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Reference to a journal publication:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. Journal of Scientific Communications, 163, 51–59.

Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon, 19, Article e00205.

Reference to a book:

Strunk, W., Jr., White, E.B., 2000. The elements of style (4th ed.). Longman (Chapter 4).

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article. In B.S. Jones, & R.Z. Smith (Eds.), Introduction to the electronic age (pp. 281–304). E-Publishing Inc.

Reference to a website:

Powertech Systems., 2015. Lithium-ion vs lead-acid cost analysis. Retrieved from http://www.powertechsystems.eu/home/tech-corner/lithium-ion-vs-lead-acid-cost-analysis/. Accessed January 6, 2016.

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1.

Reference to a conference paper or poster presentation:

Engle, E.K., Cash, T.F., Jarry, J.L., 2009, November. The Body Image Behaviours Inventory-3: Development and validation of the Body Image Compulsive Actions and Body Image Avoidance Scales. Poster session presentation at the meeting of the Association for Behavioural and Cognitive Therapies, New York, NY.

Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., Molins, S., 2020, March 25. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

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FROM THE EDITOR

International Journal of Plant BasedPharmaceuticals (IJPBP) is a peer-reviewed open access journal for original research articles, review articles and short communications related to all aspects of plant based pharmaceuticals and pharmaceutical analysis. IJPBP was launched in 2021, and published biannually by Kilis 7 Aralik University (Kilis-TURKEY).

Current areas of interest include the following areas related to plant based pharmaceuticals, but are not limited to:

- Analysis of traditional medicine (medicinal materials, patent medicine, prescription, injection)
- Pharmaceutical analysis of complex system
- Quality control and methods of biotech drugs
- Action mechanism and their metabolisms in body
- Quantitative and qualitative analysis in the drug screening process
- Tracer analysis in molecular pharmacology
- Quantitative analysis in biopharmaceutics
- Clinical laboratory and bioanalysis
- Analysis of pharmacological activity
- Analysis of drug toxicity
- Analytical chemistry techniques and methods
- New biochemistry methods for pharmaceutical analysis
- Rapid screening methods
- New analytical techniques and methods
- Others : Pharmaceutical solid materials (including biomaterials, polymers and nanoparticles), Biotechnology products (including genes, peptides, proteins and vaccines), Engineered cells

The journal has a distinguished editorial board with extensive academic qualifications, ensuring that the journal maintains high scientific standards and has a broad international coverage.

IJPBP seeks to publish original, high quality, peer-reviewed papers including original research articles and reviews as well as short communications. Submission would be encouraged on all aspects of plant based pharmaceutical analysis. The aim of this journal is to become a highly respected and trusted resource of leading knowledge in this field and to promote worldwide academic exchange.

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You are welcome to submit your manuscript to IJPBP via our manuscript submission system.

IJPBP is inviting papers for Volume 2 Issue 1, which is scheduled to be published on June, 2022. Last date of submission: May 15, 2022. However, an early submission will get preference in case of review and publication process. Please submit your manuscripts according to instructions for authors by the journal online submission system.
Sincerely,
Prof. Dr. Bektas TEPE, Ph.D.
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REVIEW

OPEN ACCESS

Traditional uses, phytochemistry, and toxic potential of *Teucrium polium* L.: A comprehensive review

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ABSTRACT

The aim of this study was to present information about the traditional use and phytochemistry of *T. polium*, to discuss contradictory views about chemotaxonomy and its toxic effect on liver and kidneys, and to make suggestions about controversial areas and gaps in the literature. Literature data showed that *T. polium* has toxic effect on kidney tissue. Moreover, in some of the studies on the liver and in all clinical reports, *T. polium* has also been proven to have toxic effect on the liver. The components responsible for toxicity are thought to be *neo*-clerodane diterpenoids. However, it has been reported that flavonoids and some polyphenols in the plant also show antioxidant and anti-inflammatory effects. It has been concluded that more attention should be paid to the use of this plant. More clinical studies are needed to better understand the effects of *T. polium* on the liver. The effects of the plant on blood serum parameters and histological changes on the liver tissue should be documented in more detail. It was also concluded that that regular consumption of *T. polium* should be avoided for long periods of time.

1. Introduction

Many sources suggest that the use of plants as medicines is as old as the written history of humanity. However, the use of plants for the treatment of various diseases probably dates back to written history (Gunes et al., 2017). The history of many active medicines today goes back to the Hellenic civilization. In Egyptian Ebers Papyrus, which is thought to date back to 1500 BC, it is known that many medicinal plants are classified according to their therapeutic properties. On the other hand, it is claimed that the Balinese and Assyrians mentioned many herbal remedies such as licorice, cinnamon and coriander. It is also known that in a work written by Chinese doctor Chou Kung in 1100 BC, the use of certain herbal medicines are described. Additionally, Sustruta, published in the early period of Christianity, contains information about 700 herbal medicines. Galen's contribution to herbal medicine is also very valuable. The herbal extract preparation methods developed by Galen are still practiced today with the term 'Galenic' (Al-Asmari et al., 2014).

Traditional medicine is a system of therapeutic methods established by local people within their own belief, socio-cultural values and varies greatly from country to country, even from region to region (Alachkar et al., 2011). Information on herbal products used for medicinal purposes has been transmitted from generation to generation for centuries and highly reliable application systems have been created with increasing experience and constantly changing information in each generation (Hayta et al., 2014). It can be argued that what percentage of the world's population actually uses local and traditional medicines. However, research on the determination of this ratio shows that a significant number of people have great interest in herbal treatment methods. Studies conducted in Australia and the United States show that 34-48.5% of the

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participants benefit from traditional treatment methods at least once in their lifetime (Hasani-Ranjbar et al., 2008). The WHO is working hard to integrate traditional medical methods into official health systems (Alachkar et al., 2011). According to the data published by WHO, approximately 70-80% of the population living in developing countries meets their treatment needs primarily by using medicinal plants (Milosevic-Djordjevic et al., 2018). The main reason for this is thought to be the economic difficulties people face. Because, in developing countries, people who have difficulty in meeting the high pharmaceutical costs are turning to herbal alternatives for health needs (Khader et al., 2010). It is estimated that approximately 50.000-70.000 plant species are used for this purpose in all over the world. Today, the international herbal product market with an annual trade volume of 62 billion dollars is estimated to reach 5 trillion dollars by 2050 (Hayta et al., 2014).

Teucrium (Lamiaceae) is a perennial, polymorphic and cosmopolitan genus, wildly distributed in temperate regions of Europe, especially the Mediterranean, and North Africa (Milosevic-Djordjevic et al., 2018). There are reports that *Teucrium* species are also distributed in Asia, America and Australia (Khaled-Khodja et al., 2014). According to some sources, this genus contains 300 plant species (El Atki et al., 2019b; Khani and Heydarian, 2014; Sadeghi et al., 2014a), while other sources state that there are 340 or more species (Boghrati et al., 2016; De Martino et al., 2010; Sabzeghabaie and Asgarpanah, 2016). *Teucrium* species have evolved considerably in both growth characteristics and aromas through natural hybridization and selection mechanisms (Asgharipour and Shabankare, 2017). They usually grow in dry and stony areas (Khani and Heydarian, 2014). *Teucrium* species have been used as medicinal plants for more than 2000 years because of their therapeutic properties (Hachicha et al., 2009; Hasani-Ranjbar et al., 2010). According to ethnopharmacological records, members of this genus have long been used in the treatment of gastrointestinal problems, absorption disorders, cough, asthma, cognitive disorders, colds, pulmonary diseases, fungal infections and parasitic diseases (Boghrati et al., 2016; Elmasri et al., 2014; Khaled-Khodja et al., 2014; Kovacevic et al., 2001; Menichini et al., 2009; Sabzeghabaie and Asgarpanah, 2016; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017). *Teucrium* species are also known as stimulant, hypoglycemic, hypolipidemic, analgesic, carminative, diuretic, perspiring, amoebicidal, antispasmodic, anti-rheumatic, antiseptic, antihelminic, anti-hypertensive, anti-inflammatory, antipyretic, anti-feedant, anticonvulsant and flavouring agents as well as their use as tonics (De Martino et al., 2010; Elmasri et al., 2014; Grubesic et al., 2012; Hachicha et al., 2009; Hasani-Ranjbar et al., 2010; Khaled-Khodja et al., 2014; Kovacevic et al., 2001; Menichini et al., 2009; Sabzeghabaie and Asgarpanah, 2016; Sadeghi et al., 2014a; Stefkov et al., 2011). These species are also preferred in the preparation of flavored wines, herbal teas and liqueurs (Menichini et al., 2009).

T. polium is a plant species belonging to Ajugoideae subfamily of Lamiaceae family. The name of this plant is known to originate from the union of the Greek terms "teúcrion", in honor of an ancient Trojan king. According to Pliny, the Roman historian and writer, the Trojan king was the first to use this plant for medical purposes. Polium, the species name of the plant, comes from the old Greek word "poliòn". This word is used to indicate that the flower colour of the plant is whitish grey (Venditti et al., 2017). It is known that *T. polium* has some subspecies or varieties such as aurasiacum, pilosum, aragonense, capitatum, gnaphalodes, cylindricum, vincentinum, expansum, polium, yalentinum *(Bahramikia and Yazdanparast, 2012)*.

T. polium is a perennial and aromatic herbaceous plant, of which base has a woody structure (Abadian et al., 2016; Venditti et al., 2017). The plant has round and pubescent stalks. The stem of the plant is erected and can extend up to 10-35 cm (Amraei et al., 2018a; Venditti et al., 2017). The plant can grow up to 30-50 cm (Abadian et al., 2016). The upper parts of the body have a fully branched anatomical appearance. The leaves of the plant are 2 cm long and 4 mm wide (Venditti et al., 2017). Some sources indicate that the leaves may be 1-3 cm long (Afifi et al., 2005; Al-Qudah et al., 2011; Asgharipour and Shabankare, 2017). The lower parts have intact and folded margins; on the contrary, the margins in the upper parts are crenate and outstretched. It blooms in different colours ranging from pink to yellow between April and August (Venditti et al., 2017). Some sources indicate that the colour of the flower may be of varying tones of white or pale cream (Abadian et al., 2016; Alizadeh et al., 2011; Asgharipour and Shabankare, 2017). It is known that bruised foliage releases a pleasant aromatic odour (Bahramikia and Yazdanparast, 2012; Mahmoudabady et al., 2018; Menichini et al., 2009). The flowering branches and leaves of the plant are known to contain essential oil (Ravan et al., 2019). The fruits are light brown to dark brown nutlets with a latticed surface (Sabzeghabaie and Asgarpanah, 2016).

T. polium is known to spread in almost all Mediterranean countries from Southeast Asia to Europe (Venditti et al., 2017). Plant shows more intensive distribution in Iran, Iraq, Saudi Arabia, Egypt, Jordan, Palestine and Turkey (Aburjai et al., 2006; Afifi et al., 2005). The plant has also been reported to distribute overseas, such as Australia and America (Chioibas et al., 2019). Dry and stony hills, calcareous soils and deserts up to 3000 m are typical habitats of the plant (Sayyad and Farahmandfar, 2017; Venditti et al., 2017). Some researchers report that the plant grows also on gravel and sandy beaches (Amraei et al., 2018a). The plant has seasonal metamorphosis to adapt to the stressful climatic conditions seen in winter and summer seasons (Lianopoulou et al., 2014).

In this review, all published studies on the ethnopharmacological properties, phytochemistry and toxic potentials of *T. polium* from 1981 to November 2019 were screened. It is known that *T. polium* is frequently used by local people in the treatment of various diseases. The aim of this study was to gather information about the traditional use and phytochemistry of *T. polium*, to discuss contradictory views about chemotaxonomy and its toxic effect on liver and kidneys, and to make suggestions about controversial areas and gaps in the literature. Although *T. polium* is one of the most important plants used by local people, scientific data show that this plant can cause serious toxicity to organs such as liver and kidney. Not only people, but also scientists have opposing views on the reliability of the use of the plant. Some scientific studies have reported that the plant has a hepatoprotective effect, while some others (especially some case reports) suggest that the plant has toxic effects due to the various phytochemical ingredients. This leads to speculative situations regarding the use of the plant. Here, the information put forward by different social groups (both local people and the scientific community) is given and an assessment of the therapeutic potential of the plant is made considering the balance of profit and loss. In addition, the gaps in this field were discussed and some suggestions were made regarding the actions to be taken to eliminate these gaps.

2. Methodology

In order to get literature data on the ethnopharmacological properties, phytochemistry and toxic potential of *T. polium*, a search was performed using the keyword 'Teucrium polium' in Web of Science, Scopus and PubMed databases. As a result of the screening, an EndNote library consisting of 379 studies containing the aforementioned keyword was created from 1981 to November 2019. Two authors screened these studies in detail at the text level and as a result of this first stage the number of records has been reduced to 276 by eliminating some of them, which does not contain *T. polium* actually as the keyword. The remaining records were then grouped according to the characteristics of Teucrium genus, geographic distribution, botanical properties and historical background of *T. polium*, ethnopharmacological uses, phytochemical composition and toxicity on kidney and liver. In addition, one of the authors identified the local names of the plant in different languages and converted them into a table (Table 1). Data obtained from ethnopharmacological studies, phytochemistry and toxicity findings were given in Tables 1, 2, 3, 4, 5, and 6, respectively. As a result of the ethnopharmacological research, controversial data regarding toxic and/or protective properties of this plant, especially on the liver, were obtained. Therefore, ethnopharmacological data and the results of scientific studies were compared and a consistent and holistic judgment was tried to be reached. Finally, the gaps created by the conflicting data about *T. polium* were pointed out, and opinions were given about what needs to be done to eliminate these gaps.

Table 1. Commonly used local names of *T. polium* in different languages.

Common name (In alphabetical order)	Language	Reference
Acı Ot	Turkish	(Erbay and Sari, 2018)
Acı Yavşan	Turkish	(Arasan and Kaya, 2015; Erbay and Sari, 2018)
Adi Yavşanotu	Turkish	(Selimoglu et al., 2015)
Ak Sedef Otu	Turkish	(Erbay and Sari, 2018)
Al-Ajrah	Arabic	(Boulila et al., 2008)
Al-Ja'adeh	Arabic	(Ben Othman et al., 2017)
Basur Otu	Turkish	(Erbay and Sari, 2018)
Bozot	Turkish	(Erbay and Sari, 2018)
Calpoureh	Persian	(Hasani-Ranjbar et al., 2010; Mahjoub et al., 2012; Mousavi et al., 2012; Nor et al., 2019)
Cat Thyme	English	(Ali-Shtayeh et al., 2000; BaniHani and Al Manasra, 2009; Khalil et al., 2009)
Ciğde	Arabic, Syriac	(Akgul et al., 2018)
Coda	Turkish	(Orhan and Aslan, 2009)
Espand	Persian	(Mashreghi and Niknia, 2012)
Felty Germander	English	(Al-Tikriti et al., 2017; Alamdar et al., 2007; Bakari et al., 2015; Dag et al., 2014b; Huseini et al., 2019; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Nor et al., 2019; Orhan and Aslan, 2009; Pellow and Nienhuis, 2018; Rad et al., 2014; Salbi et al., 2016; Stankovic et al., 2011; Stankovic et al., 2012; Venditti et al., 2017; Yaldiz et al., 2017)
Gattaba	Arabic	(Ben Othman et al., 2017)
Gattabet	Arabic	(Boulila et al., 2008)
Germander	English	(Aburjai et al., 2006; Al-Qudah et al., 2011; Bendif et al., 2018; Chioibas et al., 2019; El Atki et al., 2019a; El Atki et al., 2019b; Hasani-Ranjbar et al., 2010; Starakis et al., 2006; Suboh et al., 2004; Tadjrobehkar and Abdollahi, 2014)
Golden Germander	English	(Chitturi and Farrell, 2008; Fiorentino et al., 2011; Pacifico et al., 2012; Polymeros et al., 2002; Rahmouni et al., 2019; Stefkov et al., 2011; Vasileiadou et al., 2003)
Gurisa	Arabic	(Alachkar et al., 2011)
Ja'adah	Arabic	(Abdulrazzaq, 2017)
Ja'da	Arabic	(Jaradat et al., 2016)
Jaa'deh	Arabic	(Aburjai et al., 2006; Al-Qudah et al., 2011; Al-Tikriti et al., 2017)
Jaad	Arabic	(Al-Asmari et al., 2014)
Jaada	Arabic	(Boulila et al., 2008; El Atki et al., 2019a; El Atki et al., 2019b)
Jaadah	Arabic	(Bendif et al., 2018; Rahmouni et al., 2018)
Jaadeh	Arabic	(Alachkar et al., 2011)
Jae'dah	Arabic	(Kerbouche et al., 2015)
Jeada	Arabic	(Abu-rish et al., 2016; Alzweiri et al., 2011; Suboh et al., 2004)
Joode	Arabic	(Hosseinkhani et al., 2017)
Kalbpoureh	Persian	(Mashreghi and Niknia, 2012)
Kalpooreh	Persian	(Asgharipour and Shabankare, 2017; Dag et al., 2014b; Darabpour et al., 2010; Khani and Heydarian, 2014; Khoshnood-Mansoorkhani et al., 2010; Mahjoub et al., 2012; Mahmoudabady et al., 2018; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Nikpour et al., 2018; Raei et al., 2014; Ravan et al., 2019; Rezvannejad et al., 2019; Sadrizadeh et al., 2018; Seyyednejad and Motamedi, 2010; Shabankare et al., 2015)
Kalporeh	Persian	(Abadian et al., 2016)
Kalpoureh	Persian	(Boghrati et al., 2016; Sabzeghabaie
Kalpurak	Persian	(Sadeghi et al., 2014b)
Kalpureh	Persian	(Ghasemi et al., 2019a)
Kayatta	Arabic	(Bendif et al., 2018)
Khayata	Arabic	(Ben Othman et al., 2017; Kerbouche et al., 2015)
Kısa Mahmut	Turkish	(Polat and Satil, 2012)
Kısa Mahmut Otu	Turkish	(Uysal et al., 2012)
Koyun Otu	Turkish	(Erbay and Sari, 2018)
Koyun Yavşanı	Turkish	(Orhan and Aslan, 2009)
Mayasıl Otu	Turkish	(Erbay and Sari, 2018; Hayta et al., 2014; Tuncturk et al., 2019)
Meryem Otu	Turkish	(Erbay and Sari, 2018)
Meryem Saçı	Turkish	(Orhan and Aslan, 2009)
Mountain Germander	English	(Dababneh, 2008; Dag et al., 2014a)
Oğlan Otu	Turkish	(Erbay and Sari, 2018)
Oğul Otu	Turkish	(Erbay and Sari, 2018)
Peryavşan	Turkish	(Erbay and Sari, 2018)
Poleigamander	German	(Amraei et al., 2017b; Yousefi et al., 2018)
Poly-Germander	English	(Khoshnood-Mansoorkhani et al., 2010; Sezer and Bozaykut, 2012)
Sancı Otu	Turkish	Erbay and Sari, 2018)
Takmazzut	by Touaregs	(Baali et al., 2016)
Tiksinik Otu	Turkish	(Erbay and Sari, 2018)
Tüylü Kısamahmut	Turkish	(Coban et al., 2003; Tuncturk et al., 2019)
Ürper yavşağı	Turkish	(Cakilcioglu and Turkoglu, 2010)
Wall Germander	English	(Amraei et al., 2017a)
Yavşan	Turkish	(Erbay and Sari, 2018)

