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In vitro evaluation of antimicrobial and antioxidant activity and GC/MS profiling of the endemic species Verbascum cheiranthifolium var. asperulum

Endemik tür Verbascum cheiranthifolium var. asperulum'un antimikrobiyal ve antioksidan aktivitesinin in vitro değerlendirmesi ve GC/MS profil analizi

Begüm BİRGÜL[1*], Handan ŞAPCI SELAMOĞLU[2], Cem VURAL[3,4]

[1]Erciyes Üniversitesi, Fen Bilimleri Enstitüsü, Melikgazi, Kayseri, Türkiye
[2]Department of Agriculture, Develi Hüseyin Sahin Vocational College, Kayseri University, Kayseri, Türkiye
[3]Department of Biology, Faculty of Sciences, Erciyes University, Kayseri, Türkiye
[4]Genome and Stem Cell Center (GENKÖK) Erciyes University, Kayseri, Türkiye

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Sorumlu yazar / *Corresponding author*
Begüm BİRGÜL
e-mail: bgmbrgl@gmail.com

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Abstract

Due to their potential therapeutic applications, there has been a growing fascination in the investigation of natural bioactive compounds from medicinal plants. Verbascum cheiranthifolium var. asperulum, an endemic species in Türkiye, has been studied for its potential antimicrobial and antioxidant characteristics. Various solvents were utilized for the extraction of plant material, and the antimicrobial activity was tested against various antibiotic-resistant bacterial strains and a yeast using the minimum inhibitory concentration (MIC) and agar disc diffusion methods. Additionally, the antioxidant potential was assessed using total phenolic content, the DPPH radical-scavenging assay, and total flavonoid content methods. The extracts have exhibites significant inhibitory effects against several bacterial strains, with the methanol extract showing the most potent antimicrobial activity. The most effective inhibition activity was observed against Staphylococcus aureus at a concentration of 1.56 mg/ml. Gas chromatography-mass spectrometry (GC-MS) analysis showed numerous phytochemical compounds, including phenols, alkaloids, flavonoids, and glycosides. The study highlights the potential of V. cheiranthifolium var. asperulum as a valuable and natural bioactive compound source with antimicrobial and antioxidant characteristics, making it a promising candidate for pharmaceutical, cosmetic, and food industries.

Özet

Son yıllarda, potansiyel terapötik uygulamaları nedeniyle tıbbi bitkilerden doğal biyoaktif bileşikler arayışına artan ilgi gözlenmektedir. Türkiye'ye endemik olan *Verbascum cheiranthifolium var. asperulum*, potansiyel antimikrobiyal ve antioksidan özellikleri için incelenmiştir. Bitki materyali farklı çözücüler kullanılarak ekstrakte edilmiş ve antimikrobiyal aktivite, agar disk difüzyon ve minimum inhibisyon konsantrasyonu (MIC) yöntemleri kullanılarak çeşitli antibiyotik dirençli bakteri suşlarına ve bir mayaya karşı test edilmiştir. Ayrıca antioksidan potansiyel, DPPH radikal yakalama testi, toplam flavonoid içeriği ve toplam fenolik içerik yöntemleri kullanılarak değerlendirilmiştir. Ekstraktlar birçok bakteri suşuna karşı önemli inhibe edici etkiler göstermiş, metanol özütünün en güçlü antimikrobiyal aktiviteye sahip olduğu gözlemlenmiştir. En etkili inhibisyon aktivitesi 1.56 mg/ml konsantrasyonunda *Staphylococcus aureus*'a karşı göstermiştir. Gaz kromatografi- kütle spektrometresi (GC-MS) analizi, fenoller, flavonoidler, alkaloitler ve glikozitler dâhil olmak üzere birçok fitokimyasal bileşiğin varlığını ortaya koymuştur. Bu çalışma, *V. cheiranthifolium var. asperulum*'un doğal antimikrobiyal ve antioksidan özelliklere sahip doğal biyoaktif bileşiklerin değerli bir kaynağı olarak potansiyelini vurgulamaktadır ve bu türü, farmasötik, kozmetik ve gıda endüstrileri için umut vadeden bir aday haline getirmektedir.

