poppy flower oil on bacteria were examined using the Disk Diffusion Test and the bouillon microdilution test methods in the current study. The tests were repeated three times and the measured values were averaged. DMSO, Meropenem (MEM), amoxicillin/clavulanic acid (AMC), and Ceftriaxone (CRO) were used as negative controls for Gram (-) bacteria, Vancomycin (VA), Penicillin (P) and Ceftriaxone (CRO) were used as a positive control for Gram (+) bacteria.

2.7.1 Disk diffusion test

The poppy seeds were infused to empty the sterilized discs of 6 mm in diameter using a micropipette. Bacterial cultures were allowed to incubate at 37 °C in NB until they reached 0.5 McFarland (McF). Bacterial suspensions adjusted to 0.5 McF standard were seeded in petri dishes with agar medium and with sterile cotton swabs. After the bacteria were seeded, the disks were placed on the medium surface and kept there for half an hour at room temperature, and then incubated for 24 hours at 37°C. Diameters of the formed zones were measured in mm (Cowan 1999, Janssen *et al.* 1987).

2.7.2. Bouillon Microdilution Test

Minimum inhibition concentration (MIC) was determined with the bouillon microdilution test in the present study. The poppy seed essential oil concentration required to inhibit the propagation of the inoculated bacteria standardized under the conditions specified by this test was determined. The MIC dilution tests were used to determine the lowest concentration value (mg / L) where there was no propagation after the poppy flower essential oil was cultured in the media by monitoring the incubation. As the MIC value, after 75 µL that included poppy flower oil in different concentrations as the MIC value was added to bouillon wells, bacteria suspension based on the

0.5 McF standard was seeded. These were incubated for 24 hours at 37°C and propagation was determined based on turbidity (Dorman and Deans 2000, Janssen *et al.* 1987).

2.8. Determination of fungus activity

Antifungal activity was determined with the Agar Diffusion Plate Method. A 20 mL Potato Dextrose Agar (PDA) was poured into petri dishes of 9 cm diameter, and the 9 cm in diameter agar was removed from the middle of the petri dish, and 100 µL extract was added into this as well. A 5-mm diameter specimen from each one week fungal cultures was placed on the agar so that the spores were placed on the medium. The petri dishes were incubated at 20°C for 10 days. The distances between mycelial growth and the active substance at the center of the medium in the plates filled with distilled water as the control group were measured in millimeters in certain intervals the measurements were compared and inhibition was calculated in percentages (Kalmış and Kalyoncu 2008).

3. Results and Discussion

3.1. Physical properties of the poppy flower essential oil

The essential oil obtained with hydro-distillation method from the white poppy flowers obtained from the Afyon Alkaloids Plant Directorate cultivation sites had a characteristic fragrance and light yellow colour. The melting point of the essential oil was between 24 and 26°C and had a viscous liquid appearance (Figure 3).



Figure 3. Poppy flower essential oil

The density, refraction index and melting point of the poppy flower essential oil are presented in Table 3.

Table 3. The density, refraction index and melting point of the poppy flower essential oil

Density (g/cm ³)	Refractive index ([n] _D ²⁰)	Melting point (°C)
0.71	1.47	24.7

3.2. Chemical composition of the essential oil

The components of the essential oil were analyzed with GC-MS. The results are presented in Table 4. The GC-MS analysis indicated that the majority of the poppy flower essential oil components were not volatile (Table 4). It was determined that the main components were n-nonadecane (8.96 %), heneicosane (10.83 %), n-pentacosane (7.91 %), n-heptacosane (5.19 %), 1-heptacosanol (4.09 %), palmitic acid (7.26 %) and 1-nonadecanol (16.31 %).