3. Traditional and medicinal use

T. polium is known by different local names in different languages and cultures. Table 1 shows the local names of *T. polium* in different geographical regions of the world. Since the plant has a Eastern Mediterranean and Middle Eastern origin, it is seen that the native languages of these countries play an important role in determining the local names and the different names used in the same country are similar to each other phonetically. *T. polium* is known in Turkish, Arabic and Persian with a large number of local names that are thought to have differentiated over time from the same source. While the names 'Calpoureh' or 'Kalpoureh' are common in Persian, 'Ja'adeh' and its derivatives are used frequently in Arabic. In Tunisia, whose official language is Arabic, *T. polium* is called as 'Al-Ja'adeh', 'Khayata' or 'Gattaba'. It is stated that these terms mean 'cicatrisant' in Arabic (Ben Othman et al., 2017). In some countries, the local names of the plant may also vary regionally. For example, in the eastern regions of Algeria, *T. polium* is called as 'Kayatta', while in the western regions it is known as 'Jaadah' (Bendif et al., 2018). Because of its pharmacological and/or toxic effects on certain tissues and organs, *T. polium* attracted the attention of scientists from Western countries as well as researchers from Eastern Mediterranean and Middle East. Therefore, in Western languages, *T.*

polium is commonly known as 'Germander', 'Golden Germander' and 'Felty Germander'.

T. polium is one of the most popular herbal remedies in the world and has been used by local people for the treatment of various ailments for over 2000 years (Hasanein and Shahidi, 2012). Its use as a medicinal herb dates back to Hippocrates, Dioscorides, Palin and Galen (Ghasemi et al., 2019a). This plant has been used medicinally since ancient Greek times (Menichini et al., 2009; Sheikhbahaei et al., 2018) and medical of reputation of this plant was also noticed in traditional medicine by Socrates and Jalinous (Mahmoudi et al., 2015; Seyyednejad and Motamedi, 2010).

Table 2 gives information about the diseases which *T. polium* is used for treatment among the local people. The table also specifies which parts of the plant are used, how they are prepared and how they are used. Based on the data in the table, it is possible to make a judgment about the usage habits and frequency of use of this plant. As it is known, ethnopharmacological knowledge is transferred from past to present through generations and enriched with increasing experience in each generation. In the table, information on the use of the plant in the treatment of certain diseases is expressed by many authors, while the number of authors who comment on the use of the plant in the treatment of some other diseases is less. This shows that the effectiveness of *T. polium* in the treatment of some diseases has been verified through generations. Therefore, it can be concluded that the reliability of the information will increase as the accumulation of knowledge confirming each other about the therapeutic properties of the plant on any disease increases.

Table 2. Ethnopharmacological uses of *T. polium*.

Used as/in the treatment of (In alphabetical order)	Plant part(s)	Preparation	Suggested utilization method(s)	Reference
Amenorrhea	Aerial parts	Infusion, decoction	Not specified	(Ben Othman et al., 2017; De Marino et al., 2012)
Anorexia	Aerial parts	Infusion	Not specified	(Alzweiri et al., 2011; Bahramikia and Yazdanparast, 2012; Ben Othman et al., 2017; De Martino et al., 2010; Khaled-Khodja et al., 2014; Mashreghi and Niknia, 2012; Menichini et al., 2009; Rad et al., 2014)
Anorexia	Aerial parts	Infusion	Not specified	(Alzweiri et al., 2011; Bahramikia and Yazdanparast, 2012; Ben Othman et al., 2017; De Martino et al., 2010; Khaled-Khodja et al., 2014; Mashreghi and Niknia, 2012; Menichini et al., 2009; Rad et al., 2014)
Anti-cancer	Aerial parts	Infusion	Aerial parts are crushed and prepared as herbal tea.	(Alachkar et al., 2011; Farahmandfar et al., 2019)
Anti-convulsant	Not specified	Not specified	Not specified	(Abadian et al., 2016; Amraei et al., 2018b; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Hasani-Ranjbar et al., 2010; Khoshnood-Mansoorkhani et al., 2010; Pesaraklu et al., 2011; Rad et al., 2014; Rezvannejad et al., 2019)
Anti-diabetic, insulinotropic	Aerial parts, stems	Infusion, decoction	- Aerial parts are crushed and prepared as tea. - Aerial parts are eaten as raw material or infused in hot water to consume as tea. - Aerial parts are consumed in powder form. - Infusion taken orally three times a week. - One cup of herbal tea is consumed on an empty stomach in the morning. - One teacup herbal tea is drunk two times a day for a 1-2 weeks.	(Aburjai et al., 2006; Afifi et al., 2005; Akgul et al., 2018; Al-Qudah et al., 2011; Al-Tikriti et al., 2017; Alachkar et al., 2011; Alamdar et al., 2007; Alzweiri et al., 2011; Amini et al., 2009; Amraei et al., 2018b; Arasan and Kaya, 2015; Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009; Bahramikia and Yazdanparast, 2011, 2012; Baradaran et al., 2013; Bedir et al., 1999; Ben Othman et al., 2017; Bendif et al., 2018; Boghrati et al., 2016; Boulila et al., 2008; Bozov and Penchev, 2019; Cakilcioglu et al., 2010; Cakilcioglu and Turkoglu, 2010; Chitturi and Farrell, 2008; Coban et al., 2003; Dababneh, 2008; Dag et al., 2014a; Dag et al., 2014b; Darwish and Aburjai, 2010; De Martino et al., 2010; El Atki et al., 2019a; El Atki et al., 2019b; Elmasri et al., 2014; Esmaeili and Yazdanparast, 2004; Farahmandfar et al., 2019; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Grubesic et al., 2012; Hachicha et al., 2009; Hasani-Ranjbar et al., 2010; Hayta et al., 2014; Huseini et al., 2019; Kandouz et al., 2010; Khader et al., 2010; Khaled-Khodja et al., 2014; Khalil et al., 2009; Khodadadi et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Kiyani et al., 2011; Lianopoulou et al., 2014; Ljubuncic et al., 2005; Mahjoub et al., 2012; Mashreghi and Niknia, 2012; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Mitreski et al., 2014; Monfared and Pournourmohammadi, 2010; Mousavi et al., 2012; Movahedi et al., 2014; Niazmand et al., 2011; Niazmand et al., 2017; Nikpour et al., 2018; Oroojalian et al., 2017; Panovska and Kulevanova, 2005; Pesaraklu et al., 2011; Polat and Satil, 2012; Rad et al., 2014; Raei et al., 2014; Rahmouni et al., 2019; Rezvannejad et al., 2019; Sadeghi et al., 2014b; Scognamiglio et al., 2012; Shabankare et al., 2015; Stefkov et al., 2011; Tuncturk et al., 2019; Vasileiadou et al., 2003; Yaldiz et al., 2017; Zabihi et al., 2018)
Anti-diarrheal	Aerial parts	Infusion, decoction	- About 15 g of leaves are kept in 100 ml water for two hours; this infusion is drunk after each meal. - Infusion of the leaves and flowers is consumed as herbal tea.	(Akgul et al., 2018; Amraei et al., 2018b; Baali et al., 2016; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Jaradat et al., 2016; Krishnaiah et al., 2011; Pesaraklu et al., 2011; Rezvannejad et al., 2019; Tuncturk et al., 2019)
Anti-hemorroidal	Aerial parts	Infusion, decoction	- Dried and crushed aerial parts are used internally or externally. In the case internal use, the powdered material can be mixed to honey.	(Bedir et al., 1999; Dag et al., 2014b; Erbay and Sari, 2018; Hayta et al., 2014; Khalil et al., 2009; Stefkov et al., 2011)

Used as/in the treatment of (In alphabetical order)	Plant part(s)	Preparation	Suggested utilization method(s)	Reference
			- One cup of herbal tea is also consumed on an empty stomach in the morning.	
Anti-hyperlipidemic	Aerial parts	Infusion	One cup of herbal tea prepared from the aerial parts or powdered material is consumed on an empty stomach in the morning.	(Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009; Boghrati et al., 2016; Chitturi and Farrell, 2008; Dababneh, 2008; De Martino et al., 2010; Farahmandfar et al., 2019; Forouzandeh et al., 2013; Hachicha et al., 2009; Hayta et al., 2014; Mitreski et al., 2014; Mousavi et al., 2012; Sadeghi et al., 2014b; Shabankare et al., 2015; Stefkov et al., 2011; Vasileiadou et al., 2003; Yaldiz et al., 2017)
Anti-hypertensive	Aerial parts	Infusion	Infusion prepared from the aerial parts or powdered material is consumed as herbal tea	(Al-Tikriti et al., 2017; Amraei et al., 2018b; Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009; Bahramikia and Yazdanparast, 2012; Ben Othman et al., 2017; Boghrati et al., 2016; De Martino et al., 2010; El Atki et al., 2019a; El Atki et al., 2019b; Farahmandfar et al., 2019; Forouzandeh et al., 2013; Khaled-Khodja et al., 2014; Khoshnood-Mansoorkhani et al., 2010; Lianopoulou et al., 2014; Mashreghi and Niknia, 2012; Menichini et al., 2009; Mitreski et al., 2014; Mousavi et al., 2012; Movahedi et al., 2014; Nikpour et al., 2018; Rad et al., 2014; Rezvannejad et al., 2019; Sadeghi et al., 2014b; Scognamiglio et al., 2012; Tuncturk et al., 2019; Yaldiz et al., 2017)
Anti-inflammatory	Aerial parts, stems	Infusion	Not specified	(Al-Asmari et al., 2014; Alzweiri et al., 2011; Amraei et al., 2018b; Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009; Bahramikia and Yazdanparast, 2011, 2012; Baradaran et al., 2013; Ben Othman et al., 2017; Boghrati et al., 2016; Cakilcioglu et al., 2010; Chitturi and Farrell, 2008; Dababneh, 2008; De Martino et al., 2010; Derakhshan et al., 2011; El Atki et al., 2019b; Elmasri et al., 2014; Farahmandfar et al., 2019; Forouzandeh et al., 2013; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Grubesic et al., 2012; Hachicha et al., 2009; Huseini et al., 2019; Khader et al., 2010; Khader et al., 2007; Khaled-Khodja et al., 2014; Khalil et al., 2009; Khodadadi et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Lianopoulou et al., 2014; Ljubuncic et al., 2005; Mashreghi and Niknia, 2012; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Mitreski et al., 2014; Mousavi et al., 2012; Movahedi et al., 2014; Niazmand et al., 2011; Niazmand et al., 2017; Panovska and Kulevanova, 2005; Pesaraklu et al., 2011; Rad et al., 2014; Raei et al., 2014; Rezvannejad et al., 2019; Scognamiglio et al., 2012; Shabankare et al., 2015; Stefkov et al., 2011; Yaldiz et al., 2017; Zabihi et al., 2018)
Anti-mutagenic	Not specified	Not specified	Not specified	(Farahmandfar et al., 2019)
Anti-nociceptive, analgesic, anti-spasmodic on abdominal colic/pains, headache, body and joint pains, dysmenorrhea, toothache and visceral pains	Aerial parts	Infusion, decoction	- Aerial parts are consumed in powder form or as herbal tea. - Fresh leaves can be chewed. - Aerial parts are crushed and prepared as tea. - Infusion taken orally three times a week. - One cup of herbal tea is consumed three times a day or aerial parts can be cooked. - Infusion prepared from the aerial parts or powdered material is consumed as herbal tea.	(Abadian et al., 2016; Abdollahi et al., 2003; Aburjai et al., 2006; Afifi et al., 2005; Akgul et al., 2018; Al-Qudah et al., 2011; Al-Tikriti et al., 2017; Alachkar et al., 2011; Alamdar et al., 2007; Alzweiri et al., 2011; Ardestani and Yazdanparast, 2007; Baali et al., 2016; Bahramikia et al., 2009; Bahramikia and Yazdanparast, 2011, 2012; Bakari et al., 2015; Baradaran et al., 2013; Ben Othman et al., 2017; Bendif et al., 2018; Boghrati et al., 2016; Bozov and Penchev, 2019; Dababneh, 2008; Dag et al., 2014a; Dag et al., 2014b; Darwish and Aburjai, 2010; Elmasri et al., 2014; Farahmandfar et al., 2019; Forouzandeh et al., 2013; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Grubesic et al., 2012; Gunes et al., 2017; Hachicha et al., 2009; Hasani-Ranjbar et al., 2010; Huseini et al., 2019; Kandouz et al., 2010; Kerbouche et al., 2015; Khaled-Khodja et al., 2014; Khazaei et al., 2018; Khodadadi et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Kiyani et al., 2011; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mashreghi and Niknia, 2012; Masoudi, 2018; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Mitreski et al., 2014; Mosaddegh et al., 2012; Movahedi et al., 2014; Niazmand et al., 2011; Niazmand et al., 2017; Nikpour et al., 2018; Oroojalian et al., 2017; Pacifico et al., 2012; Pesaraklu et al., 2011; Rad et al., 2014; Raei et al., 2014; Rahmouni et al., 2019; Rezvannejad et al., 2019; Sabzeghabaie and Asgarpanah, 2016; Sadeghi et al., 2014a; Sadeghi et al., 2014b; Scognamiglio et al., 2012; Sevindik et al., 2016; Shabankare et al., 2015; Tuncturk et al., 2019; Venditti et al., 2017; Yaldiz et al., 2017; Zendehdel et al., 2011)
Anti-parasitic (amoebicidal, anti-helmintic, vermifuge)	Not specified	Infusion	Not specified	(Aburjai et al., 2006; Al-Qudah et al., 2011; Alamdar et al., 2007; Bendif et al., 2018; El Atki et al., 2019a; El Atki et al., 2019b; Elmasri et al., 2014; Grubesic et al., 2012; Sadeghi et al., 2014a)
Anti-pyretic, febrifuge	Aerial parts	Infusion, decoction	- Infusion of the leaves and flowers or their powdered forms is consumed as herbal tea.	(Abdollahi et al., 2003; Bahramikia and Yazdanparast, 2012; Bakari et al., 2015; Ben Othman et al., 2017; Boghrati et al., 2016; Cakilcioglu and Turkoglu, 2010; Chitturi and Farrell, 2008; Dababneh, 2008; El Atki et al., 2019a; El Atki et al.,