INTRODUCTION

Due to their potential therapeutic applications, there has been a growing attention to the investigation of natural bioactive compounds from plant sources. Medicinal plants, particularly endemic species, have gained attention as valuable reservoirs of diverse secondary metabolites with significant antimicrobial and antioxidant characteristics (Kalarija et al. 2018, Vaou et al. 2021). These bioactive compounds hold immense promise as potential alternatives to conventional antibiotics and antioxidants, especially in the face of emerging drug-resistant pathogens and increasing oxidative stress-related diseases. Because of their therapeutic potential,

humans have used medicinal plants in traditional medicines for centuries (Alan et al. 2009, Vital and Rivera 2009, Yabalak et al. 2020, Punetha et al. 2022). Approximately 80% of people worldwide consider traditional medicine their main healthcare provider. As a result, it is critical to look for bioactive compounds in medicinal plants or conduct additional pharmacological studies. Secondary metabolites, present abundantly in plants, display various biological effects (De-Fatima et al. 2006, Saltan et al. 2011). The distinctiveness of Türkiye arises from its exceptional representation as the exclusive nation substantially covered by three out of the world's 34 biodiversity hotspots (Iran-Anatolian, Caucasus, and Mediterranean), thereby showcasing remarkable ecological variety throughout its jurisdiction (Parolly and Tan 2007, Şekercioğlu et al. 2011). As a result, Türkiye is one of the most important biodiversity hotspots on the planet. More than 30% of its natural vascular plant taxa are unique to Türkiye (Davis 1971, Avcı 2005). *Verbascum* L. belongs to the Scrophulariaceae family and has 242 species divided into 13 groups and 129 hybrids (Huber-Morath 1978, Yılmaz and Dane 2008), and 192 of them are native to Turkish Flora (Huber Davis et al. 1987, Ekim 2000, Kaynak et al. 2006, Özhatay 2006, Karavelioğulları and Aytaç 2008). Some *Verbascum* species including *V. thapsus* L., *V. densiflorum* Bertol., *V. phlomoides* L., *V. bugulifolium* Lam., *V. prusianum* Boiss., *V. olympicum* Boiss., *V. bombyciferum* Boiss., *V. salviifolium* Boiss. are being studied and used in different traditional medicine to treat rheumatic disorders, eczema, spasmodic coughs (Sutor'y 2001, Dülger et al. 2002, Karavelioğulları et al. 2004, Sutor'y 2004, Karavelioğulları et al. 2006, Klimek et al. 2010, Gökmen et al. 2020, Prakash and Sagar 2021). *Verbascum cheiranthifolium* var. *asperulum* is a widespread endemic species of the genus *Verbascum* distributed in Türkiye (Küçük et al. 2016). This study aimed to investigate the antioxidant and antimicrobial characteristics of *V. cheiranthifolium* var. *asperulum* (Figure 1). The total phenolic content, DPPH radical-scavenging assay, and total flavonoid content methods were used to assess antioxidant activity. Antimicrobial activity against various prevalent human pathogenic bacterial strains was determined with the aid of agar disc diffusion and MIC assays. The phytochemical components of *V. cheiranthifolium var. asperulum* were also identified using GC-MS.

MATERIAL AND METHOD

Plant Material and Preparation of Extracts

Fresh leaves of *V. cheiranthifolium* var. *asperulum* were collected in Kayseri. The leaves were air dried and cut into small pieces. Hexane, methanol, and chloroform were used to extract the powdered substance. A rotavapor (Heidolph Laborota 4000) was used to concentrate the extract at a pressure less than 50 °C.

Bacterial Strains

The inhibitory effects of V. *cheiranthifolium* var. *asperulum* on eight gram-positive bacteria, six gram-negative bacteria, and one fungus were investigated. The test organism used were "*Enterococcus faecalis* ATCC 29212 (gram-possitive)", "*Staphylococcus aureus* ATCC 25923 (gram-possitive)", "*Pseudomonas fluorescens* ATCC 49838 (gram-negative)", "*Staphylococcus epidermidis* ATCC 12228 (gram-possitive)", "*Micrococcus luteus ATCC 10240* (gram-possitive)", "*Listeria monocytogenes* ATCC 19115 (gram-possitive)", "*Klebsiella pneumonia* ATCC 13883 (gram-negative)", "*Bacillus cereus* ATCC 11778 (gram-possitive)", "*Escherichia coli* ATCC 25922 (gram-negative)", "*Enterobacter aerogenes* ATCC 13048 (gram-negative)", "*Proteus mirabilis* ATCC 25933 (gram-negative)", "*Bacillus subtilis* ATCC 6633 (gram-possitive)", "*Corynebacterium renale*ATCC 19412 (gram-possitive)", "*Pseudomonas aeruginosa* ATCC 27853 (gram-negative)", and "*Candida albicans* ATCC 90028". A deliberate choice was made to include human pathogens that exhibit resistance to antibiotics or are resistant to multiple antibiotics.