Table 4. The components of the poppy flower essential oil and relative percentages

Peak No	Retention time	Components	Relative percentage
1	23.22	C ₉ n-Pelargonaldehyde	3.47
2	32.84	C ₁₇ n-Heptadecane	3.26
3	38.12	C ₁₉ n-Nonadecane	8.96
4	43.20	C ₂₁ Heneicosane	10.83
5	46.84	C ₁₉ 1-Nonadecanol	16.31
6	47.83	C ₂₃ n-Tricosane	1.39
7	51.36	C ₂₂ n-Docosane	1.69

8	52.12	**	1.97
9	52.49	C ₂₅ n-Pentacosane	7.91
10	58.73	C ₂₇ n-Heptacosane	5.19
11	59.18	C ₂₇ 1-Heptacosanol	4.09
12	66.00	C ₁₆ Palmitic acid	7.26
13	67.85	C ₂₉ Nonacosanol	1.42
14	72.24	**	7.62
15	95.73	**	1.48
16	97.15	**	1.11

As shown in Table 4, long and straight chained hydrocarbon fractions and saturated hydrocarbons with high molecular weight as well as aldehydes and alcohols with high molecular weight were also found in the poppy flower essential oil structure. Analysis of the composition of poppy flower oil demonstrated that the number of single carbon compounds (C₉-C₂₉) was higher than that of double carbon compounds (C₁₆, C₂₂). It was determined that the poppy flower oil was mostly in the form of stearoptene (the solid part of the essential oil) rather than volatile oil. These results were similar to the compounds found in the GC and GC-MS analysis of the solid residue of rose concrete and absolute conducted in 2005 by Aycı *et al.* In contrast, components of the poppy oil included hydrocarbons with lower carbon count, aldehydes, and alcohols. These aldehydes and alcohols provided the characteristic odour of the poppy flower oil. Similar results were found in a study by Mladenova *et al.* (1983) on the solid residue of Bulgarian rose concrete.

3.3 Saturated and Unsaturated Fatty Acids in Poppy Flower Essential Oil

The essential oil fatty acid content was determined by GC. The opium poppy volatile oil was first transformed into methyl esters with the BF₃ methanol method. The fatty acid methyl esters standard (37 Component FAME mix) was used to determine the fatty acid content. Fatty acids in the essential oil were determined by comparing the poppy flower oil peaks with fatty acid standard peaks. The fatty acid composition of the poppy flower oil is presented in Table 5. As shown in Table 5, poppy flower oil was included saturated and unsaturated fatty acids. It was found that the double-carbon fatty acid count was higher than the single carbon fatty acids. The fatty acid

components in the poppy flower oil were compared to those of the Eastern Anatolian poppy seed (*Papaver bractearum*). It was found that there were similarities between the poppy flower fatty acids and Eastern Anatolia poppy seed fatty acids. The main fatty acid content of the Eastern Anatolia poppy included palmitic acid, stearic acid, oleic acid, and linoleic acid. It was found that these fatty acids were also present in the poppy flower essential oil (Şen et al. 2008).

3.4 Antibacterial effect of the poppy flower essential oil

Antibacterial effect of the poppy flower essential oil was determined by the disc diffusion technique. The results are presented in Table 6. It was demonstrated that the poppy extract exhibited antimicrobial activities on both Gram (+) and Gram (-) bacteria. The highest antimicrobial effect was observed on *Micrococcus luteus* (ATCC-9341) with 12 mm. This was followed by the effects on *Proteus vulgaris* (ATCC-13315) (10 mm) and *Klebsiella pneumoniae* (ATCC-700603) (8 mm) (Table 6). In a study conducted on *Papaver macrostomum* extracts by Khanafari et al. (2013), it was reported that these extracts had antimicrobial properties against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, yeasts and *A. niger*, and flower extracts could be used in bedsores. Manju et al. (2011) investigated the antimicrobial effect of *Papaver somniferum* ethanol extracts by disk diffusion technique and reported that they could be used in the treatment of diseases caused by various microorganisms since they have antimicrobial, anti-inflammatory, and anti-

Table 6. Findings on antibacterial properties of the poppy flower essential oil

Test Organism	MEM	AMC	CRO	VA	P	DMSO	Papaver Somniferum
Gram (-)							
<i>Salmonella typhimurium</i> (NRRLB-4420)	37	12	15			-	6
<i>Proteus vulgaris</i> (ATCC-13315)	35	25	35			-	10
<i>Klebsiella pneumoniae</i> (ATCC-700-603)	27	13	21			-	8

helminthic effects. Çağlayan et al. (2009) determined that the ether and diethyl-ether extracts of *Papaver* (*Papaveraceae*) species exhibited antimicrobial activities against *Staphylococcus aureus* (MIC: 9.76 and 19.52 [µg] / mL).