Used as/in the treatment of (In alphabetical order)	Plant part(s)	Preparation	Suggested utilization method(s)	Reference
			- One teacup of herbal tea is drunk two times a day before meal until recovery.	2019b; Elmasri et al., 2014; Forouzandeh et al., 2013; Grubesic et al., 2012; Hasani-Ranjbar et al., 2010; Khaled-Khodja et al., 2014; Khazaei et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Krishnaiah et al., 2011; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mashreghi and Niknia, 2012; Menichini et al., 2009; Mitreski et al., 2014; Movahedi et al., 2014; Niazmand et al., 2011; Niazmand et al., 2017; Pacifico et al., 2012; Rad et al., 2014; Sabzeghabaie and Asgarpanah, 2016; Sadeghi et al., 2014b; Sevindik et al., 2016; Tuncturk et al., 2019; Uysal et al., 2012; Yaldiz et al., 2017; Zabihi et al., 2018)
Anti-rheumatic	Aerial parts	Infusion, decoction	Not specified	(Akgul et al., 2018; Al-Asmari et al., 2014; Bahramikia and Yazdanparast, 2011, 2012; Bendif et al., 2018; Dababneh, 2008; El Atki et al., 2019a; El Atki et al., 2019b; Farahmandfar et al., 2019; Grubesic et al., 2012; Huseini et al., 2019; Khaled-Khodja et al., 2014; Khazaei et al., 2018; Lianopoulou et al., 2014; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Raei et al., 2014; Tuncturk et al., 2019; Yaldiz et al., 2017)
Antibacterial, antifungal	Aerial parts	Not specified	Not specified	(Amraei et al., 2018b; Ardestani and Yazdanparast, 2007; Bahramikia et al., 2009; Bahramikia and Yazdanparast, 2012; Dababneh, 2008; De Martino et al., 2010; Farahmandfar et al., 2019; Forouzandeh et al., 2013; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Huseini et al., 2019; Khaled-Khodja et al., 2014; Khazaei et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Menichini et al., 2009; Mousavi et al., 2012; Movahedi et al., 2014; Nikpour et al., 2018; Pesaraklu et al., 2011; Rad et al., 2014; Rezvannejad et al., 2019; Shabankare et al., 2015; Tuncturk et al., 2019; Yaldiz et al., 2017)
Antioxidant	Aerial parts	Not specified	Not specified	(Dababneh, 2008; De Martino et al., 2010; Farahmandfar et al., 2019; Shabankare et al., 2015; Tuncturk et al., 2019)
Antiseptic	Not specified	Not specified	Not specified	(Grubesic et al., 2012; Sevindik et al., 2016)
Appetizer	Aerial parts	Infusion	It is consumed as herbal tea	(Amraei et al., 2017a; Bendif et al., 2018; Elmasri et al., 2014; Goulas et al., 2012; Sharififar et al., 2009; Stankovic et al., 2011; Stankovic et al., 2012; Tepe et al., 2011; Tepe et al., 2012)
Arthritis, gout	Aerial parts	Infusion, decoction	Not specified	(Ben Othman et al., 2017; De Marino et al., 2012; Khader et al., 2010)
Astringent	Not specified	Not specified	Not specified	(Bendif et al., 2018; Sadeghi et al., 2014a)
Body weight loss agent	Not specified	Not specified	Not specified	(Farahmandfar et al., 2019; Tuncturk et al., 2019)
Cholagogic, bile stimulator	Aerial parts	Not specified	Not specified	(Abdollahi et al., 2003; Bakari et al., 2015; Dababneh, 2008; Ghasemi et al., 2019a; Grubesic et al., 2012; Hasani-Ranjbar et al., 2010; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mitreski et al., 2014; Pacifico et al., 2012; Sabzeghabaie and Asgarpanah, 2016; Yaldiz et al., 2017)
Chronic bronchitis, asthma, cough, expectorant, common cold, flu, grippe	Aerial parts	Infusion, decoction	- One cup of herbal tea is consumed three times a day. - One teacup of herbal tea is drunk two times a day before meal until recovery. - For the treatment of flu, one cup of herbal tea is consumed on an empty stomach in the morning. - Infusion of the leaves and flowers is consumed as herbal tea.	(Akgul et al., 2018; Bahramikia and Yazdanparast, 2011, 2012; Ben Othman et al., 2017; Bendif et al., 2018; Boghrati et al., 2016; De Marino et al., 2012; Elmasri et al., 2014; Farahmandfar et al., 2019; Gunes et al., 2017; Hayta et al., 2014; Krishnaiah et al., 2011; Masoudi, 2018; Oroojalian et al., 2017; Raei et al., 2014; Sadeghi et al., 2014a; Sheikhbahaei et al., 2018; Tuncturk et al., 2019; Uysal et al., 2012; Venditti et al., 2017)
Condiment, spice	Aerial parts	Not specified	Not specified	(Amraei et al., 2017a; Baali et al., 2016; Bendif et al., 2018; Goulas et al., 2012; Mahjoub et al., 2012; Pacifico et al., 2012; Sharififar et al., 2009; Stankovic et al., 2012)
Dementia, mental performance	Not specified	Not specified	Not specified	(Ghasemi et al., 2019a; Ghasemi et al., 2019b; Hasanein and Shahidi, 2012; Milosevic-Djordjevic et al., 2018; Orhan and Aslan, 2009; Simonyan and Chavushyan, 2015)
Depurative	Aerial parts	Infusion	Not specified	(Aburjai et al., 2006; Al-Qudah et al., 2011; Alamdar et al., 2007; Ben Othman et al., 2017; Bendif et al., 2018; Sadeghi et al., 2014a)
Diaphoretic, sweat gland activator	Aerial parts	Infusion, decoction	Not specified	(Abdollahi et al., 2003; Bakari et al., 2015; Ben Othman et al., 2017; Dababneh, 2008; De Marino et al., 2012; Ghasemi et al., 2019a; Hasani-Ranjbar et al., 2010; Kerbouche et al., 2015; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mashreghi and Niknia, 2012; Mitreski et al., 2014; Movahedi et al., 2014; Pacifico et al., 2012; Rad et al., 2014; Sabzeghabaie and Asgarpanah, 2016; Yaldiz et al., 2017)
Diuretic	Aerial parts	Infusion, decoction	Not specified	(Abdollahi et al., 2003; Amraei et al., 2018b; Bakari et al., 2015; Ben Othman et al., 2017; Boghrati et al., 2016; Chitturi and Farrell, 2008; Dababneh, 2008; De Marino et al., 2012; Ghasemi et al., 2019a; Grubesic et al., 2012; Hasani-Ranjbar et al., 2010; Huseini et al., 2019; Kerbouche et al., 2015; Khazaei et al., 2018; Khoshnood-Mansoorkhani et al., 2010; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mashreghi and Niknia, 2012; Mitreski et al., 2014; Movahedi

Used as/in the treatment of (In alphabetical order)	Plant part(s)	Preparation	Suggested utilization method(s)	Reference
				et al., 2014; Pacifico et al., 2012; Rad et al., 2014; Sabzeghabaie and Asgarpanah, 2016; Tuncturk et al., 2019; Yaldiz et al., 2017)
Eczema	Not specified	Not specified	Not specified	(Khader et al., 2010; Milosevic-Djordjevic et al., 2018; Tuncturk et al., 2019)
Emesis	Aerial parts	Infusion	Infusion prepared from the aerial parts or powdered material is consumed as herbal tea	(Sadeghi et al., 2014b)
Fertility, feminine sterility	Not specified	Not specified	Not specified	(Al-Tikriti et al., 2017; Bendif et al., 2018; Sadeghi et al., 2014a)
Flavouring	Not specified	Not specified	Not specified	(Bendif et al., 2018; Grubesic et al., 2012)
Gastrointestinal disorders (indigestion, dyspepsia, stomachache, gastralgia, gastric inflammation, enteritis) and effect on intestinal motility and abdominal tension as carminative and purgative agents	Aerial parts, stems	Infusion, decoction	- Infusion of the leaves and flowers is consumed as herbal tea. - Aerial parts or stems are consumed as powdered material. - Infusion of the aerial parts or powdered material is taken orally three times a week. - Aerial parts are crushed and prepared as tea.	(Akgul et al., 2018; Al-Asmari et al., 2014; Al-Tikriti et al., 2017; Alachkar et al., 2011; Ali-Shtayeh et al., 2000; Alzweiri et al., 2011; Amraei et al., 2018b; Baali et al., 2016; Bahramikia and Yazdanparast, 2011, 2012; Bedir et al., 1999; Ben Othman et al., 2017; Bendif et al., 2018; Boghrati et al., 2016; Boulila et al., 2008; Bozov and Penchev, 2019; Cakilcioglu et al., 2010; Cakilcioglu and Turkoglu, 2010; Chizzola, 2006; Coban et al., 2003; Dag et al., 2014a; Dag et al., 2014b; Darwish and Aburjai, 2010; De Marino et al., 2012; De Martino et al., 2010; Derakhshan et al., 2011; El Atki et al., 2019a; El Atki et al., 2019b; Farahmandfar et al., 2019; Ghasemi et al., 2019a; Ghasemi et al., 2019b; Grubesic et al., 2012; Hasani-Ranjbar et al., 2010; Jaradat et al., 2016; Kandouz et al., 2010; Khader et al., 2010; Khaled-Khodja et al., 2014; Khalil et al., 2009; Khazaei et al., 2018; Khodadadi et al., 2018; Krishnaiah et al., 2011; Ljubuncic et al., 2005; Masoudi, 2018; Menichini et al., 2009; Milosevic-Djordjevic et al., 2018; Mosaddegh et al., 2012; Nikpour et al., 2018; Oroojalian et al., 2017; Panovska and Kulevanova, 2005; Rad et al., 2014; Raei et al., 2014; Rahmouni et al., 2019; Rezvannejad et al., 2019; Sadeghi et al., 2014a; Sadeghi et al., 2014b; Stankovic et al., 2011; Stankovic et al., 2012; Tuncturk et al., 2019; Venditti et al., 2017)
Gynaecological infections, leucorrhoea, urogenital diseases, urinary tract inflammations	Aerial parts	Infusion, decoction	Aerial parts are consumed as herbal tea or they can be cooked.	(Al-Tikriti et al., 2017; Bahramikia and Yazdanparast, 2011, 2012; Ben Othman et al., 2017; De Marino et al., 2012; Khader et al., 2010; Masoudi, 2018; Milosevic-Djordjevic et al., 2018; Mosaddegh et al., 2012; Raei et al., 2014; Venditti et al., 2017)
Heart failure, alleviating heart pain	Not specified	Not specified	Not specified	(Khodadadi et al., 2018; Niazmand et al., 2011; Niazmand et al., 2017)
Insect repellent, anti-feedant	Aerial parts	Infusion	Infusion prepared from the aerial parts or the powdered material is applied topically.	(Sadeghi et al., 2014b)
Kidney stones, pains and other kidney disorders	Aerial parts, stems	Infusion, decoction	- Infusion taken orally three times a week. - One teacup herbal tea is drunk two times a day for a 1-2 weeks.	(Aburjai et al., 2006; Akgul et al., 2018; Al-Qudah et al., 2011; Al-Tikriti et al., 2017; Alamdar et al., 2007; Alzweiri et al., 2011; Bendif et al., 2018; Darwish and Aburjai, 2010; Elmasri et al., 2014; Khader et al., 2010; Khader et al., 2007; Khalil et al., 2009; Ljubuncic et al., 2005; Milosevic-Djordjevic et al., 2018; Polat and Satil, 2012; Stefkov et al., 2011)
Liver disorders, anti-hepatitis	Aerial parts, stems	Infusion, decoction	Infusion of the leaves and flowers is consumed as herbal tea.	(Akgul et al., 2018; Al-Asmari et al., 2014; Bedir et al., 1999; Cakilcioglu et al., 2010; El Atki et al., 2019a; El Atki et al., 2019b; Khader et al., 2010; Khader et al., 2007; Khalil et al., 2009; Krishnaiah et al., 2011; Ljubuncic et al., 2005; Milosevic-Djordjevic et al., 2018)
Neurotonic disorders	Aerial parts	Not specified	Not specified	(Chizzola, 2006)
Obesity	Not specified	Not specified	Not specified	(Al-Tikriti et al., 2017; Chitturi and Farrell, 2008)
Refreshing beverage	Aerial parts	Infusion	Infusion of the leaves and flowers is consumed as herbal tea.	(Goulas et al., 2012; Krishnaiah et al., 2011; Movahedi et al., 2014; Sharififar et al., 2009; Tepe et al., 2011; Tepe et al., 2012)
Sedative	Aerial parts	Infusion	Infusion prepared from the aerial parts or powdered material is consumed as herbal tea	(Sadeghi et al., 2014b)
Skin erythema	Not specified	Not specified	Not specified	(Stefkov et al., 2011)
Snake/scorpion bite	Aerial parts	Infusion	Infusion prepared from the aerial parts or the powdered material is applied topically.	(Sadeghi et al., 2014b)
Stimulant	Aerial parts	Infusion, decoction	Not specified	(Ben Othman et al., 2017; Bendif et al., 2018; De Marino et al., 2012; Grubesic et al., 2012; Sadeghi et al., 2014a; Tuncturk et al., 2019)
Tea	Not specified	Not specified	Not specified	(Baali et al., 2016; Goulas et al., 2012; Mahjoub et al., 2012; Pacifico et al., 2012; Sharififar et al., 2009; Stankovic et al., 2012; Tepe et al., 2011; Tepe et al., 2012)
Tonic	Aerial parts	Infusion, decoction	One teacup of herbal tea two times a day before meal until recovery is drunk.	(Abdollahi et al., 2003; Baali et al., 2016; Bakari et al., 2015; Ben Othman et al., 2017; Bendif et al., 2018; Coban et al., 2003; Dababneh, 2008; De Marino et al., 2012; Ghasemi et al., 2019a; Hasani-Ranjbar et al., 2010; Kerbouche et al., 2015; Khazaei et al., 2018; Ljubuncic et al., 2006; Mahmoudabady et al., 2018; Mashreghi and Niknia, 2012;

Used as/in the treatment of (In alphabetical order)	Plant part(s)	Preparation	Suggested utilization method(s)	Reference
				Movahedi et al., 2014; Pacifico et al., 2012; Rad et al., 2014; Sabzeghabaie and Asgarpanah, 2016; Sadeghi et al., 2014a; Sevindik et al., 2016; Stankovic et al., 2012; Uysal et al., 2012; Yaldiz et al., 2017)
Vulnerary, wound healing agent	Aerial parts	Infusion	Aerial parts are consumed as herbal tea or in powder form.	(Bendif et al., 2018; Dag et al., 2014a; Elmasri et al., 2014; Sadeghi et al., 2014a; Sadeghi et al., 2014b; Tuncturk et al., 2019)

As far as our literature survey could ascertain, since 1981, 120 studies have been reported providing information on the use of *T. polium* in traditional medicine. Among these studies, those who stated that the plant has anti-diabetic and/or insulinotropic effect are in the first place (79 reports, 65.8% of the total). This is followed by the plant's anti-nociceptive effect (71 reports, 59.2% of the total), therapeutic potential on gastrointestinal diseases (59 reports, 49.2% of the total), and anti-inflammatory activity (50 reports, 41.7% of the total). The plant is also often used for its anti-pyretic, anti-hypertensive, diuretic, antimicrobial (i.e. anti-bacterial and anti-fungal) activities and its effectiveness on upper respiratory tract infections (chronic bronchitis, asthma, cough, expectorant, common cold, and flu). There is a detailed discussion of the toxic effects of the plant under the heading 'Toxicity on kidney and liver'. However, it is worth mentioning that the plant has some therapeutic properties on liver and kidney disorders. Although there is some scientific evidence that the plant has toxic effects on the liver and kidney, it is known that the plant is frequently used by the local people in the treatment of liver diseases, hepatitis, kidney stones, kidney pain and other kidney diseases. Therefore, a profit-loss balance regarding the use of the plant should be considered. It is believed that both health authorities and scientists should provide a satisfactory explanation of the therapeutic properties of the plant without ignoring toxic effects.

As can be seen from Table 1, there are very few reports on the therapeutic properties of the plant on certain diseases. For example, one or two reports claims that *T. polium* is effective in the treatment of amenorrhea, obesity, emesis, neurotonic disorders, skin erythema and snake/scorpion bite or can be used as anti-cancer, antiseptic, astringent, flavouring, anti-mutagenic, insect repellent, anti-feedant, sedative and body weight loss agent agents. Therefore, the therapeutic potential of the plant on these disorders should be considered with suspicion. It would be a more realistic approach to have doubts before obtaining satisfactory information about the therapeutic potential of the plant on these ailments.

It was previously stated that the plant is a species of Eastern and Central Mediterranean origin. Therefore, this plant is mostly used by people living in the Middle East for therapeutic purposes. It is seen that especially the Iranians frequently use this plant in the treatment of type 2 diabetes (Alamdar et al., 2007; Amini et al., 2009; Bahramikia and Yazdanparast, 2011, 2012; Esmaeili and Yazdanparast, 2004; Farahmandfar et al., 2019; Ghasemi et al., 2019a; Khodadadi et al., 2018; Movahedi et al., 2014), gastrointestinal disorders (Bahramikia and Yazdanparast, 2011, 2012; Boghrati et al., 2016; Farahmandfar et al., 2019; Khodadadi et al., 2018; Raei et al., 2014), inflammation (Ardestani and Yazdanparast, 2007; Bahramikia and Yazdanparast, 2011; Forouzandeh et al., 2013; Ghasemi et al., 2019a; Movahedi et al., 2014), abdominal colic, pain and tension (Alamdar et al., 2007; Bahramikia and Yazdanparast, 2012; Farahmandfar et al., 2019; Ghasemi et al., 2019a; Raei et al., 2014), common cold, grippe (Bahramikia and Yazdanparast, 2012; Boghrati et al., 2016; Farahmandfar et al., 2019; Raei et al., 2014), bacterial infections (Ardestani and Yazdanparast, 2007; Forouzandeh et al., 2013; Ghasemi et al., 2019a; Movahedi et al., 2014), hypertension (Ardestani and Yazdanparast, 2007; Forouzandeh et al., 2013; Movahedi et al., 2014), urogenital diseases (Bahramikia and Yazdanparast, 2012; Raei et al., 2014), rheumatism (Bahramikia and Yazdanparast, 2011; Farahmandfar et al., 2019), hyperlipidaemia (Ardestani and Yazdanparast, 2007; Forouzandeh et al., 2013), heart failure (Khodadadi et al., 2018; Niazmand et al., 2017), headache (Abadian et al., 2016; Alamdar et al., 2007), convulsion (Abadian et al., 2016; Ghasemi et al., 2019a), kidney stones (Alamdar et al., 2007), dysmenorrhea (Abadian et al., 2016), diarrhoea (Ghasemi et al., 2019a), dementia (Ghasemi et al., 2019a) and as anti-spasmodic (Alamdar et al., 2007; Forouzandeh et al., 2013; Movahedi et al., 2014), anti-pyretic (Forouzandeh et al., 2013; Movahedi et al., 2014), anti-nociceptive (Ardestani and Yazdanparast, 2007; Movahedi et al., 2014), visceral pain killer (Zendehdel et al., 2011), vermifuge (Alamdar et al., 2007), diuretic (Movahedi et al., 2014), diaphoretic (Movahedi et al., 2014) and depurative agents (Alamdar et al., 2007). It is also known that this plant is one of the first remedies for the treatment of type 2 diabetes, especially in the southern parts of Iran (Bahramikia and Yazdanparast, 2011, 2012).