Antimicrobial Activity

The techniques employed to estimate the antimicrobial activity of *V. cheiranthifolium* var. *asperulum* encompassed agar disc diffusion and MIC. The disc diffusion tests were conducted under the National Committee for Clinical Laboratory Standards methodology (Vural and Aydoğdu 1993, NCCLS 1997 and

1999). Dimethyl sulfoxide (DMSO) was added to the dried plant extracts for dissolution. Tryptic Soy Broth (TSB) was used to suspend the bacterial strains, and the fungal strains were suspended in Yeast peptone Dextrose Broth (YPDB). The optical density was set to 0.1 at a wavelength of 570 nm using a Shimadzu UV 1800 spectrophotometer. The microorganism under investigation was dispersed onto the surface of the solid media plates. An amount of 30 µl of the extracts was absorbed by a Whatman paper disc measuring 1 mm in thickness. Subsequently, the infected discs were positioned on the plate. After being exposed to 4 °C for 2 hours, the bacteria were later incubated at 37 °C for 24 hours. The negative control in this study consisted of pure dimethyl sulfoxide (DMSO), while the positive control included conventional antibiotics such as ampicillin, tetracycline, and nystatin. In millimeters, the inhibitory zones were measured. The experiments were conducted in triplicate.

The National Committee for Clinical Laboratory Standards technique was employed to conduct Minimum Inhibition Concentration (MIC) testing, utilizing sterile 96-well microplates (Karavelioğulları et al. 2004). Extracts were made at 1000 µg/ml concentrations for chloroform, methanol, and hexane solvents. Subsequently, the obtained extracts were transferred to individual wells of a microplate to achieve a serial dilution with two folds, ranging from 1:2 to 1:64. The experiments were conducted using Mueller Hinton Broth (MHB) as the test medium, with the exception of the yeast, which was cultured in YPDB. Incubation of the microplates occurred at 37 °C for 24 hours in the case of bacteria, and 48 hours for yeast. The growth of bacteria was observed by forming a white "pellet" at the bottom of the well.

Antioxidant Activity

The antioxidant potential of *V. cheiranthifolium var. asperulum* was assessed by the utilization of the DPPH radical-scavenging assay and the determination of the total flavonoid and total phenolic content. The DPPH radical-scavenging assay was conducted following the protocol outlined by Cuendet et al. (1997). In this experiment, dimethyl sulfoxide (DMSO) was incorporated at a concentration of 0.2 percent (v/v), and to achieve a final volume of 3 ml, a 100% ethanol solution was introduced, altering the overall quantity. After 30 minutes of incubation at ambient temperature, absorbance was measured at a wavelength of 517 nm relative to a blank sample. The extract was ultimately weakened to 200 µg/ml concentration. The ethanol sample was assessed as if it were a blank. The computation of DPPH radical inhibition was carried out by employing the formula I (percent) = 100 x (A0-AS)/A0, where A0 represents the absorbance of the control solution containing all reagents except for the test substance, and AS represents the absorbance of the test sample. BHT was employed as a positive control in this study. The IC_{50} value, representing the percentage of inhibition, was computed. The experiment was conducted three times, and the average value was recorded.

The total phenolic content quantification was conducted by employing the Folin Ciocalteu reagent, as indicated in 28. A 10-ml reaction mixture was prepared using 0.06 mg/ml extracts of *V. cheiranthifolium* var. *asperulum*, 0.5 ml of Folin Ciocalteu reagent, and 0.05 ml of 10% Na_2CO_3. The solution was permitted to remain undisturbed at ambient temperature for 2 hours. Following this, the mixture was analyzed by measuring absorbance at 760 nm. To establish a control, a Folin Ciocalteu reagent including a 10% concentration of Na_2CO_3 was employed. The standard utilized in the experiment was Gallic acid. The quantification of the overall phenolic content in the extracts was conducted by measuring the amount of gallic acid equivalents found in one gram of the extract being tested.

The quantification of the flavonoid concentration in the extracts was conducted utilizing the aluminum chloride colorimetric technique (Chang et al. 2002). A solution was prepared by combining 10 mg/ml extracts of *V. cheiranthifolium* var. *asperulum* with 1.5 ml of ethanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of potassium acetate (1 M), and 2.8 ml of distilled water. The combination was permitted to remain at room temperature for 40 minutes. Afterwards, the absorbance of the mixture was determined at 417 nm. Quercetin was employed as a reference compound.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The chemical composition of *V. cheiranthifolium* var. *asperulum* extracts in methanol, chloroform, and hexane was determined using GC-MS (Shimadzu QP2010 Ultra). The conditions pertaining to the experiment using the GC-MS system were outlined as follows: column measuring 30m, inner diameter of 0.25 mm, and film thickness of 0.25 µm. At a rate of 0.90 ml/min, helium served as the carrier gas. Initially, the oven temperature was sustained at 60 °C for 2 minutes, and subsequently escalated to 280 °C with a heating rate of 1 °C per minute.