Table 5. Saturated and unsaturated fatty acids in poppy flower essential oil

Peak No	Karbon number	Fatty acids
1	C _{13:0}	Tridecanoic acid
2	C _{14:0}	Myristic acid
3	C _{16:0}	Palmitic acid
4	C _{18:0}	Stearic acid
5	C _{18:1n9c}	Oleic acid
6	C _{18:2n6c}	Linoleic acid
7	C _{18:3n3}	Linolenic acid
8	C _{20:2n6}	Eicosadienoic acid
9	C _{20:3n3}	Eicosatrienoic acid

3.5 Poppy flower essential oil buyyon microdilution test findings

The findings obtained in the bouillon microdilution test conducted on the poppy flower essential oil are presented in Table 7. It was determined that *Klebsiella pneumoniae* (ATCC-4352) and *Salmonella typhimurium* (NRRLB-4420) were present in minimum inhibition poppy saturated solution, and *Proteus vulgaris* (ATCC-13315) was present in 1/12 dilution (Table 7).

<i>Escherichia coli</i> (ATCC-25922)	30	20	16	-	-	-
<i>Pseudomonas aeruginosa</i> (ATCC-7664)	Dilution Rate 20	-	-	-	-	-
Organism	Saturated solution	1/3D	1/9D	1/12D	1/15D	1/18D
<i>Escherichia coli</i> (ATCC-25922)	+	+ 8	+ 19	+ 12	+-	7
<i>Staphylococcus aureus</i> (ATCC-12228)	+	+ 8	+ 19	+ 10	+-	6
<i>Micrococcus luteus</i> (ATCC-9341)	-	+ 40	+ 36	+ 42	+-	12
<i>Pseudomonas aeruginosa</i> (ATCC-7664)	-	- 9	- 21	- 10	+-	7
<i>Staphylococcus aureus</i> (ATCC-12228)	+	+ 30	+ 20	+ 34	+-	+
Diphenhydramine (D), Clonazepam (MEM), Amoxicillin/clavulanic acid (AMC), Ceftriaxone (CRO), Vancomycin (VA), Penicillin (P)						

(+) Reproductive, (-) No reproduction, (D) Dilution

Table 7. Poppy flower essential oil MIC values obtained in Bouillon Microdilution test

3.6 Antifungal effect of poppy flower essential oil

Findings on the antifungal properties of poppy flower essential oil are presented in Table 8.

Table 8. Antifungal properties of poppy flower essential oil

Test organism	% Inhibition			
	2. Day	4. Day	7. Day	10. Day
<i>Aspergillus flavus</i> (ATCC-10124)	13.06	30.90	17.18	3.19
<i>Penicillium expansum</i> (ATCC-7861)	12.41	40.74	8.03	16.10
<i>Alternaria alternata</i> (ATCC-6663)	3.82	12.55	24.95	0.00
<i>Mucor racemosus</i> (ATCC-42647)	7.69	5.66	5.63	24.50
<i>Cladosporium herborum</i> (ATCC-201364)	6.02	3.94	0.85	12.8

The maximum inhibition was observed in *Aspergillus flavus* (ATCC-10124) and *Penicillium expansum* (ATCC-7861), on the 4th day, while it was observed in *Alternaria alternata* (ATCC-6663) on the 7th day and in *Mucor racemosus* (ATCC-42647) and *Cladosporium herborum* (ATCC-201364) on the 10th day. In general, it was determined that the poppy extract inhibited the mycelial development in the tested fungi (Table 8).

4. Conclusion

In the present study, chemical composition and antimicrobial activities of the essential oil obtained from poppy flowers were determined. The relative rates of the components in the poppy essential oil

were determined by identifying the structure of 13 compounds. The poppy flower essential oil demonstrated the highest antimicrobial effect on *Micrococcus luteus* (ATCC-9341) (12 mm) and *Proteus vulgaris* (ATCC-13315) (10 mm). Concurrently, it was determined that the essential oil had some antimicrobial effects on all fungal species tested. In the current study, the flowers of the poppy plant, the capsule of which is a significant product in Turkey, were investigated as a source of potential antimicrobial compounds, and it is considered that the present study will shed light on future studies.

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