As a result of ethnopharmacological researches, it was understood that *T. polium* is not only used for the treatment of various diseases, but is also consumed for certain purposes in kitchens. The plant is especially valuable in the Middle Eastern cuisine as appetizer, condiment and spice, flavouring agent, tonic and tea. Especially in Iran, it is widely used as appetizer and spice (Sharififar et al., 2009). Although the issue of safety is one of the most debated issues, it is reported that the plant does not have any critical side effects although it is consumed so often in the Middle East (Derakhshan et al., 2011). The plant is also used as a refreshing beverage in personal care (Goulas et al., 2012; Krishnaiah et al., 2011; Movahedi et al., 2014; Sharififar et al., 2009; Tepe et al., 2011; Tepe et al., 2012).

In addition to the purposes for which the plant is used, it is necessary to mention which parts are consumed and in what way. Almost all of the sources evaluated indicate that the aerial parts of the plant are used. While some studies do not specify the form of preparation, the majority of the studies state that an infusion or a decoction is prepared from the plant. It is seen that the aerial parts of the plant or the powder obtained from these parts are often consumed as tea or raw material. Sometimes the aerial parts of the plant are consumed by cooking. There are also cases where the aerial parts of the plant are chewed in the mouth or mixed with honey to relieve stomach ailments. When the usage behaviour of the local people is evaluated, it is seen that there is no standardized use of the plant. There are those who use one tea cup tea or infusion on an empty stomach in the morning, after each meal, one or two times a day before meals until recovery and three times a week, as well as those who consume the same amount of tea or infusion twice a day for 1-2 weeks. It has been reported that the plant can be administered topically for snake/scorpion bites or as insect repellent/anti-feedant agent as well as the above-mentioned oral uses.

4. Phytochemistry

Data on the chemical composition of essential oils isolated from *T. polium*, subspecies or varieties were given in Table 3. The main components of essential oils and other phytochemicals were also given in Tables 4 and 5 respectively.

Table 3. A comprehensive list of the chemical constituents isolated from the essential oils *T. polium* together with its subspecies and varieties[1].

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
1	(+)-3-Carene	-	Jordan	TCM[4]	(Al-Qudah et al., 2011)
2	*(+)*-Aromadendrene	-	Jordan	TCM	(Al-Qudah et al., 2011)
3	*(+)*-Spathulenol	-	Jordan	TCM	(Al-Qudah et al., 2011)
4	*(+)*-Cycloisosativene	-	Iran	Aerial parts	(Nikpour et al., 2018)
5	*(-)*-Myrtenol	-	Jordan	TCM	(Al-Qudah et al., 2011)
6	*(-)*-Globulol	-	Iran	Aerial parts	(Nikpour et al., 2018)
7	*(-)-trans*-Pinocarvyl acetate	-	Iran	Aerial parts	(Nikpour et al., 2018)
8	*(-)*-α-Panasinsen	-	Iran	Aerial parts	(Nikpour et al., 2018)
9	*(1R)-(-)*-Myrtenal	-	Iran	Aerial parts	(Nikpour et al., 2018)
10	*(1R)-endo-(+)*-Fenchyl alcohol	-	Iran	Aerial parts	(Nikpour et al., 2018)
11	*(1S)-(-)*-Verbenone	-	Iran	Aerial parts	(Nikpour et al., 2018)
12	(3*E*,5*E*)-2,6-Dimethyl-1,3,5,7-octatetraene	-	Iran	Aerial parts	(Nikpour et al., 2018)
13	*(E,E)*-1,3,5-Undecatriene	-	Iran	Aerial parts	(Boroomand et al., 2018)
14	*(E,E)*-2,4-Decadienal	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
15	*(E)*-2-Hexenal	-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Iran	Leaves	(Masoudi, 2018)
16	*(E)*-3-Caren-2-ol	-	Saudi Arabia	Aerial parts	(Ibrahim et al., 2017)
17	*(E)*-9-Octadecanoic acid	-	Iran	Flowers	(Masoudi, 2018)
18	*(E)*-Anethole	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
19	*(E)*-Caryophyllene	-	Iran	Aerial parts	(Keykavousi et al., 2016; Sadeghi et al., 2014a)
		-	Algeria	Flowers	(Bendif et al., 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
20	*(E)*-Decaline	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
21	*(E)*-Hex-2-en-1-ol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
22	*(E)*-Isoelemicin	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
23	*(E)*-Linalool oxide	-	Algeria	Vegetative parts	(Bendif et al., 2018)
24	*(E)*-Nerolidol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
25	*(E)*-Phytol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
26	*(E)*-Piperitenone oxide	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
27	*(E)*-α-Bergamotene	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
28	*(E)*-β-Damascenone	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
29	*(E)*-β-Farnesene	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
30	*(E)*-β-Ionone	-	Algeria	Vegetative parts	(Bendif et al., 2018)
31	*(E)*-β-Ocimene	-	Serbia and Montenegro, Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Gholivand et al., 2013; Kovacevic et al., 2001)
		ssp. *capitatum*	Corsica, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Iran, France	Leaves	(Chizzola, 2006; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
32	*(E)*-γ-Bisabolene	-	Iran	Aerial parts	(Gholivand et al., 2013; Sadeghi et al., 2014a)
33	*(Z,E)*-Farnesol	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
34	*(Z,Z)*-9,12-Octadecadienoic acid	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
35	*(Z,Z)*-Farnesol	-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
36	*(Z)*-9-Octadecenamide	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
37	*(Z)*-9,17-Octadecadienal	-	Iran	Flowers	(Masoudi, 2018)
38	*(Z)*-Hex-3-en-1-ol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
39	*(Z)*-Nerolidol	-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Iran	Leaves	(Masoudi, 2018)
40	*(Z)*-α-Bisabolene	-	Amman	Aerial parts	(Aburjai et al., 2006)
41	*(Z)*-α-Caryophyllene	-	Iran	Aerial parts	(Gholivand et al., 2013)
42	*(Z)*-α-Santalol	-	Amman	Aerial parts	(Aburjai et al., 2006)
43	*(Z)*-β-Caryophyllene	-	Iran	Aerial parts	(Gholivand et al., 2013)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
44	*(Z)*-β-Farnesene		Croatia, Turkey, Iran	Aerial parts	(Bezic et al., 2011; Heydarzade and Moravvej, 2012; Sevindik et al., 2016)
		ssp. *capitatum*	Serbia and Montenegro	Aerial parts	(Mitic et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
45	*(Z)*-β-Ocimene	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Heydarzade and Moravvej, 2012)
		ssp. *capitatum*	Serbia and Montenegro	Aerial parts	(Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
46	*(Z)*-γ-Bisabolene	-	Amman	Aerial parts	(Aburjai et al., 2006)
47	1-Methoxynaphthalene	-	Iran	Aerial parts	(Gholivand et al., 2013)
48	1-*nor*-Bourbonanone	ssp. *capitatum*	Bulgaria	Aerial parts	(Mitic et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
49	1-Octen-3-ol	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Gholivand et al., 2013; Nikpour et al., 2018)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
50	1-Octen-3-yl-acetate	-	Iran	Aerial parts	(Nikpour et al., 2018)
		-	Iran	Leaves	(Masoudi, 2018)
51	1,2,3-Trimethyl-cyclopentene	-	Iran	Aerial parts	(Nikpour et al., 2018)
52	1,2,3,6,7,7a-hexahydro-5-h-inden-5-one	-	Iran	Aerial parts	(Boroomand et al., 2018)
53	1,2,4,4-Tetramethylcyclopentene	-	Iran	Aerial parts	(Nikpour et al., 2018)
54	1,3,8-*p*-Menthatriene	-	Iran	Aerial parts	(Gholivand et al., 2013; Nikpour et al., 2018)
55	1,5-Epoxysalvial-4(14)-ene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
56	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
57	1,8-Cineole	-	Iran, Tunisia, Greece	Aerial parts	(Asgharipour and Shabankare, 2017; Boulila et al., 2008; Essid et al., 2015; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Serbia and Montenegro	Aerial parts	(Djabou et al., 2012; Mitic et al., 2012)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
58	1,8-Dehydro-cineole	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
59	11-Acetoxyeudesman-4-α-ol	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
60	14-Hydroxy-9-*epi*-*trans*-caryophyllene	-	Amman	Aerial parts	(Aburjai et al., 2006)
61	14-Hydroxy-α-muurolene	-	Algeria	Flowers	(Bendif et al., 2018)
62	1H-3a,7-Methanoazulene	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
63	1H-Cycloprop-[e]-azulene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
64	2-Methyl naphthalene	-	Iran	Stems	(Masoudi, 2018)
65	2-Methylbutyl butyrate	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
66	2-Naphthalene methanol	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
67	2-Pentyl furan	-	Algeria	Vegetative parts	(Bendif et al., 2018)
68	2-Undecanone	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
69	2-(4-Methyl-3-cyclohexen-1-yl)-2-propanamine	-	Iran	Aerial parts	(Nikpour et al., 2018)
70	2-Benzyl-1,3-dimethyl-guanidine	-	Iran	Aerial parts	(Nikpour et al., 2018)
71	2-Bromo-1-phenyl-1-propanone	-	Iran	Aerial parts	(Nikpour et al., 2018)
72	2-Menthene	-	Iran	Aerial parts	(Nikpour et al., 2018)
73	2-Methyl-3-hexyne	-	Iran	Aerial parts	(Nikpour et al., 2018)
74	2-Methyl-5-(1-methylethyl), *(S)*-2-cyclohexen-1-one	-	Iran	Aerial parts	(Nikpour et al., 2018)
75	2-Methylene bornane	-	Iran	Aerial parts	(Nikpour et al., 2018)
76	2-Pentanone	-	Iran	Aerial parts	(Nikpour et al., 2018)
77	2,3,3-Trimethyl-3-cyclopentene acetaldehyde	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
78	2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane	-	Iran	Aerial parts	(Nikpour et al., 2018)
79	2,4-Hexadiene	-	Iran	Aerial parts	(Nikpour et al., 2018)
80	2*E*-Hexenol	-	Algeria	Vegetative parts	(Bendif et al., 2018)
81	2H-Cycloprop-[e]-azulene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
82	3-Cyclohexene-1-methanol,α,4-dimethyl	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
83	3-Dodecarone	-	Iran	Aerial parts	(Gholivand et al., 2013)
84	3-Octanol	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
		-	Iran	Fruits	(Oroojalian et al., 2017)
85	3-Methyl butanal	-	Iran	Aerial parts	(Nikpour et al., 2018)
86	3-Methyl cyclohexene	-	Iran	Aerial parts	(Nikpour et al., 2018)
87	3,7-Dimethyl-2,6-octadien-1-ol	-	Iran	Aerial parts	(Nikpour et al., 2018)
88	3β-Hydroxy-α-muurolene	ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
89	4-Epicubebol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
90	4-Methylacetophenone	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
91	4-Vinyl guaiacol	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
92	4-Amino furazan-3-carboximide acid	-	Iran	Aerial parts	(Nikpour et al., 2018)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
	hydrazide				
93	4-Isopropyl-1-methyl-2-cyclohexen-1-ol	-	Iran	Aerial parts	(Nikpour et al., 2018)
94	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	-	Iran	Aerial parts	(Nikpour et al., 2018)
95	4,6-Dimethylhept-5-en-2-one	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
96	4α-Hydroxy dihydro agarofuran	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
97	5-*iso*-Cedranol	-	Amman	Aerial parts	(Aburjai et al., 2006)
98	5-Isopropyl-2-methyl bicyclo[3.1.0]hexan-2-ol	-	Iran	Aerial parts	(Nikpour et al., 2018)
99	5,6-Dimethyl-1,3-cyclohexadiene	-	Iran	Aerial parts	(Nikpour et al., 2018)
100	5*E*,9*E*-Farnesyl acetone	-	Algeria	Vegetative parts	(Bendif et al., 2018)
101	6-Methyl-5-heptene-2-one	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
102	6-Camphenol	-	Iran	Aerial parts	(Nikpour et al., 2018)
103	6,10,14-Trimethyl-2 pentadecanone	-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
104	6,6-Dimethyl-2-methylene bicyclo[3.1.1]heptan-3-one	-	Iran	Aerial parts	(Nikpour et al., 2018)
105	6,7-Bisepoxy-sec-calamenene	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
106	7-*epi*-α-Eudesmol	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
107	7-*epi*-α-Selinene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
108	7-*epi*-γ-Eudesmol	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
109	7-Methyl-1-octene	-	Iran	Aerial parts	(Nikpour et al., 2018)
110	8-Cedren-13-ol	-	Amman	Aerial parts	(Aburjai et al., 2006)
111	8-(1-Methylethylidene)bicyclo[5.1.0]octane	-	Iran	Aerial parts	(Nikpour et al., 2018)
112	Acetic acid	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
113	Agarospirol	-	Iran	Aerial parts	(Nikpour et al., 2018)
114	*allo*-Aromadendrene	-	Amman, Tunisia, Greece, Algeria	Aerial parts	(Aburjai et al., 2006; Bendjabeur et al., 2018; Boulila et al., 2008; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
115	*ar*-Curcumene	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
116	Aristolene	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
117	Aromadendrene	-	Algeria, Serbia and Montenegro, Iran	Aerial parts	(Bendjabeur et al., 2018; Kovacevic et al., 2001; Nikpour et al., 2018; Sadrizadeh et al., 2018)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
118	Aromadendrene oxide	-	Iran	Aerial parts	(Nikpour et al., 2018)
119	Benzene	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
120	Benzene, 1-methyl	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
121	Benzenemethanol, 4-(1-methylethyl)	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
122	Benzyl benzoate	-	Iran	Aerial parts	(Gholivand et al., 2013)
123	Bicyclo[3.1.1]Hept-2-ene-2-methanol	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
124	Bicyclo[3.1.1]Hept-3-en-2-one	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
125	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
126	Bicyclogermacrene	-	Iran, Algeria, Serbia and Montenegro	Aerial parts	(Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Sadeghi et al., 2014a; Sadrizadeh et al., 2018; Shabankare et al., 2015)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Greece, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
127	Bicyclosesquiphellandrene	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
128	Borneol	-	Tunisia, Croatia,	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Bezic

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
			Iran, Serbia and Montenegro, Greece		et al., 2011; Gholivand et al., 2013; Kovacevic et al., 2001; Raei et al., 2014; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Crete, Corsica, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
129	Bornyl acetate	-	Iran, Algeria, Croatia	Aerial parts	(Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Djabou et al., 2012; Keykavousi et al., 2016; Nikpour et al., 2018; Raei et al., 2014; Shabankare et al., 2015)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
130	Bornyl propionate	-	Iran	Aerial parts	(Gholivand et al., 2013)
131	Bourbonanone	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
132	Bulnesol	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
133	Bulnesyl acetate	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
134	Butanoic acid ethyl ester	-	Iran	Aerial parts	(Nikpour et al., 2018)
135	Butyl hydroxy toluene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
136	Cadalene	-	Amman, Algeria, Iran	Aerial parts	(Aburjai et al., 2006; Bendjabeur et al., 2018; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica, Crete, Iran, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Khani and Heydarian, 2014; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
137	Cadina-1,4-diene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
138	Cadina-4,10(15)-dien-3-one	-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
139	Cadinol	-	Serbia and Montenegro	Aerial parts	(Kovacevic et al., 2001)
140	Camphene	-	Iran, Tunisia, Algeria, Serbia and Montenegro	Aerial parts	(Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Nikpour et al., 2018; Shabankare et al., 2015)
		ssp. *capitatum*	Corsica, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
141	Camphenilone	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
142	Camphor	-	Tunisia, Croatia, Iran, Serbia and Montenegro	Aerial parts	(Ben Othman et al., 2017; Bezic et al., 2011; Boulila et al., 2008; Essid et al., 2015; Keykavousi et al., 2016; Kovacevic et al., 2001)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
143	Car-3-ene	-	Tunisia	Aerial parts	(Boulila et al., 2008)
144	Carotol	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
145	Carvacrol	-	Iran, Tunisia, Greece	Aerial parts	(Asgharipour and Shabankare, 2017; Essid et al., 2015; Keykavousi et al., 2016; Menichini et al., 2009; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	France	Leaves	(Chizzola, 2006)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
146	Carvone	-	Algeria, Iran, Greece	Aerial parts	(Bendjabeur et al., 2018; Heydarzade and Moravvej, 2012; Nikpour et al., 2018; Vokou and