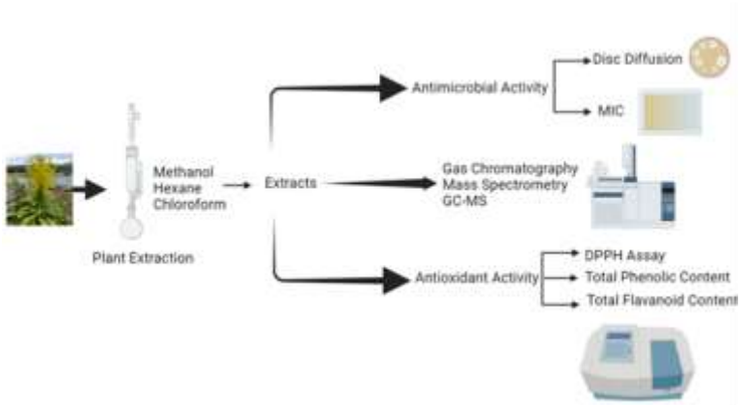


Figure 1. Layout of GC-MS analysis, antioxidant and antimicrobial activity of *V. cheiranthifolium* var. *asperulum*

RESULT AND DISCUSSION

The study conducted in vitro experiments to investigate the antioxidant (DPPH, total flavonoid concentration, and total phenolic content) and antibacterial properties of extracts obtained from *Verbascum cheiranthifolium* var. *asperulum* using hexane, methanol, and chloroform solvents. The methanol extracts yielded a proportion of 19.23% of usable compounds, while chloroform and hexane extracts yielded 2.743% and 2.213% of useful compounds, respectively.

Antimicrobial Activity

This study evaluated the antibacterial activity of *V. cheiranthifolium var. asperulum* extracts using the MIC and agar disc diffusion methods in vitro. A total of 14 bacterial strains and one yeast strain were subjected to antimicrobial testing. The in vitro assessment of the antibacterial activity of *V. cheiranthifolium* var. *asperulum* extracts involved agar disc diffusion and the MIC method was assessed to evaluate their effects on gram-positive and gram-negative bacteria strains. The findings of the disc diffusion and minimum inhibitory concentration (MIC) experiments are presented in Table 1 and 2.

The disc diffusion assay outcome demonstrated that the extracts obtained from *V. cheiranthifolium* var. *asperulum* had inhibitory effects on the growth of *S. aureus, S. epidermidis, B. cereus,* and *K. pneumoniae*. The hexane extract demonstrates a higher degree of inhibition compared to the other disc diffusion assay extracts. The inhibitory effects of methanol, hexane, and chloroform extracts of *V. cheiranthifolium* var. *asperulum* against various bacteria were observed at varied concentrations, as indicated by the minimum inhibitory concentration (MIC) results presented in Table 2. The methanol extracts derived from *V. cheiranthifolium* var. *asperulum* had the most potent inhibitory effect against *S. aureus* at a 1.56 mg/ml concentration. Conversely, the chloroform extracts demonstrated the highest inhibitory activity against *E. aerogenes* at 8.95 mg/ml. Furthermore, it was observed that hexane extracts derived from *V. cheiranthifolium* var. *asperulum* exhibited the highest level of inhibitory efficacy against *L. monocytogenes*. Table 1-2 displays the outcomes of the disc diffusion and MIC experiments.

The efficacy of *V. cheiranthifolium* var. *asperulum* in inhibiting the growth of eight gram-positive bacteria, six gram-negative bacteria, and one fungus was investigated. The test organism used were "*Staphylococcus aureus* ATCC 25923", "*Enterococcus faecalis* ATCC 29212", "*Pseudomonas fluorescens* ATCC 49838",

"*Staphylococcus epidermidis* ATCC 12228", "*Micrococcus luteus ATCC 10240*", "*Listeria monocytogenes* ATCC 19115", "*Bacillus cereus* ATCC 11778", "*Klebsiella pneumonia* ATCC 13883", "*Enterobacter aerogenes* ATCC 13048", "*Escherichia coli* ATCC 25922", "*Bacillus subtilis* ATCC 6633", "*Proteus mirabilis* ATCC 25933", "*Pseudomonas aeruginosa* ATCC 27853", "*Corynebacterium renale*ATCC 19412", and "*Candida albicans* ATCC 90028". The human pathogens selected were resistant to antibiotics or had multiple resistances (Table 2).

Ozcan et al. (2011) examined the antimicrobial activities of extracts from *Verbascum pinetorum* samples and reported that they had varying effects on different microorganisms tested. Sen-Utsukarcı et al. (2018) extracted samples from five *Verbascum* species (*V. densiflorum* Bertol., *V. gnaphalodes* Bieb., *V. lagurus*, *V. phlomoides L*., *V. xanthophoeniceum* Griseb.) using methanol, petroleum ether, toluene, chloroform, and ethyl acetate. They tested the antimicrobial activities of all extracts against resistant bacteria and yeasts and reported that the extracts (except for *V. xanthophoeniceum* - petroleum ether) had varying levels of antibacterial activity against all bacteria. Arabacı et al. (2021) conducted studies to determine the antimicrobial activities of methanol extracts from *Verbascum thapsus* and found that the extracts exhibited inhibition zones ranging from 8 to 25 mm. The findings of our study, indicating the antimicrobial effects of extracts from *V. cheiranthifolium var. asperulum* samples, parallel with studies conducted on other *Verbascum* species.

Table 1. Results of antimicrobial activity: diameter of ınhibition zone in disc diffusion assay (mm)

Extracts of *V. cheiranthifolium* var. *asperulum*				**Antibiotics**		
	Hexane	**Methanol**	**Chloroform**	**Trimethoprim (30 µg/disk)**	**Tetracycline (30 µg/disk)**	**Ampicillin 50 µg/disk**
S. aureus	-	7.7	-	25.6	20.09	52.4
E.faecalis	-	-	-	26.1	8.3	32.5
P. fluores.	-	-	-	-	13.6	-
S. epider.	-	11.9	-	38.8	-	20.06
M. luteus	-	-	-	33.2	26.3	43.5
L. monoc.	-	-	-	40	28.2	33.8
B. cereus	9.5	10.0	-	-	23.3	10.6
K. pneum.	-	8.7	-	29	20.5	12.9
E. aeroge.	-	-	-	25.2	17	-
E. coli	-	-	-	26.4	12.5	23.1
B. subtilis	-	-	-	39.7	25.3	-
P. mirabi.	-	-	-	20.2	-	28.3
P. aerogi.	-	-	-	-	8.9	-
C. renale	-	-	-	29.2	28.4	60.2
C. albicans	-	-	-	**Nystatin (30 µg/disk)**		**Miconazole (40 µg/disk)**
				17.4		23.7

-: No inhibition zone. (A: "ampicillin", T: "tetracycline", N: "nystatin")

Table 2. Results for antimicrobial potency: MIC analysis (mg/ml)

Extracts of *V. cheiranthifolium* var. *asperulum*			
	Hexane	**Methanol**	**Chloroform**
S. aureus	25	1.56	71.6
E. faecalis	25	50	71.6
P. fluorescens	12.5	50	71.6
S. epidermidis	12.5	50	71.6
M. luteus	6.25	50	71.6
L. monocytogenes	3.13	50	71.6
B. cereus	6.25	50	71.6
K. pneumoniae	12.5	50	71.6
E. aerogenes	12.5	12.5	8.95
E. coli	12.5	12.5	17.9
B. subtilis	25	12.5	17.9
P. mirabilis	6.25	25	17.9
P. aeroginosa	6.25	25	17.9
C. renale	6.25	25	17.9
C. albicans	6.25	12.5	17.9

-: No MIC value measured. (A: "ampicillin", T: "tetracycline", N: "nystatin")

Antioxidant Activity

Table 3 exhibits the effect of V. *cheiranthifolium* var. *asperulum* and BHT, a synthetic antioxidant, on the scavenging of DPPH free radicals. The results of the analysis indicated that the hexane extract of *V. cheiranthifolium var. asperulum* exhibited DPPH scavenging activity. However, significant DPPH scavenging activity was not detected in the chloroform and methanol extracts.

By utilizing the Folin-Ciocalteu reagent, the researchers estimated the total phenolic content. The amount of phenolic compounds in the various fractions of *V. cheiranthifolium var. asperulum* was influenced by the solvent used and represented in terms of milligrams of Gallic acid equivalent (GAE). The hexane, chloroform, and methanol extracts of *V. cheiranthifolium* var. *asperulum* had phenolic contents of 59.2, 49.5, and 12.7 mg Gallic acid equivalent/g, respectively, as shown in Table 3. The hexane presented the greatest phenol concentration. The lowest phenol concentration was detected in the methanol extracts.

Utilizing the aluminum chloride colorimetric approach, the determination of the total flavonoid content was conducted. The flavonoid content of different fractions of *V. cheiranthifolium var. asperulum* was reliant on the solvent and indicated in milligrams corresponding to Quercetin. The total flavonoid contents of the chloroform, methanol, and hexane extracts of. *V. cheiranthifolium var. asperulum* were found to be 58, 18, and 17.3 mg Quercetin equivalent/g, respectively, as shown in Table 3. The highest concentration of flavonoid was detected in the chloroform extract.