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
					Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
147	Caryophylladienol I	ssp. *capitatum*	Greece	Aerial parts	(Menichini et al., 2009)
148	Caryophyllene	-	Iran	Aerial parts	(Nikpour et al., 2018; Sadrizadeh et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
149	Caryophyllene alcohol	-	Iran	Aerial parts	(Gholivand et al., 2013)
150	Caryophyllene oxide	-	Tunisia, Iran, Amman, Serbia and Montenegro, Greece	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Nikpour et al., 2018; Raei et al., 2014; Sadeghi et al., 2014a; Sadrizadeh et al., 2018; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Crete, Corsica, Iran, Serbia and Montenegro	Aerial parts	(De Martino et al., 2010; Djabou et al., 2012; Khani and Heydarian, 2014; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
151	Caryophyllenol II	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
152	Cedrenol	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
153	Cedrol	-	Iran, Serbia and Montenegro	Aerial parts	(Gholivand et al., 2013; Kovacevic et al., 2001)
154	Chrysanthenone	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
155	Cinerolon	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
156	Cinerone	-	Iran	Aerial parts	(Nikpour et al., 2018)
157	*cis-(Z)*-α-Bisabolene epoxide	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
158	*cis*-Carveol	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
159	*cis*-Carvone oxide	-	Iran	Aerial parts	(Nikpour et al., 2018)
160	*cis*-Chrysanthenyl acetate	-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
161	*cis*-Decaline	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
162	*cis*-Geraniol	-	Iran	Aerial parts	(Nikpour et al., 2018)
163	*cis*-Jasmone	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
164	*cis*-Linalool oxide, furanoid	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
165	*cis*-Muurola-4(14),5-diene	ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
166	*cis*-Pinocamphone	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
167	*cis*-Pinocarveol	ssp. *capitatum*	Bulgaria	Aerial parts	(Mitic et al., 2012)
168	*cis*-Piperitone epoxide	ssp. *capitatum*	Serbia and Montenegro	Aerial parts	(Mitic et al., 2012)
169	*cis*-Sabinene hydrate	ssp. *capitatum*	Crete, Corsica, Greece	Aerial parts	(De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
170	*cis*-Sabinol	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
171	*cis*-Thujopsenal	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
172	*cis*-Verbenol	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Corsica, Greece	Aerial parts	(De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
173	*cis*-Verbenone	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
174	*cis*-α-Bisabolene	-	Tunisia	Aerial parts	(Boulila et al., 2008)
175	*cis*-β-Farnesene	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
176	*cis*-β-Guaiene	-	Amman	Aerial parts	(Aburjai et al., 2006)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
177	*cis*-β-Ocimene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
178	Citral	-	Iran	Aerial parts	(Nikpour et al., 2018)
179	Citronellol	-	Iran	Aerial parts	(Gholivand et al., 2013)
180	Cryptomerione	-	Iran	Aerial parts	(Keykavousi et al., 2016)
181	Cryptone	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
182	Cubenol	-	Iran, Serbia and Montenegro	Aerial parts	(Kovacevic et al., 2001; Nikpour et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
183	Cumin aldehyde	ssp. *capitatum*	Crete, Greece, Corsica	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
184	Cuminol	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
185	Cuparene	-	Iran	Aerial parts	(Nikpour et al., 2018)
186	Cyclohexene, 1-methyl-4-(1-methylethenyl)	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
187	Cyclolongifolene oxide, dehydro	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
188	Cyclosativene	-	France	Inflorescence	(Chizzola, 2006)
189	Cyperene	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
190	Decanal	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
191	Dehydro sabina ketone	ssp. *capitatum*	Bulgaria	Aerial parts	(Mitic et al., 2012)
192	Dehydro sesquicineol	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
193	*diepi*-α-Cedrene epoxide	-	Iran	Aerial parts	(Nikpour et al., 2018)
194	Diethyl phthalate	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
195	Diisobutyl phthalate	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
196	Dillapiol	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
197	Dodecanoic acid	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Stems	(Masoudi, 2018)
198	Dotriacontane (C32)	-	Algeria	Flowers	(Bendif et al., 2018)
199	Eicosane (C20)	-	Iran, Algeria	Flowers	(Bendif et al., 2018; Masoudi, 2018)
200	Elemol	-	Serbia and Montenegro	Aerial parts	(Kovacevic et al., 2001)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
201	Elemol acetate	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
202	Endobornyl acetate	-	Jordan	TCM	(Al-Qudah et al., 2011)
203	*epi*-Bicyclosesquiphellandrene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
204	*epi*-α-Cadinol	-	Iran	Aerial parts	(Gholivand et al., 2013)
		-	France	Leaves	(Chizzola, 2006)
205	*epi*-α-Muurolol	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
206	Epicubenol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
207	Epiglobulol	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
208	Epizonaren	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
209	Eucarvone	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
210	Eudesma-4(15)-ene-6-ol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
211	Eudesma-3,7(11)-diene	-	Iran	Aerial parts	(Nikpour et al., 2018)
212	Eugenol	-	Croatia, Iran	Aerial parts	(Bezic et al., 2011; Heydarzade and Moravvej, 2012; Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
213	Farnesene	-	Iran	Aerial parts	(Raei et al., 2014)
214	Fenchol	-	Tunisia	Aerial parts	(Ben Othman et al., 2017; Essid et al., 2015)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
215	Fencholenic aldehyde	-	Tunisia	Aerial parts	(Bakari et al., 2015)
216	Fenchone	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
217	Fenchyl acetate	-	Iran, Tunisia	Aerial parts	(Ben Othman et al., 2017; Heydarzade and Moravvej, 2012)
		-	Iran	Fruits	(Djabou et al., 2012)
218	Folifolone	ssp. *capitatum*	Corsica	Aerial parts	(Sabzeghabaie and Asgarpanah, 2016)
219	Gauilyl acetate	-	Jordan	TCM	(Djabou et al., 2012)
220	Geranial	ssp. *polium*	Algeria	Aerial parts	(Al-Qudah et al., 2011)
221	Geraniol	-	Iran	Aerial parts	(Djabou et al., 2012)
		ssp. *capitatum*	Corsica	Aerial parts	(Gholivand et al., 2013)
		ssp. *polium*	Algeria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		-	Algeria	Flowers	(Djabou et al., 2012)
222	Geranyl acetate	-	Iran	Aerial parts	(Bendif et al., 2018)
		ssp. *capitatum*	Corsica	Aerial parts	(Nikpour et al., 2018)
		-	Iran	Fruits	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
223	Geranyl acetone	-	Iran	Stems	(Masoudi, 2018)

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
224	Geranyl-*n*-butyrate	-	Iran	Stems	(Masoudi, 2018)
225	Germacra-4(15),5,10(14)-trien-1-α-ol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
226	Germacrene	-	Iran, Tunisia	Aerial parts	(Alamdar et al., 2007; Essid et al., 2015)
227	Germacrene A	-	Iran	Aerial parts	(Gholivand et al., 2013)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
228	Germacrene B	-	Iran, Amman, Turkey, Tunisia, Serbia and Montenegro	Aerial parts	(Aburjai et al., 2006; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Sadeghi et al., 2014a; Saltan et al., 2019; Sayyad and Farahmandfar, 2017)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
229	Germacrene D	-	Amman, Iran, Algeria, Croatia, Tunisia, Serbia and Montenegro, Turkey	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Sadrizadeh et al., 2018; Sevindik et al., 2016; Shabankare et al., 2015)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Greece, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
230	Germacrene D 4-ol	-	Amman	Aerial parts	(Aburjai et al., 2006)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
231	Globulol	ssp. *capitatum*	Crete, Iran, Greece	Aerial parts	(De Martino et al., 2010; Khani and Heydarian, 2014; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
232	Glycerol-2-palmitate	-	Algeria	Flowers	(Bendif et al., 2018)
233	Gossonerol	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
234	Guaiol	-	Amman	Aerial parts	(Aburjai et al., 2006)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		-	Iran	Stems	(Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
235	Heneicosane (C21)	-	Algeria	Flowers	(Bendif et al., 2018)
236	Hentriacontane	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
237	Heptacosane (C27)	-	Croatia	Aerial parts	(Bezic et al., 2011)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
238	Heptadecane	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
239	Hexadecanoic acid	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
240	Hexahydrofarnesyl acetone	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
241	Hexanal	-	Iran	Aerial parts	(Nikpour et al., 2018)
242	Hinesol	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
243	Humulene epoxide II	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
244	*Iso*-3-thujanol	-	Iran	Aerial parts	(Gholivand et al., 2013)
245	*Iso*-Menthone	ssp. *capitatum*	Serbia and Montenegro	Aerial parts	(Mitic et al., 2012)
246	Isoaromadendrene epoxide	-	Iran	Aerial parts	(Nikpour et al., 2018)
247	Isobornylacetate	ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
248	Isochrysanthenone	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
249	Isophorone	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
250	Isopiperitenone	-	Iran	Aerial parts	(Nikpour et al., 2018)
251	Isopropylsulfonyl chloride	-	Iran	Aerial parts	(Nikpour et al., 2018)
252	Isospathulenol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
253	Junipene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
254	Kaurene	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
255	Khusinol	-	Iran	Leaves	(Masoudi, 2018)
256	Ledene	-	Turkey	Aerial parts	(Sevindik et al., 2016)
257	Ledol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
258	Limonene	-	Amman, Iran, Tunisia, Algeria, Croatia, Serbia and Montenegro, Turkey, Greece	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Greece, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
259	Linalool	-	Iran, Tunisia, Croatia, Greece, Tunisia	Aerial parts	(Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bezic et al., 2011; Gholivand et al., 2013; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Nikpour et al., 2018; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
260	Linalool oxide	-	Tunisia	Aerial parts	(Bakari et al., 2015)
261	Linalyl acetate	-	Croatia, Tunisia	Aerial parts	(Bezic et al., 2011; Boulila et al., 2008)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
262	Longipinanol	-	Algeria	Vegetative parts	(Bendif et al., 2018)
263	Longiverbenone	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
264	Manoyl oxide	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
265	Menthone	-	Iran	Aerial parts	(Keykavousi et al., 2016)
266	Menthyl acetate	-	Iran	Aerial parts	(Gholivand et al., 2013)
267	Methyl cyclopentane	-	Iran	Aerial parts	(Nikpour et al., 2018)
268	Mint sulfide	-	Algeria	Vegetative parts	(Bendif et al., 2018)
269	Mustakone	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
270	Myrcene	-	Amman, Iran, Tunisia, Croatia, Serbia and Montenegro,	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Ben Othman et al., 2017; Bezic et al., 2011; Boulila et al., 2008; Essid et al., 2015; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016;

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
			Turkey, Greece		Kovacevic et al., 2001; Purnavab et al., 2015; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
271	Myrtenal	-	Tunisia, Algeria, Iran, Serbia and Montenegro, Greece	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mitic et al., 2012; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
272	Myrtenol	-	Iran, Greece	Aerial parts	(Gholivand et al., 2013; Nikpour et al., 2018; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
273	Myrtenyl acetate	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
274	Naphthalene	-	Iran	Aerial parts	(Mahmoudi et al., 2014; Sadrizadeh et al., 2018)
275	*neo*-Intermedeol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
276	*neo*-*iso*-3-Thujanol acetate	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
277	Nerol	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
278	Neryl acetate	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
279	Nonacosane (C29)	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
280	Nonanal	-	Tunisia, Iran	Aerial parts	(Ben Othman et al., 2017; Gholivand et al., 2013; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica, Crete, Greece, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
281	*o*-Cymene	-	Tunisia	Aerial parts	(Essid et al., 2015)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
282	*o*-Cymol	-	Iran	Aerial parts	(Nikpour et al., 2018)
283	*o*-Menth-8-ene	-	Iran	Aerial parts	(Mahmoudi et al., 2014)
284	Ocimene	-	Iran	Aerial parts	(Nikpour et al., 2018)
285	Octacosane (C28)	-	Croatia	Aerial parts	(Bezic et al., 2011)
		-	Algeria	Flowers	(Bendif et al., 2018)
286	Octane	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
287	Oplopanone	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
288	*p*-Acetyltoluene	-	Iran	Aerial parts	(Nikpour et al., 2018)
289	*p*-Cymen-7-ol	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
290	*p*-Cymen-8-ol	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
291	*p*-Cymene	-	Amman, Iran, Tunisia, Algeria, Greece	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Nikpour et al., 2018; Raei et al., 2014; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
292	*p*-Cymenene	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
293	*p*-Mentha-1-en-7-al	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
294	*p*-Mentha-1,3-dien-7-al	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
295	*p*-Mentha-1,4-dien-7-ol	-	Iran	Aerial parts	(Nikpour et al., 2018)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
296	*p*-Mentha-1,5-dien-8-ol	-	Iran, Tunisia	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Gholivand et al., 2013; Keykavousi et al., 2016; Sadrizadeh et al., 2018)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
297	*p*-Mentha-3-en-8-ol	-	Iran	Aerial parts	(Gholivand et al., 2013)
298	*p*-Menthane-1,2,3-triol	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
299	*p*-Mentha-1-en-9-al	-	Iran	Aerial parts	(Nikpour et al., 2018)
300	*p*-Methoxyacetophenone	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
301	Palmitic acid	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
302	Pentacosane (C25)	-	Croatia	Aerial parts	(Bezic et al., 2011)
		ssp. *capitatum*	Serbia and Montenegro, Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
303	Perillaldehyde	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
304	Phellandral	-	Iran	Aerial parts	(Nikpour et al., 2018)
305	Phenylacetaldehyde	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
306	Phytol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
307	Phytone	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
308	Pinocarveol	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
309	Pinocarvone	-	Tunisia, Algeria, Iran, Serbia and Montenegro	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Gholivand et al., 2013; Kovacevic et al., 2001)
		ssp. *capitatum*	Corsica, Crete, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
310	Piperitenone	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
311	Piperitenone oxide	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
312	Pulegone	-	Iran	Aerial parts	(Keykavousi et al., 2016)
313	Rosifoliol	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
314	Sabina ketone	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
315	Sabinene	-	Iran, Amman, Algeria, Tunisia, Serbia and Montenegro	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Nikpour et al., 2018; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015)
		ssp. *capitatum*	Corsica, Crete, Iran, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Khani and Heydarian, 2014; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Greece	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
316	Salicylic acid butyl ester	-	Iran	Aerial parts	(Gholivand et al., 2013)
317	Salvial-4(14)-en-1-one	-	Algeria, Iran	Aerial parts	(Bendjabeur et al., 2018; Sadrizadeh et al., 2018)
		ssp. *capitatum*	Bulgaria	Aerial parts	(Mitic et al., 2012)
318	Sesquisabinene hydrate	-	Iran	Aerial parts	(Mahmoudi et al., 2014; Mahmoudi et al., 2015)
		ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
319	Shyobunol	ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
320	Sorbaldehyde	-	Iran	Aerial parts	(Nikpour et al., 2018)
321	Spathulenol	-	Amman, Iran, Tunisia, Algeria, Serbia and Montenegro	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Raei et al., 2014; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015)
		ssp. *capitatum*	Crete, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France, Greece, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
322	Spathulenol, 1h-Cycloprop	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
323	Squalene	-	Algeria	Flowers	(Bendif et al., 2018)
324	*t*-Cadinol	-	Turkey, Tunisia, Algeria, Greece	Aerial parts	(Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Saltan et al., 2019; Vokou and Bessiere, 1985)
325	*t*-Muurolol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
326	*tau*-Cadinol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
327	Terpinen-4-ol	-	Tunisia, Croatia, Iran, Greece	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Bezic et al., 2011; Boulila et al., 2008; Gholivand et al., 2013; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	Iran, Greece	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
328	Terpinolene	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
329	Tetradecanal	-	Iran	Stems	(Masoudi, 2018)
330	Tetradecanoic acid	-	Iran	Stems	(Masoudi, 2018)
331	Thuja-2,4(10)-diene	ssp. *capitatum*	Bulgaria	Aerial parts	(Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
332	Thymol	-	Iran	Aerial parts	(Gholivand et al., 2013)
		ssp. *capitatum*	Crete, Corsica, Greece	Aerial parts	(De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France	Leaves	(Chizzola, 2006)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
333	Toluene	-	Iran	Aerial parts	(Nikpour et al., 2018)
334	Torreyol	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
335	*trans-(+)*-Carveol	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
336	*trans*-2-Hexenal	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
337	*trans*-Calamenene	-	Serbia and	Aerial parts	(Kovacevic et al., 2001)