Jamshidi-Kia et al. (2020) examined the four *Verbascum* species' (V. *songaricum, V. macrocarpum, V. pseudo-digitalis, V. sinuatum*) hydro-ethanolic extracts' phenolic profiles and antioxidant activities. They found that the total phenolic component values ranged from 27.35 to 55.62 mg GAE/g, while the total flavonoid component values ranged from 10.01 to 19.88 mg RE/g. The antioxidant capacity was analyzed using the DPPH radical, and they reported IC_{50} values ranging from 45.12 to 226.62 µg/ml. Ozcan et al. (2011) examined the antioxidant capacity of methanol extracts from *V. pinetorum* samples using the DPPH radical and calculated the IC_{50} value of *V. pinetorum*'s methanol extracts as 13.04 mg/ml. Hazman et al. (2021), in their studies on the antioxidant activities of acetone and methanol extracts of *Verbascum lasianthum*, calculated total phenolic component values of 64.47 µg GAE/mg and 30.82 µg GAE/mg, respectively. They found that the IC_{50} values for antioxidant capacities were 17.07 mg/ml and 6.44 mg/ml, respectively. The literature review is consistent with our study.

Table 3. DPPH radical scavenging activity, Total phenolic and total flavanoid content of extracts of *V. cheiranthifolium* var. *asperulum*

Solvent	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	DPPH radical scavenging activity (IC_{50}) (mg/ml)
Hexane extract	59.2	17.3	38.64 ±0.003
Chloroform extract	49.5	58	-
Methanol extract	12.7	18	-
BHT	-	-	48.03 ±0.24

GC-MS Analysis

The utilization of GC-MS for phytochemical analysis is considered a very sensitive identification approach due to its rapid screening rate and exceptional mass accuracy. The GC-MS analysis revealed that the hexane extract of *V. cheiranthifolium var. asperulum* had a total of 39 phytochemical compounds, whereas the methanol extract contained 20 phytochemical compounds. The chloroform extract contains a total of 38 phytochemical components. The investigation of *Verbascum* extracts using GC-MS identified a multitude of noteworthy chemicals. The methanol extract derived from *V. cheiranthifolium* var. *asperulum* exhibited the lowest number of bioactive chemicals. The primary constituents identified in the extracts consist of fatty acids and esters, as presented in Table 4. Several substances, including ginsenol, were identified using GC-MS analysis and have been documented to possess antiviral and antifungal properties (Volobueva et al. 2021). Moreover, a significant abundance (36.6%) of hexadenoic acid methyl ester was observed, namely in the methanol extract. According to Suseem and Mary Saral (2013), it has been demonstrated that hexadecenoic acid methyl ester, 9-octadecanone, octadecanoic acid, and octadenoic acid methyl ester possess anti-inflammatory and anti-arthritic characteristics. Taşgın and Asil (2021) conducted a GC-MS analysis to determine the volatile components of the endemic *V. amanum* and *V. caesareum* species. When they examined the volatile compounds they found, they

reported that these compounds possessed medicinal plant properties due to their bioactivity, pharmaceutical, and pharmacological characteristics. Nadeem et al. (2021) conducted a GC-MS analysis of extracts from *V. thapsus* and found significant components such as Hexadecanoic acid methyl ester, Stigmasterol (with antibacterial activity), and 2(5H)-Furanone (an appetite suppressant). This study highlighted the notable antibacterial and antioxidant potential of *Verbascum* thapsus extracts. Furthermore, with the use of GC-MS, various chemicals like glycerin, oxalic acid, coumarine, heptadecane, and tetradecane were determined to be present in *V. cheiranthifolium* var. *asperulum*. Numerous research have also been published regarding the advantageous properties exhibited by these phenolics in various applications such as surfactants, detergents, plasticizers, softeners, cosmetic bases, and medicines.

Table 4. Chemical compounds identified in extracts of *V. cheiranthifolium* var. *asperulum* by GC-MS.

		Chloroform		Hexane		Methanol	
	Compound name	**Area%**	**RT**	**Area%**	**RT**	**Area%**	**RT**
1	"2,2-Dimethoxybutane"	0.32	4.03	0.93	4.02		
2	"9-Octadecanone"	1.26	41.36	-	-	-	-
3	"5-Hyodroxy-2-Decenoic AcidLactone"	0.45	41.65	-	-	-	-
4	"1-Dodecanol, 3,7,11-trimethyl-"	0.65	42.30	-	-	-	-
5	"Hexadecanal"	2.56	45.55	3.11	45.70	-	-
6	"2-Pentadecanone, 6,10,14-trimethyl"	4.39	46.60	3.37	46.64	-	-
7	"1-Tetradecanol"	0.16	48.11	-	-	-	-
8	"Hexadecanoicacid, methyl ester"	5.92	49.37	14.90	49.42	36.66	49.38
9	"Methylstrearate"	3.62	55.83	-	-	14.74	55.86
10	"Palmitaldehyde, diallylacetal"	2.02	56.43	-	-	-	-
11	"Hexen-1-ylcyclohexane"	0.80	56.64	-	-	-	-
12	"Octadecanoicacid"	7.31	57.37	-	-	-	-
13	"Fumaricacid, 3-methylbut-2-yl undecyl ester"	1.97	61.05	-	-	-	-
14	"Dodecanamide, N-(2-hydroxyethyl)"	2.58	62.40	-	-	-	-
15	"(-)-Loliolide"	0.53	64.83	-	-	-	-
16	"Methyl 3-oxo-5-(1-nitro-2-oxocycloheptyl) pentanoate"	0.87	66.52	-	-	-	-
17	"Isolongifol-8-ol"	2.15	66.78	-	-	-	-
18	"1,5-Dithiaspirol [5,5] undecane-2-pentanoic acid, 10-methyl-7-(1-methylethyl)"	0.24	67.05	-	-	-	-
19	"2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-(1-cyclohexen-1-yl)-5-ethyl-	1.25	67.81	-	-	-	-
20	Di-n-octylphthalate"	4.25	67.96	-	-	-	-
21	"i-Propyl 9-tetradecenoate"	0.26	69.33	-	-	-	-
22	"9-epi-19-norambrox"	1.24	69.59	-	-	-	-
23	"Glycerine-1,3-diolen"	0.53	69.69	-	-	-	-
24	"(3aS,9aS,9bR)-6,6,9aa-trimethyl-cis-perhydronaphtho[2,1-b]furan"	4.92	70.80	-	-	0.82	75.32
25	"Oxalicacid, 6-ethyloct-3-yl propyl ester"	7.20	71.00	-	-	-	-
26	"14-Beta-H-Pregna"	9.88	71.08	1.73	74.36		
27	"Coumarine, 3,4,5,6-tetrahydro-6,8a-epidioxy-4a-methyl"	4.34	71.54	-	-	-	-
28	"4-(1,1-Dimethyl-propyl)-2,6-bis-thiophen-2ylmethylene-cyclohexanone"	2.18	72.23	-	-	-	-
29	"1-(Hydroxymethyl)-2,5,5,8A-Tetramethyldecahydro-2-Naphthalenol"	3.17	72.51	-	-	-	-
30	"Driman-8,11-diol"	0.59	72.71	-	-	-	-
31	"1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester"	3.62	73.96	-	-	-	-
32	"Borinicacid, diethyl-, 1-cyclododecen-1-1yl-ester"	3.62	73.99	-	-	-	-
33	"N-(2-Acetylcyclopentylidene) cyclohexylamine"	1.07	74.47	-	-	-	-
34	"Ginsenol"	4.47	75.03	0.31	73.78	0.82	74.08
35	"Desacyl-Kondurangogenins A"	0.20	75.16	0.88	74.74	1.91	72.98
36	"2,2,6,8,12-Pentamethyl-7,9,10,trioxa-tricyclo[6.2.2.0(1,6)] dodec-11-ene"	6.49	75.55	-	-	-	-
37	"1,4-Benzenediol, 2,5-bis (1,1-d,methylethyl)"	1.73	75.77	0.88	70.92	-	-
38	" (1S,2S,5R)-1-(3-[2-(1S,2S,5R)-1-Hydroxy-2-2isopropyl-5-methylcyclohexyl] but-3-enyl)-2-isopropyl-5-methylcylohexan-1-ol"	1.18	75.85	-	-	-	-
39	"3,3-Dimethoxy-2-butanone"	-	-	0.33	5.78	-	-
40	"Tetradecanol"	-	-	1.50	39.12	-	-
41	"Neophytadiene"	-	-	2.68	47.16	-	-
42	"3,7,11,15-Tetramethyl-2-hexadecen-1-ol"	-	-	6.72	47.79	-	-
43	"n-pentadecan"	-	-	2.28	54.66	-	-
44	"Octadecanoicacid, methyl ester"	-	-	8.17	55.87	30.16	55.09
45	"Heptadecane"		-	3.12	57.89	-	-
46	"2,5-Furandione, 3-dodecyl-"	-	-	0.34	66.17	-	-
47	"Naphtho[2,1-d][1,3] dioxepin, dodecahydro-5a,8,8,11a-tetramethyl-,[5aR-(5a.alpha.,7a.beta.,11a.alpha.,11b.beta9]"	-	-	0.35	67.59	-	-
48	"Odoroside-H"	-	-	0.45	68.40	-	-
49	"2,6-di-t-butyloctahydroazulene-3a,8-diol"	-	-	2.63	69.66	-	-