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
			Montenegro		
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
338	*trans*-Carveol	-	Tunisia, Iran, Greece	Aerial parts	(Ben Othman et al., 2017; Gholivand et al., 2013; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
339	*trans*-Carvyl acetate	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
340	*trans*-Caryophyllene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
341	*trans*-Chrysanthenyl acetate	-	Iran	Aerial parts	(Nikpour et al., 2018)
342	*trans*-Linalool oxide, furanoid	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
343	*trans*-*p*-Mentha-2,8-dien-1-ol	-	Algeria	Flowers	(Bendif et al., 2018)
344	*trans*-Pinocamphone	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
345	*trans*-Pinocarveol	-	Tunisia, Serbia and Montenegro, Greece	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Kovacevic et al., 2001; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
346	*trans*-Sabinene hydrate	-	Tunisia	Aerial parts	(Boulila et al., 2008)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
347	*trans*-Sabinol	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
348	*trans*-Sabinyl acetate	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
349	*trans*-Sesquisabinene hydrate	-	Iran	Stems	(Masoudi, 2018)
350	*trans*-Thujone	-	Algeria	Vegetative parts	(Bendif et al., 2018)
351	*trans*-Verbenol	-	Greece, Iran	Aerial parts	(Keykavousi et al., 2016; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Bulgaria, Corsica, Serbia and Montenegro	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
352	*trans*-α-Bergamotene	-	Croatia, Serbia and Montenegro	Aerial parts	(Bezic et al., 2011; Kovacevic et al., 2001)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005)
		-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
353	*trans*-β-Caryophyllene	-	Tunisia	Aerial parts	(Boulila et al., 2008)
354	*trans*-β-Farnesene	-	Iran	Aerial parts	(Gholivand et al., 2013; Sadrizadeh et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
355	*trans*-β-Guaiene	-	Algeria, Iran	Aerial parts	(Bendjabeur et al., 2018; Gholivand et al., 2013)
356	*trans*-β-Ocimene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
357	Triacontane	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
358	Umbellulone	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
359	Undecanal	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
360	Untriacontane (C31)	-	Algeria	Flowers	(Bendif et al., 2018)
361	Valencene	-	Amman, Serbia and Montenegro, Iran	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Kovacevic et al., 2001; Shabankare et al., 2015)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
362	Valerianol	-	Iran	Aerial parts	(Keykavousi et al., 2016)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		-	Iran	Stems	(Masoudi, 2018)
363	Verbenene	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Gholivand et al., 2013; Mahmoudi et al., 2014)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
364	Verbenol	-	Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Nikpour et al., 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
365	Verbenone	-	Iran, Tunisia, Greece	Aerial parts	(Ben Othman et al., 2017; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
					al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
366	Viridiflorol	-	Serbia and Montenegro, Iran	Aerial parts	(Kovacevic et al., 2001; Sadrizadeh et al., 2018)
		ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
367	Widdrol	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
368	α-Agarofuran	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
369	α-Amorphene	-	Serbia and Montenegro	Aerial parts	(Kovacevic et al., 2001)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
370	α-Bisabolene	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
371	α-Bisabolol	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
372	α-Bisabolol oxide	-	Jordan	TCM	(Al-Qudah et al., 2011)
373	α-Bisabolol oxide B	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
374	α-Bourbonene	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
375	α-Bulnesene	-	Iran	Aerial parts	(Nikpour et al., 2018)
376	α-Cadinene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
377	α-Cadinol	-	Greece, Serbia and Montenegro, Turkey, Amman, Tunisia	Aerial parts	(Aburjai et al., 2006; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Kovacevic et al., 2001; Saltan et al., 2019; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Iran, Crete, Greece, Bulgaria	Aerial parts	(De Martino et al., 2010; Khani and Heydarian, 2014; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
378	α-Calacorene	-	Algeria	Aerial parts	(Bendjabeur et al., 2018)
		ssp. *capitatum*	Corsica, Crete, Greece	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
379	α-Camphene	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
		-	Iran	Fruits	(Oroojalian et al., 2017)
380	α-Campholenal	-	Tunisia, Iran	Aerial parts	(Ben Othman et al., 2017; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
381	α-Campholenaldehyde	-	Algeria, Tunisia	Aerial parts	(Bakari et al., 2015; Bendjabeur et al., 2018)
382	α-Caryophyllene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
383	α-Copaene	-	Iran, Greece, Serbia and Montenegro, Algeria, Croatia, Tunisia	Aerial parts	(Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Kovacevic et al., 2001; Nikpour et al., 2018; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
		-	France	Inflorescence	(Chizzola, 2006)
		-	Iran	Leaves	(Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
384	α-Cubebene	-	Algeria, Tunisia	Aerial parts	(Bendjabeur et al., 2018; Boulila et al., 2008)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
385	α-Cubenol	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
386	α-Curcumene	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
387	α-Cyperone	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
388	α-Elemene	-	Serbia and Montenegro	Aerial parts	(Kovacevic et al., 2001)
389	α-Farnesene	-	Turkey	Aerial parts	(Sevindik et al., 2016)
390	α-Fenchyl acetate	-	Iran	Stems	(Masoudi, 2018)
391	α-Funebrene	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
392	α-Guaiene	-	Iran	Aerial parts	(Gholivand et al., 2013)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
393	α-Gurjunene	-	Amman, Tunisia	Aerial parts	(Aburjai et al., 2006; Boulila et al., 2008)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
394	α-Humulene	-	Iran, Greece, Serbia and Montenegro, Algeria, Croatia, Tunisia, Amman	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Nikpour et al., 2018; Raei et al., 2014; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
395	α-Longipinene	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
396	α-Muurolene	-	Greece, Serbia and Montenegro, Algeria, Iran	Aerial parts	(Bendjabeur et al., 2018; Kovacevic et al., 2001; Nikpour et al., 2018; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Stems	(Masoudi, 2018)
397	α-Muurolol	-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
398	α-Phellandrene	-	Tunisia, Iran	Aerial parts	(Boulila et al., 2008; Gholivand et al., 2013; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
399	α-Pinene	-	Iran, Turkey, Greece, Serbia and Montenegro, Amman, Tunisia, Algeria	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Sadeghi et al., 2014a; Saltan et al., 2019; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
400	α-Selinene	-	Algeria	Flowers	(Bendif et al., 2018)
401	α-Terpinene	-	Amman, Tunisia, Algeria, Iran	Aerial parts	(Aburjai et al., 2006; Bakari et al., 2015; Bendjabeur et al., 2018; Boulila et al., 2008; Gholivand et al., 2013)
		ssp. *capitatum*	Corsica, Serbia and Montenegro	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
402	α-Terpineol	-	Serbia and Montenegro, Tunisia, Iran	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Gholivand et al., 2013; Kovacevic et al., 2001; Nikpour et al., 2018)
		ssp. *capitatum*	Corsica, Greece	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
403	α-Terpinolene	-	Tunisia, Algeria, Iran	Aerial parts	(Bakari et al., 2015; Bendjabeur et al., 2018; Gholivand et al., 2013)
		-	France	Leaves	(Chizzola, 2006)
404	α-Terpinyl acetate	-	Serbia and Montenegro, Iran	Aerial parts	(Boroomand et al., 2018; Gholivand et al., 2013; Kovacevic et al., 2001)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
405	α-Thujene	-	Iran, Turkey, Amman, Tunisia, Algeria	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Gholivand et al., 2013; Nikpour et al., 2018; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015)
		ssp. *capitatum*	Corsica, Crete, Serbia and Montenegro	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	France	Leaves	(Chizzola, 2006)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
406	α-Thujenol	-	Jordan	TCM	(Al-Qudah et al., 2011)
407	α-Thujone	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
408	α-Ylangene	-	Serbia and Montenegro, Iran	Aerial parts	(Gholivand et al., 2013; Kovacevic et al., 2001)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
409	α-Zingiberene	-	Tunisia	Aerial parts	(Boulila et al., 2008)
410	β-Bisabolene	-	Iran, Turkey, Tunisia	Aerial parts	(Asgharipour and Shabankare, 2017; Boulila et al., 2008; Nikpour et al., 2018; Raei et al., 2014; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015)
411	β-Bisabolenol	-	Algeria	Flowers	(Bendif et al., 2018)
412	β-Bisabolol	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
413	β-Bourbonene	-	Iran, Greece, Serbia and Montenegro, Amman, Algeria, Croatia, Tunisia	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Sadrizadeh et al., 2018; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
414	β-Cadinene	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
415	β-Calacorene	-	Serbia and Montenegro, Greece	Aerial parts	(Kovacevic et al., 2001; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
416	β-Caryophyllene	-	Iran, Greece, Serbia and Montenegro, Amman, Algeria, Croatia, Tunisia	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Essid et al., 2015; Heydarzade and Moravvej, 2012; Kovacevic et al., 2001; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
417	β-Copaene	-	Croatia	Aerial parts	(Bezic et al., 2011)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
418	β-Cubebene	-	Serbia and Montenegro, Amman, Tunisia	Aerial parts	(Aburjai et al., 2006; Boulila et al., 2008; Kovacevic et al., 2001)
		ssp. *capitatum*	Crete, Greece, Serbia and Montenegro	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009; Mitic et al., 2012)
		-	France	Inflorescence	(Chizzola, 2006)
419	β-Cyclocitral	ssp. *capitatum*	Crete	Aerial parts	(De Martino et al., 2010)
420	β-Dihydroagarofuran	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
421	β-Elemene	-	Iran	Aerial parts	(Gholivand et al., 2013)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
422	β-Eudesmol	-	Iran, Amman	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016)
		ssp. *capitatum*	Corsica, Serbia and Montenegro	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
423	β-Eudesmol acetate	-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
424	β-Farnesene	-	Iran, Tunisia	Aerial parts	(Essid et al., 2015; Nikpour et al., 2018)
425	β-Guaiene	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
426	β-Gurjunene (Calarene)	-	Turkey, Algeria	Aerial parts	(Bendjabeur et al., 2018; Sevindik et al., 2016)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		-	France	Leaves	(Chizzola, 2006)
427	β-Humulene	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
428	β-Myrcene	-	Iran, Tunisia, Algeria	Aerial parts	(Bakari et al., 2015; Bendjabeur et al., 2018; Gholivand et al., 2013; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Nikpour et al., 2018)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
429	β-Oplopenone	-	Amman	Aerial parts	(Aburjai et al., 2006)
		-	Greece	Leaves	(Lianopoulou et al., 2014)
430	β-Patchoulene	-	Iran	Aerial parts	(Nikpour et al., 2018)
431	β-Phellandrene	-	Amman, Iran, Turkey	Aerial parts	(Aburjai et al., 2006; Boroomand et al., 2018; Sevindik et al., 2016)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
432	β-Pinene	-	Iran, Turkey, Greece, Serbia and Montenegro, Amman, Tunisia, Algeria, Croatia	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mahmoudi et al., 2014;

No	Chemical compound	ssp.²/var.³	Locality	Part/Extract	Reference
					Mahmoudi et al., 2015; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Sevindik et al., 2016; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *capitatum*	Corsica, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
433	β-Selinene	-	Algeria, Iran, Turkey	Aerial parts	(Bendjabeur et al., 2018; Gholivand et al., 2013; Sevindik et al., 2016)
		ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	France	Leaves	(Chizzola, 2006)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
434	β-Thujone	-	Tunisia, Croatia	Aerial parts	(Bakari et al., 2015; Ben Othman et al., 2017; Bezic et al., 2011)
		ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
435	β-Ylangene	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
436	γ-Cadinene	-	Iran, Greece, Serbia and Montenegro, Amman, Algeria, Tunisia	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Kovacevic et al., 2001; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Crete, Serbia and Montenegro, Bulgaria	Aerial parts	(De Martino et al., 2010; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Oroojalian et al., 2017)
		-	Iran	Leaves	(Masoudi, 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
437	γ-Elemene	-	Iran	Aerial parts	(Alamdar et al., 2007; Gholivand et al., 2013; Nikpour et al., 2018)
		ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
438	γ-Muurolene	-	Iran, Algeria	Aerial parts	(Alamdar et al., 2007; Bendjabeur et al., 2018)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
439	γ-Terpinene	-	Amman, Tunisia, Algeria, Iran	Aerial parts	(Aburjai et al., 2006; Bakari et al., 2015; Bendjabeur et al., 2018; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013)
		ssp. *capitatum*	Corsica, Serbia and Montenegro	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria	Flowers	(Bendif et al., 2018)
		-	Greece, Iran	Leaves	(Lianopoulou et al., 2014; Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
440	δ-Amorphene	ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
441	δ-Cadinene	-	Iran, Turkey, Greece, Amman, Algeria, Tunisia	Aerial parts	(Aburjai et al., 2006; Bendjabeur et al., 2018; Boulila et al., 2008; Essid et al., 2015; Gholivand et al., 2013; Nikpour et al., 2018; Sadeghi et al., 2014a; Sadrizadeh et al., 2018; Sevindik et al., 2016; Vokou and Bessiere, 1985)
		ssp. *capitatum*	Corsica, Crete, Greece, Serbia and Montenegro, Bulgaria	Aerial parts	(Cozzani et al., 2005; De Martino et al., 2010; Djabou et al., 2012; Menichini et al., 2009; Mitic et al., 2012)

No	Chemical compound	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Algeria, Iran	Flowers	(Bendif et al., 2018; Masoudi, 2018)
		-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
		-	France	Inflorescence	(Chizzola, 2006)
		-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
		ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
		-	Iran	Stems	(Masoudi, 2018)
		-	Algeria	Vegetative parts	(Bendif et al., 2018)
442	δ-Cadinol	-	Tunisia	Aerial parts	(Boulila et al., 2008)
		ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
443	δ-Calacorene	-	Greece	Aerial parts	(Vokou and Bessiere, 1985)
444	δ-Elemene	-	Iran	Flowers	(Masoudi, 2018)
		-	Iran	Leaves	(Masoudi, 2018)
445	δ-Guaiene	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
446	τ-Cadinol	-	Amman	Aerial parts	(Aburjai et al., 2006)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
		-	Jordan	TCM	(Al-Qudah et al., 2011)
447	τ-Muurolol	-	Iran	Aerial parts	(Nikpour et al., 2018)
		ssp. *capitatum*	Serbia and Montenegro, Bulgaria	Aerial parts	(Mitic et al., 2012)
		ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)

[1] Essential oil components found in the trace amounts in essential oils have been ignored. The components are listed in alphabetical order.
[2] ssp : subspecies,
[3] var: variety,
[4] TCM: Tissue culture material

As can be seen from Table 3, the chemical composition of the essential oil of *T. polium* has been studied by many researchers. In addition to *T. polium* itself, the subspecies aurasicum, capitatum, and polium have also been studied many times. As stated in section 3, *T. polium* is a plant species of Middle East origin. For this reason, most of the studies on this plant have been carried out in Middle Eastern countries such as Iran, Amman, Jordan, and Saudi Arabia. In addition, researchers from North African countries, such as Algeria and Tunisia, have been shown to be intensely interested in this plant species. Chemical composition of the essential oil of *T. polium* was also analysed by several research teams in Turkey. Due to the nature of a bridge between Europe and the Middle East, Turkey has a great importance in the comparison of data between east and west. In addition, essential oil compositions of samples collected from European countries such as Greece, Croatia, Bulgaria, France, Serbia and Montenegro, and islands in the Mediterranean such as Corsica and Crete were studied. In essential oil analysis, it was understood that the most ideal sample is aerial parts. In addition, flowers, fruits, inflorescence, leaves, stems, and vegetative parts have also been used in essential oil isolation. Interestingly, even tissue culture material was used to obtain the essential oil (Al-Qudah et al., 2011).

Since 1982, a total of 447 essential oil components have been identified from *T. polium* and its subspecies. It is understood that monoterpenes such as limonene, myrcene, β-Pinene, linalool, α-pinene, *(E)*-β-ocimene, borneol, *p*-cymene, sabinene, α-terpineol, α-thujene and sesquiterpenoids such as bicyclogermacrene, caryophyllene oxide, germacrene D, α-copaene, α-humulene, δ-cadinene, β-bourbonene, elemol, spathulenol, γ-cadinene are frequently identified in essential oil samples in the majority of these studies.

The main components of essential oils isolated from *T. polium* and its subspecies were given in Table 4. Compounds with a rate of more than 5 % in the oil samples were given in the table. According to literature data, as in Table 3, the main components of the essential oil of both *T. polium* and its subspecies (ssp. *capitatum*, ssp. *aurasicum*, and ssp. *polium*) were monoterpenes or sesquiterpenes. In samples collected from Iran, Tunisia, Amman, Croatia, Algeria and Greece, almost half of the essential oils were found to be composed of carvacrol (monoterpene), β-caryophyllene and β-bisabolol (sesquiterpenes). In addition, β-pinene, 11-acetoxyeudesman-4-a-ol, α-bisabolol, 1,2,3,6,7,7α-hexahydro-5-h-inden-5-one, α-pinene, germacrene D, 8-cedren-13-ol, γ-muurolene, 3β-hydroxy-α-muurolene, piperitenone oxide, t-cadinol and *(Z)*-α-caryophyllene were also higher than 20% in oil samples. It was found that the major compounds mentioned above were generally determined in the oils isolated from aerial parts of the samples. The main components were also found in the leaves, inflorescences, stems, fruits, flowers, and tissue culture materials.

Other components isolated from *T. polium* and its subspecies were given in Table 5. In addition to the subspecies given Tables 3 and 4, *expansum*, *gnaphalodes*, *pilosum*, *aureum*, and *vincentinum* were also among the subspecies where the compounds in Table 5 was isolated.

According to the data in the table, a total of 172 compounds belonging to flavonoids, *neo*-clerodane diterpenoids, phenolic compounds, phenylpropanoid glycosides, iridoid glycosides, abietane diterpenoids, sterols, triterpenic alcohols, abeo-abietanes, phenylethanol glycosides, and saponin glycosides were identified. In addition to aerial parts of the plant, roots, leaves, stems, and seeds were also used for the isolation of these compounds. Solvents used in the isolation of these compounds were EtOAc, acetone, petroleum ether, chloroform, MeOH, water, *n*-hexane, and CH_2Cl_2. The most frequently isolated components in the studies presented in Table 5 were flavonoids (apigenin, luteolin, cirsimaritin, routine). In addition, poliumoside, verbascoside (phenylpropanoid glycosides), teucardoside (iridoid glycoside), caffeic acid (phenolic compound) and teulamifin B (*neo*-clerodane diterpenoid) were also frequently identified. Aerial parts of the samples were mostly used for the isolation of these components. It was understood that the variety components in both Tables 3, 4, and 5 were not affected by the localities where the plants were collected and similar components were detected in the samples collected from both the Middle Eastern and European countries.