Table 4(continued)

50	"10-Hydroxydecanoic acid"	-	-	1.45	69.74	-	-
51	"Capnellane-5.Alpha-ol"	-	-	1.28	70.34	-	-
52	"1-Bromoeicosane"	-	-	1.31	70.97	-	-
53	"AllylCyclohexylcarbonate"	-	-	5.24	71.12	2.84	71.44
54	"5-hydroxy-6,7-dehydromatrine"	-	-	0.79	71.63	-	-
55	"Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene"	-	-	1.08	72.18	-	-
56	"17.alfa.,21.beta.-28,30-Bisnorhopane"	-	-	0.60	72.30	-	-
57	"2H-3,9a-Methano-1benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9alpha,9a.alpha.)]"	-	-	4.52	72.59	-	-
58	"Propiolicacid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-, ethyl ester"	-	-	0.37	72.66	-	-
59	"1-monolinoleoylglycerol trimethylsilylether"	-	-	2.79	72.91	1.48	70.34
60	"2-(N-Methylpyrrolyl) thienoate"	-	-	1.43	72.97	-	-
61	" (+-)-1-(3,4,4a.alpha.,5,6,7,8,8octahydro-2,8a.brta.-dimethyl-1-naphthyl)-3-hexanol"	-	-	1.28	73.11	-	-
62	"1,5,9,9-Tetramethyl-2-oxatricyclo[6.4.0.0(4,8)] dodecane"	-	-	0.83	73.26	-	-
63	".alpha.-Tocopherylacetate"	-	-	7.82	73.62	-	-
64	"2,5-Furandione, 3-dodecyl-"	-	-	0.93	73.68	-	-
65	"2-Myristynoyl-glycinamide"	-	-	4.08	75.44	-	-
66	"6H-Furol[2',3',4,5]oxazolo[3,2-a] pyrimidin-6-one, 3-(acetyloxy)-2-[(acetyloxy) methyl)]-2,3,3a,9a-tetrhydro-"	-	-	4.89	75.56	-	-
67	"Acetamide, N-86-acetylaminobenzothiazol-2-yl)2-(adamantan-1-yl)- "	-	-	1.83	75.84	-	-
68	"14.alpha.-Cheilanth-12-enic Methyl Ester"	-	-	0.83	76.00	0.64	74.73
69	"2-N-Butyl-3-N-Hexyldecahydronaphthalene"	-	-	-	-	0.99	61.68
70	"1H-Indene, 5-decyloctahydro"	-	-	-	-	0.69	71.57
71	"Cholesta-5,17 (20)-dien-3-ol, acetate, (3.beta., 17E)- "	-	-	-	-	0.75	72.52
72	"1,2-di-but-2-enyl-cyclohexane"	-	-	-	-	1.96	72.76
73	"Cheilanth-13(14)-enicMethyl Ester"	-	-	-	-	0.74	74.11
74	"2,2 Dimethyl-6-methylene-1-[3,5-dixydroxy-1-pentenyl] cyclohexan-1-perhydrol"	-	-	-	-	0.86	74.31
75	"7-(2-Hydroxy-1-methylethyl)-1,4a-dimethyldecahydronaphthalen-2-ol"	-	-	-	-	0.76	74.67
76	"7-Heptadecanol, 7-methyl-"	-	-	-	-	1.20	75.58
77	"Hexabarbital"	-	-	-	-	0.66	76.09

CONCLUSION

The photochemical composition and antimicrobial and antioxidant activity of *V. cheiranthifolium* var. *asperulum* were investigated in this study.

In antimicrobial assays, extracts of *V. cheiranthifolium* var. *asperulum* showed remarkable inhibition against the majority of bacterial strains.

GC-MS analyses revealed significant phytochemical compounds in *V. cheiranthifolium* var. *asperulum* extracts, including phenols, flavonoids, alkaloids, and glycosides.

Finally, our findings indicate that extracts of V. cheiranthifolium var. asperulum may be an important potential antioxidant. Furthermore, *V. cheiranthifolium* var. *asperulum* appears to be a good supplier of ingredients for the pharmaceutical, cosmetic, and food sectors.

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