In addition to the classification performed according to morphological and/or anatomical features, plants can also be subjected to chemical classification, taking into account chemical variations. In this classification, called chemotaxonomy, the distribution of chemical compounds or biosynthetically related compound groups in plants is examined. Although traditionalist researchers insist that they do not accept chemical taxonomy against morphological classification, since ancient times, chemotaxonomic data are believed to be important, since some of the main components of essential oils have been used in the cosmetic, food and pharmaceutical industries (Bhargava et al., 2013). Kamel and Sandra (1994) suggested that sesquiterpenoids, particularly sesquiterpene alcohols, can be used as chemotaxonomic markers for the essential oils of *T. polium*. The data in Table 4 show that the suggestion that sesquiterpenes can be used as chemotaxonomic markers is correct. However, monoterpenes can also be evaluated as important chemotaxonomic markers for *T. polium* and its subspecies.

Table 4. Major compounds of the essential oils of *T. polium* together with its subspecies and varieties[1]

Chemical compound	Percentage (%)	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
(-)-Myrtenol	5.20	-	Jordan	TCM[2]	(Al-Qudah et al., 2011)
(+)-3-Carene	6.80	-	Jordan	TCM	(Al-Qudah et al., 2011)
(+)-Aromadendrene	8.70	-	Jordan	TCM	(Al-Qudah et al., 2011)
(+)-Spathulenol	8.60	-	Jordan	TCM	(Al-Qudah et al., 2011)
(E,E)-1,3,5-Undecatriene	8.97	-	Iran	Aerial parts	(Boroomand et al., 2018)
(E)-3-Caren-2-ol	12.10	-	Saudi Arabia	Aerial parts	(Ibrahim et al., 2017)
(E)-Caryophyllene	8.0-12.90	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
(E)-β-Farnesene	10.05	ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
(Z)-Nerolidol	7.13	-	Iran	Leaves	(Masoudi, 2018)
	6.23	-	Iran	Fruits	(Oroojalian et al., 2017)
(Z)-α-Caryophyllene	18.91-20.10	-	Iran	Aerial parts	(Gholivand et al., 2013)
(Z)-β-Farnesene	15.49	-	Turkey	Aerial parts	(Sevindik et al., 2016)
1,2,3,6,7,7a-Hexahydro-5-h-inden-5-one	25.80	-	Iran	Aerial parts	(Boroomand et al., 2018)
1,8-Cineole	6.26	-	Tunisia	Aerial parts	(Essid et al., 2015)
11-Acetoxyeudesman-4-α-ol	30.20	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
	26.30	-	Iran	Aerial parts	(Sayyad and Farahmandfar, 2017)
3β-Hydroxy-α-muurolene	22.50	ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
8-Cedren-13-ol	24.75	-	Amman	Aerial parts	(Aburjai et al., 2006)
Bicyclo[3.1.1]Hept-3-en-2-one	6.76	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
Bicyclogermacrene	5.00-12.00	-	Iran, Algeria	Aerial parts	(Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Purnavab et al., 2015; Raei et al., 2014; Shabankare et al., 2015)
	6.2	ssp. *capitatum*	Serbia	Aerial parts	(Mitic et al., 2012)
	5.5	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	5.80	-	France	Inflorescence	(Chizzola, 2006)
	6.20	-	France	Leaves	(Chizzola, 2006)
	9.11	ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
Camphene	6.40	-	Jordan	TCM	(Al-Qudah et al., 2011)
Camphor	6.21	-	Iran	Fruits	(Oroojalian et al., 2017)
Carvacrol	8.00-56.06	-	Iran, Tunisia	Aerial parts	(Asgharipour and Shabankare, 2017; Essid et al., 2015; Keykavousi et al., 2016; Shabankare et al., 2015)
	9.60-10.10	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
Carvone	11.29	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
Caryophyllene	9.80-10.10	ssp. *capitatum*	Crete, Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
Caryophyllene oxide	5.70-6.70	-	Iran, Greece	Aerial parts	(Keykavousi et al., 2016; Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017; Vokou and Bessiere, 1985)
	5.00-25.90	ssp. *capitatum*	Crete, Iran	Aerial parts	(De Martino et al., 2010; Khani and Heydarian, 2014)
	6.49	-	Iran	Stems	(Masoudi, 2018)
Cedrol	14.52-15.26	-	Iran	Aerial parts	(Gholivand et al., 2013)
cis-Verbenol	6.25	-	Iran	Aerial parts	(Nikpour et al., 2018)
cis-β-Farnesene	5.60-18.40	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
Cubenol	10.00	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
Elemol	14.50	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
	5.53	-	Iran	Stems	(Masoudi, 2018)
	8.20	-	Jordan	TCM	(Al-Qudah et al., 2011)
Endobornyl acetate	5.90	-	Jordan	TCM	(Al-Qudah et al., 2011)
epi-α-Muurolol	8.10	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
Epizonaren	9.62	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
Eugenol	6.50	-	Jordan	TCM	(Al-Qudah et al., 2011)
Farnesene	13.00	-	Iran	Aerial parts	(Raei et al., 2014)
Gauilyl acetate	9.50	-	Jordan	TCM	(Al-Qudah et al., 2011)
Germacrene B	8.70-10.11	-	Turkey, Iran	Aerial parts	(Mahmoudi et al., 2014; Mahmoudi et al., 2015; Saltan et al., 2019)
Germacrene D	6.33-25.00	-	Amman, Iran, Algeria, Croatia, Tunisia ,Serbia and Montenegro, Turkey	Aerial parts	(Aburjai et al., 2006; Asgharipour and Shabankare, 2017; Bendjabeur et al., 2018; Bezic et al., 2011; Boulila et al., 2008; Gholivand et al., 2013; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Purnavab et al., 2015; Raei et al., 2014; Sadrizadeh et al., 2018; Sevindik et al., 2016;

Chemical compound	Percentage (%)	ssp.²/var.³	Locality	Part/Extract	Reference
					Shabankare et al., 2015)
	17.70-31.80	ssp. *capitatum*	Serbia, Bulgaria	Aerial parts	(Mitic et al., 2012)
	14.80	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	7.80-12.50	-	Algeria	Flowers	(Bendif et al., 2018)
	7.36	-	Iran	Fruits	(Oroojalian et al., 2017)
	12.70-34.40	-	France	Inflorescence	(Chizzola, 2006)
	8.70-35.00	-	France, Greece	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014)
	53.68	ssp. *capitatum*	Greece	Leaves and inflorescences	(Fanouriou et al., 2018)
	13.80	-	Algeria	Vegetative parts	(Bendif et al., 2018)
Guaiol	8.70	-	Jordan	TCM	(Al-Qudah et al., 2011)
Hexadecanoic acid	16.37	-	Iran	Flowers	(Masoudi, 2018)
	5.17	-	Iran	Stems	(Masoudi, 2018)
Ledene	6.33	-	Turkey	Aerial parts	(Sevindik et al., 2016)
Limonene	5.03-9.20	-	Iran, Tunisia, Croatia, Turkey	Aerial parts	(Alamdar et al., 2007; Bakari et al., 2015; Bezic et al., 2011; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Purnavab et al., 2015; Sevindik et al., 2016)
	5.20-6.40	ssp. *capitatum*	Corsica, Bulgaria	Aerial parts	(Djabou et al., 2012; Mitic et al., 2012)
	5.60	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	5.00	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
Linalool	15.65-15.65	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
	14.00	ssp. *capitatum*	Serbia	Aerial parts	(Mitic et al., 2012)
	7.80	-	Greece	Leaves	(Lianopoulou et al., 2014)
Myrcene	12.50-15.50	-	Tunisia, Iran	Aerial parts	(Boulila et al., 2008; Purnavab et al., 2015)
	6.20-9.90	-	France	Inflorescence	(Chizzola, 2006)
	5.00-11.50	-	France	Leaves	(Chizzola, 2006)
Nonacosane (C29)	6.30	-	Algeria	Vegetative parts	(Bendif et al., 2018)
o-Cymene	6.13	-	Tunisia	Aerial parts	(Essid et al., 2015)
p-Cymene	5.25	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
	7.00	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005)
Phytol	9.50	-	Algeria	Vegetative parts	(Bendif et al., 2018)
Piperitenone oxide	21.72	-	Iran	Aerial parts	(Heydarzade and Moravvej, 2012)
Sabinene	5.24	-	Amman	Aerial parts	(Aburjai et al., 2006)
	21.80	-	France	Inflorescence	(Chizzola, 2006)
	25.50	-	France	Leaves	(Chizzola, 2006)
Sesquisabinene hydrate	5.26	-	Iran	Aerial parts	(Mahmoudi et al., 2014; Mahmoudi et al., 2015)
Shyobunol	5.60-8.40	-	Algeria	Flowers	(Bendif et al., 2018)
Spathulenol	5.80-15.06	-	Iran, Algeria	Aerial parts	(Alamdar et al., 2007; Bendjabeur et al., 2018; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Mahmoudi et al., 2014; Mahmoudi et al., 2015)
	6.40	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	6.70	-	Greece	Leaves	(Lianopoulou et al., 2014)
Spathulenol, 1h-Cycloprop	18.39	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
t-Cadinol	9.30-21.00	-	Turkey, Greece	Aerial parts	(Saltan et al., 2019; Vokou and Bessiere, 1985)
Terpinen-4-ol	6.20	ssp. *capitatum*	Corsica	Aerial parts	(Djabou et al., 2012)
	5.30-6.10	-	Greece	Leaves	(Lianopoulou et al., 2014)
Terpineol	5.00	-	Jordan	TCM	(Al-Qudah et al., 2011)
Terpinyl acetate	19.60	-	Iran	Aerial parts	(Boroomand et al., 2018)
Thymol	7.90	-	France	Leaves	(Chizzola, 2006)
Torreyol	6.50-7.60	ssp. *capitatum*	Crete Greece	Aerial parts	(De Martino et al., 2010; Menichini et al., 2009)
trans-Caryophyllene	6.17	-	Iran	Aerial parts	(Sadrizadeh et al., 2018)
trans-Caryophyllene	8.80	ssp. *capitatum*	Serbia	Aerial parts	(Mitic et al., 2012)
trans-Verbenol	6.30	-	Iran	Aerial parts	(Keykavousi et al., 2016)
Valenene	5.40	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
Verbenone	5.03	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
	5.30	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Bisabolol	24.60-27.10	-	Iran	Aerial parts	(Sadeghi et al., 2014a; Sayyad and Farahmandfar, 2017)
	9.60	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Bisabolol oxide	9.70	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Bisabolol oxide B	7.40	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
α-Cadinol	5.10-8.80	-	Greece, Turkey, Tunisia	Aerial parts	(Boulila et al., 2008; Saltan et al., 2019; Vokou and Bessiere, 1985)
	46.80	ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
	46.20	ssp. *capitatum*	Iran	Aerial parts	(Khani and Heydarian, 2014)
	13.01	-	Iran	Flowers	(Masoudi, 2018)
	8.11	-	Iran	Leaves	(Masoudi, 2018)
	15.72	-	Iran	Stems	(Masoudi, 2018)
	9.40	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Camphene	6.10	-	Iran	Aerial parts	(Asgharipour and Shabankare, 2017; Shabankare et al., 2015)
	5.73	-	Iran	Fruits	(Oroojalian et al., 2017)
α-*epi*-Cadinol	5.27-5.44	-	Iran	Aerial parts	(Gholivand et al., 2013)
α-Farnesene	10.71	-	Turkey	Aerial parts	(Sevindik et al., 2016)
α-Humulene	7.90	-	France	Inflorescence	(Chizzola, 2006)

Chemical compound	Percentage (%)	ssp.[2]/var.[3]	Locality	Part/Extract	Reference
	5.80	-	France	Leaves	(Chizzola, 2006)
α-Muurolol	19.53	-	Iran	Flowers	(Masoudi, 2018)
	20.03	-	Iran	Leaves	(Masoudi, 2018)
	25.02	-	Iran	Stems	(Masoudi, 2018)
α-Pinene	5.02-25.76	-	Iran, Serbia and Montenegro, Turkey, Tunisia	Aerial parts	(Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Boulila et al., 2008; Essid et al., 2015; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Nikpour et al., 2018; Purnavab et al., 2015; Raei et al., 2014; Saltan et al., 2019; Shabankare et al., 2015)
	9.50	ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
	9.30-28.80	ssp. *capitatum*	Corsica, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
	7.20	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	18.20	-	Iran	Fruits	(Sabzeghabaie and Asgarpanah, 2016)
	9.70-14.80	-	France	Inflorescence	(Chizzola, 2006)
	6.30-20.00	-	France	Leaves	(Chizzola, 2006)
α-Terpineol	5.20	-	Iran	Aerial parts	(Nikpour et al., 2018)
	5.10	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Thujene	8.46	-	Tunisia	Aerial parts	(Ben Othman et al., 2017)
	5.00-8.10	ssp. *capitatum*	Corsica	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012)
α-Thujenol	5.20	-	Jordan	TCM	(Al-Qudah et al., 2011)
α-Ylangene	5.00	-	Greece	Leaves	(Lianopoulou et al., 2014)
β-Bisabolol	45.60	-	Iran	Aerial parts	(Sadeghi et al., 2014a)
β-Caryophyllene	7.70-52.00	-	Iran, Amman, Croatia, Tunisia, Greece	Aerial parts	(Aburjai et al., 2006; Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bezic et al., 2011; Essid et al., 2015; Raei et al., 2014; Sayyad and Farahmandfar, 2017; Shabankare et al., 2015; Vokou and Bessiere, 1985)
	10.64	-	Iran	Flowers	(Masoudi, 2018)
	7.94	-	Iran	Fruits	(Oroojalian et al., 2017)
	5.40-14.70	-	France	Inflorescence	(Chizzola, 2006)
	10.11-16.70	-	France, Iran	Leaves	(Chizzola, 2006; Masoudi, 2018)
	10.86	-	Iran	Stems	(Masoudi, 2018)
β-Eudesmol	5.70-7.51	-	Iran	Aerial parts	(Alamdar et al., 2007; Keykavousi et al., 2016)
	8.70	-	Algeria	Vegetative parts	(Bendif et al., 2018)
β-Gurjunene	7.50	-	Turkey	Aerial parts	(Sevindik et al., 2016)
β-Myrcene	6.07-10.05	-	Iran, Tunisia	Aerial parts	(Bakari et al., 2015; Mahmoudi et al., 2014; Mahmoudi et al., 2015)
β-Phellandrene	6.62-10.77	-	Turkey, Iran	Aerial parts	(Boroomand et al., 2018; Sevindik et al., 2016)
β-Pinene	5.77-35.97	-	Iran, Serbia and Montenegro, Tunisia, Algeria	Aerial parts	(Alamdar et al., 2007; Asgharipour and Shabankare, 2017; Bakari et al., 2015; Ben Othman et al., 2017; Bendjabeur et al., 2018; Boulila et al., 2008; Heydarzade and Moravvej, 2012; Keykavousi et al., 2016; Kovacevic et al., 2001; Mahmoudi et al., 2014; Mahmoudi et al., 2015; Purnavab et al., 2015; Raei et al., 2014; Shabankare et al., 2015)
	8.30	ssp. *aurasiacum*	Algeria	Aerial parts	(Kabouche et al., 2007)
	7.20-26.80	ssp. *capitatum*	Corsica, Serbia, Bulgaria	Aerial parts	(Cozzani et al., 2005; Djabou et al., 2012; Mitic et al., 2012)
	16.60	ssp. *polium*	Algeria	Aerial parts	(Djabou et al., 2012)
	6.09-10.10	-	Iran	Fruits	(Oroojalian et al., 2017; Sabzeghabaie and Asgarpanah, 2016)
	12.20-22.70	-	France	Inflorescence	(Chizzola, 2006)
	6.65-19.30	-	Greece, France, Iran	Leaves	(Chizzola, 2006; Lianopoulou et al., 2014; Masoudi, 2018)
β-Thujone	5.70	-	Croatia	Aerial parts	(Bezic et al., 2011)
γ-Cadinene	6.26	-	Iran	Fruits	(Oroojalian et al., 2017)
γ-Elemene	16.80	-	Iran	Aerial parts	(Alamdar et al., 2007)
γ-Muurolene	23.15	-	Iran	Aerial parts	(Alamdar et al., 2007)
δ-Cadinene	7.70	-	Algeria	Flowers	(Bendif et al., 2018)
τ-Cadinol	9.20	-	Jordan	TCM	(Al-Qudah et al., 2011)

[1] Compounds of greater than 5.0% in oil samples were considered. [2] ssp : subspecies, [3] var: variety, [4] TCM: Tissue culture material

In providing information on chemotaxonomic markers, it is considered that in addition to the general names of the chemical compound groups, the authors should clearly document the compounds included in these groups (eg carvacrol, β-caryophyllene, β-bisabolol, β-pinene, α-bisabolol, α-pinene, germacrene D etc.). It has also been suggested in the literature that phenylethanoid and iridoid glycosides can be used as chemotaxonomic markers (Mitreski et al., 2014; Venditti et al., 2017). However, in addition to these groups, flavonoids, neo-clerodane diterpenoids, phenolic compounds, and phenylpropanoid glycoside were thought to be chemotaxonomic markers for T. polium.

5. Toxicity on kidney and liver

As discussed in detail in section 3, the use of *T. polium* among the people is very common. However, as with all herbal products consumed for various purposes, *T. polium* should be questioned in terms of possible toxic effects. In the literature, there are some data on the beneficial properties of this plant, as well as researchers who argue that it has various levels of toxic effects. Unfortunately, the researchers are not yet in consensus on whether *T. polium* has a toxic effect. Literature data on the effects of various extracts from *T. polium* on kidney and liver are given in Table 6 and 7.

Table 5. Other phytochemicals isolated from *T. polium* together with its subspecies and varieties[1].

Chemical class	No	Compound	ssp.[2]/var.[3]	Plant part	Extract	Reference
abeo-Abietanes	448	12,16-epoxy-6,11,14-trihydroxy-17(15→16)-abeo-3a,18cyclo-5,8,11,13,15-abietapentaen-7-one	-	Roots	EtOAc	(Fiorentino et al., 2010)
	449	12,16-epoxy-6,11,14-trihydroxy-17(15→16)-abeo-5,8,11,13,15-abietapentaen-7-one	-	Roots	EtOAc	(Fiorentino et al., 2010)
	450	12,16-epoxy-6,11,14,17-tetrahydroxy-17(15→16)-abeo5,8,11,13,15-abietapentaen-7-one	-	Roots	EtOAc	(Fiorentino et al., 2010)
	451	12,16-epoxy-6,11,14,17-tetrahydroxy-17(15→16)abeo-3a,18-cyclo-5,8,11,13,15-abietapentaen-7-one	-	Roots	EtOAc	(Fiorentino et al., 2010)
Abietane diterpenoids	452	Ferruginol	ssp. *expansum*	Roots	Acetone	(Cuadrado et al., 1992)
	453	Teuvincenone A	ssp. *expansum*	Roots	Acetone	(Cuadrado et al., 1992)
			-	Roots	EtOAc	(Fiorentino et al., 2010)
	454	Teuvincenone B	ssp. *expansum*	Roots	Acetone	(Cuadrado et al., 1992)
			-	Roots	EtOAc	(Fiorentino et al., 2010)
	455	Teuvincenone C	-	Roots	EtOAc	(Fiorentino et al., 2010)
	456	Teuvincenone D	-	Roots	EtOAc	(Fiorentino et al., 2010)
	457	Teuvincenone H	ssp. *expansum*	Roots	Acetone	(Cuadrado et al., 1992)
	458	Teuvincenone I	ssp. *expansum*	Roots	Acetone	(Cuadrado et al., 1992)
Flavonoids	459	3′,6-Dimethoxyapigenin	-	Aerial parts	Petroleum ether, chloroform, MeOH, water	(Sharififar et al., 2009)
	460	3′,4′,5trihydroxy-6,7-dimethoxy-flavone	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	461	4′,7-Dimethoxyapigenin	-	Aerial parts	Petroleum ether, chloroform, MeOH, water	(Sharififar et al., 2009)
	462	5,3′,4′-trihydroxy-3,7-dimethoxyflavone	-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
	463	5,4′-dihydroxy-3,7-dimethoxyflavone	-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
	464	7,4′-*O*-dimethylscutellar-ein(5,6-dihydroxy-7,4′-dimethoxyflavone)	-	Aerial parts	*n*-Hexane, CH_2Cl_2, and MeOH	(Elmasri et al., 2014)
	465	7-*O*-β-D-(5-*O*-syringyl)apiofuranosyl-(1→2)-β-D-glucopyranoside	-	Leaves	MeOH	(D'Abrosca et al., 2013)
	466	Acacetin	-	Stems and leaves	EtOH	(Venditti et al., 2017)
	467	Apigenin	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
			-	Leaves	MeOH	(D'Abrosca et al., 2013)
			-	Aerial parts	MeOH	(Esmaeili et al., 2009b)
			-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Leaves	MeOH	(Pacifico et al., 2012)
			-	Leaves	MeOH	(Proestos et al., 2006)
			-	Aerial parts	Petroleum ether, chloroform, MeOH, water	(Sharififar et al., 2009)
			-	Stems and leaves	EtOH	(Venditti et al., 2017)
	468	Apigenin 5-galloylglucoside	-	Leaves and stems	EtOH	(Kawashty et al., 1999)
	469	Apigenin 7-glucoside	-	Leaves and stems	EtOH	(Kawashty et al., 1999)
	470	Apigenin 7-*O*-glucoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
	471	Apigenin 7-*O*-glucuronide	-	Not specified	H_20	(Tepe et al., 2011)
	472	Apigenin-7-*O*-rutinoside	-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)

Chemical class	No	Compound	ssp.[2]/var.[3]	Plant part	Extract	Reference
	473	Apigenin 7-*O*-β-glucoside	-	Stems and leaves	EtOH	(Venditti et al., 2017)
	474	Apigenin glucoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	475	Apigenin-4',7-dimethylether	-	Not specified	Not specified	(Verykokidouvitsaropoulou and Vajias, 1986)
	476	Apigenin-4'-*O*-glucoside	-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
	477	Cirsilineol	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
	478	Cirsiliol	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	Not specified	(Stefova et al., 2007)
			-	Not specified	Not specified	(Verykokidouvitsaropoulou and Vajias, 1986)
	479	Cirsimaritin	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Stems and leaves	EtOH	(Venditti et al., 2017)
			-	Not specified	Not specified	(Verykokidouvitsaropoulou and Vajias, 1986)
	480	Dihydroxymethoxyflavone glycoside	-	Not specified	H_20	(Tepe et al., 2011)
	481	Diosmetin	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
			-	Not specified	H_20	(Tepe et al., 2011)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
	482	Diosmetin 7-*O*-glycoside	-	Not specified	H_20	(Tepe et al., 2011)
	483	Diosmetin 7-*O*-rutinoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	484	Eriodictyol	-	Leaves	MeOH	(Proestos et al., 2006)
	485	Eupatorin	-	Not specified	Not specified	(Verykokidouvitsaropoulou and Vajias, 1986)
	486	Isorhoifolin	ssp. *gnaphalodes*	Aerial parts	MeOH	(Boghrati et al., 2016)
	487	Jaranol	ssp. *gnaphalodes*	Aerial parts	MeOH	(Boghrati et al., 2016)
	488	Kaempferol	-	Leaves	EtOH	(Chioibas et al., 2019)
	489	Kaempferol 7-*O*-diglucoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	490	Luteolin	spp. *capitatum*	Aerial parts	EtOH	(Stefkov et al., 2011)
			-	Leaves	MeOH	(D'Abrosca et al., 2013)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Leaves	MeOH	(Pacifico et al., 2012)
			-	Leaves	MeOH	(Proestos et al., 2006)
			-	Not specified	H_20	(Tepe et al., 2011)
			-	Stems and leaves	EtOH	(Venditti et al., 2017)
	491	Luteolin 7-glucoside	-	Leaves and stems	EtOH	(Kawashty et al., 1999)
	492	Luteolin-7-*O*-glucoside	-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	H_20	(Tepe et al., 2011)
	493	Luteolin-7-*O*-rutinoside	-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	H_20	(Tepe et al., 2011)
	494	Luteolin-4'-*O*-glucoside	-	Aerial parts	MeOH	(De Marino et al., 2012)
	495	Luteolin-7-*O*-neohesperidoside	-	Aerial parts	MeOH	(De Marino et al., 2012)
	496	Luteolin-rutinoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	497	Myricetin	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
	498	Naringenin	-	Leaves	MeOH	(Proestos et al., 2006)
	499	*p*-Coumaroylglucoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
	500	Quercetin	-	Leaves	EtOH	(Chioibas et al., 2019)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Leaves	MeOH	(Proestos et al., 2006)
	501	Quercetin 3-*O*-rutinoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	502	Quercetin-3-rutinoside	-	Not specified	Not specified	(Esmaeili et al., 2009a)
	503	Rutin	-	Leaves	EtOH	(Chioibas et al., 2019)
			-	Aerial parts	MeOH	(Esmaeili et al., 2009b)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Leaves	MeOH	(Proestos et al., 2006)
			-	Aerial parts	Petroleum ether, chloroform, MeOH, water	(Sharififar et al., 2009)
	504	Salvigenin	-	Aerial parts	*n*-Hexane, CH_2Cl_2, and MeOH	(Elmasri et al., 2014)
	505	Tetrahydroxyflavone 7-*O*-glycoside	-	Not specified	H_20	(Tepe et al., 2011)
	506	Vicenin-2	-	Leaves and stems	EtOH	(Kawashty et al., 1999)
Iridoid glycosides	507	(1*R*,4*S*,10*R*) 10,11-dimethyl-dicyclohex-5(6)-en-1,4-diol-7-one	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016a)
	508	(7*S*,8*R*)-4-(*O*-*β*-D-	-	Aerial parts	CH_2Cl_2–	(Elmasri et al., 2015b)

Chemical class	No	Compound	ssp.²/var.³	Plant part	Extract	Reference
		glucopyranosyl)dehydrodiconiferyl alcohol			MeOH	
	509	(7*S*,8*R*)-5-methoxy-4-(*O*-β-D-glucopyranosyl)dehydrodiconiferyl alcohol	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	510	1α-(β-D-glucopyranoxy)-6α,7α-epoxy-4aβ,5α-dihydroxy-7methyl-1,4a,5,6,7,7aβ-hexahydrocyclopenta[c]pyran	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	511	1α-(β-D-glucopyranoxy)-7α,8α-epoxy-5β,6α-dihydroxy-8-methyl-1,5,6,7,8,9β-hexahydrocyclopenta[c]pyran	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
	512	4-[(β-D-glucopyranosyloxy)methylene]-5α-(2-hydroxyethyl)-5-(α-L-rhamnopyranosyloxy)-3-methylcyclopent-2en-1-one	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
				Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
	513	4α-[(β-Dglucopyranosyloxy)methyl]-5α-(2-hydroxyethyl)-3-methylcyclopent-2-en-1-one	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	514	5,6,7,3′,4′-pentahydroxyflavone	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	515	5α-(2hydroxyethyl)-4α-hydroxymethyl-3-methylcyclopent-2-en-1one	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	516	5α-[2(β-D-glucopyranosyloxy)ethyl]-4α-hydroxymethyl-3-methylcyclopent-2-en-1-one	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	517	Teucardoside	-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Aerial parts	*n*-Hexane, CH_2Cl_2, and MeOH	(Elmasri et al., 2014)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
			-	Not specified	EtOH	(Rizk et al., 1986)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2016b)
			-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
			-	Not specified	EtOH	(Rizk et al., 1986)
neo-clerodane diterpenoids	518	19-Acetyl gnaphalin	ssp. *aureum*	Aerial parts	Acetone	(Eguren et al., 1981)
	519	19-Acetylteupolin-Iv	ssp. *pilosum*	Not specified	Not specified	(Delatorre et al., 1986)
	520	19-Deacetylteuscorodol	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	521	20-*epi*-Auropolin	ssp. *polium*	Aerial parts	Acetone	(Bruno et al., 2003)
	522	20-*O*-Acetyl-teucrasiatin	-	Stems and leaves	EtOH	(Venditti et al., 2017)
	523	3,20-bis-deacetylteupyreinidine	ssp. *aurasianum*	Aerial parts	CH_2Cl_2–H_20	(Ladjel et al., 1994)
	524	3,6,20-tri-Deacetylteupyreinidine	ssp. *aurasianum*	Aerial parts	CH_2Cl_2–H_20	(Ladjel et al., 1994)
	525	3-Deacetylteumicropodine	ssp. *aurasianum*	Aerial parts	CH_2Cl_2–H_20	(Ladjel et al., 1994)
	526	6,20-bis-Deacetylteupyreinidine	ssp. *aurasianum*	Aerial parts	CH_2Cl_2–H_20	(Ladjel et al., 1994)
	527	7-Epicapitatin	-	Not specified	Not specified	(Alhazimi and Miana, 1993)
	528	Acetyl auropolin	ssp. *polium*	Aerial parts	Acetone	(Bruno et al., 2003)
	529	Auropolin	ssp. *aureum*	Aerial parts	Acetone	(Eguren et al., 1981)
			ssp. *polium*	Aerial parts	Acetone	(Bruno et al., 2003)
	530	Capitatin	ssp. *polium*	Aerial parts	Acetone	(Bruno et al., 2003)
	531	Gnaphalidin	ssp. *aureum*	Aerial parts	Acetone	(Eguren et al., 1981)
	532	Montanin B	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	533	Montanin D	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)
			-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	534	Montanin E	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	535	Montanin F	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	536	Polivincin A	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)
	537	Polivincin B	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)
	538	Polivincin C	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)

Chemical class	No	Compound	ssp.[2]/var.[3]	Plant part	Extract	Reference
	539	Teubutilin A	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	540	Teuchamaecrin C	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	541	Teucrasiatin	-	Stems and leaves	EtOH	(Venditti et al., 2017)
	542	Teucrin P_1	ssp. *aureum*	Aerial parts	Acetone	(Eguren et al., 1981)
	543	Teucroxylepin	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	544	Teukotschyn	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	545	Teulamifin B	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)
			-	Not specified	Not specified	(Malakov et al., 1988)
			-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	546	Teulolin A	-	Aerial parts	MeOH	(Bedir et al., 1999)
	547	Teulolin B	-	Aerial parts	MeOH	(Bedir et al., 1999)
	548	Teumicropodine	ssp. *aurasianum*	Aerial parts	CH_2Cl_2–H_20	(Ladjel et al., 1994)
	549	Teupolin I	-	Not specified	Not specified	(Alhazimi and Miana, 1993)
	550	Teupolin III	-	Not specified	Not specified	(Malakov et al., 1982)
	551	Teupolin IX	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	552	Teupolin VI	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	553	Teupolin VII	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	554	Teupolin VIII	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	555	Teupolin X	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	556	Teupolin XI	-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	557	Teupolin XII	ssp. *vincentinum*	Aerial parts	Acetone	(Bozov and Penchev, 2019)
			-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	558	Teusalvin C	-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Leaves	EtOAc, MeOH	(Fiorentino et al., 2011)
	559	Teuvincentin A	ssp. *vincentinum*	Not specified	Not specified	(Alhazimi and Miana, 1993)
	560	Teuvincentin B	ssp. *vincentinum*	Not specified	Not specified	(Alhazimi and Miana, 1993)
	561	Teuvincentin C	ssp. *vincentinum*	Not specified	Not specified	(Alhazimi and Miana, 1993)
Phenolic compounds	562	*(+)*-Catechin	-	Leaves	MeOH	(Proestos et al., 2006)
	563	3,4-Dihydroxybenzoic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	564	3-Nitro-phthalic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	565	5-Caffeoylquinic acid	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	566	8-*O*-Acetylharpagide	-	Aerial parts	MeOH	(De Marino et al., 2012)
	567	Arteincultone	-	Aerial parts	*n*-Hexane, CH_2Cl_2, and MeOH	(Elmasri et al., 2014)
	568	Caffeic acid	-	Leaves	EtOH	(Chioibas et al., 2019)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Leaves	MeOH	(Proestos et al., 2006)
			-	Not specified	H_20	(Tepe et al., 2011)
			-	Not specified	Not specified	(Vladimir-Knezevic et al., 2014)
	569	Catechin	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
	570	Chlorogenic acid	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Not specified	Not specified	(Vladimir-Knezevic et al., 2014)
	571	Coumaric acid	-	Leaves	EtOH	(Chioibas et al., 2019)
	572	Coumarin	-	Leaves	MeOH	(Proestos et al., 2006)
	573	Epicatechin	-	Leaves	EtOH	(Chioibas et al., 2019)
	574	Ferulic acid	-	Leaves	EtOH	(Chioibas et al., 2019)
			-	Leaves	MeOH	(Proestos et al., 2006)
			-	Not specified	Not specified	(Vladimir-Knezevic et al., 2014)
	575	Gallic acid	-	Leaves	EtOH	(Chioibas et al., 2019)
			-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)

Chemical class	No	Compound	ssp.[2]/var.[3]	Plant part	Extract	Reference
			-	Leaves	MeOH	(Proestos et al., 2006)
	576	Gentisic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	577	Hydroxycaffeic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	578	Hydroxytyrosol	-	Leaves	MeOH	(Proestos et al., 2006)
	579	*o*-Coumaric acid	-	Leaves	MeOH	(Proestos et al., 2006)
	580	*o*-Hydroxybenzoic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	581	*p*-Coumaric acid	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Leaves	MeOH	(Proestos et al., 2006)
	582	Pheophorbide A	-	Stems and leaves	EtOH	(Venditti et al., 2017)
	583	*p*-Hydroxybenzoic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	584	*p*-Hydroxyphenylpropionic acid	-	Leaves	MeOH	(Proestos et al., 2006)
	585	Protocatechuic acid	-	Leaves	EtOH	(Chioibas et al., 2019)
	586	Resveratrol	-	Leaves	EtOH	(Chioibas et al., 2019)
	587	Rosmarinic acid	-	Leaves	EtOH	(Chioibas et al., 2019)
	588	Sinapinic acid	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
	589	Tyrosol	-	Leaves	MeOH	(Proestos et al., 2006)
	590	Vanillic acid	-	Aerial parts	MeOH	(Milosevic-Djordjevic et al., 2018)
			-	Leaves	MeOH	(Proestos et al., 2006)
Phenylethanol glycosides	591	2-(3,4dihydroxyphenyl)ethanol	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	592	3-(O-β-D-glucopyranosyl)α-(O-β-D-glucopyranosyl)-4-hydroxyphenylethanol	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
	593	3,4-dihydroxy-3(O-β-D-glucopyranosyl)phenethanol	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015b)
Phenylpropanoid glycosides	594	Allysonoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	595	Caerulescenoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	596	Castanoside A	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	597	Echinacoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	598	Forsythoside A	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	599	Forsythoside B	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	600	Leucoseptoside A	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	601	Poliumoside	ssp. *gnaphalodes*	Aerial parts	MeOH	(Boghrati et al., 2016)
			-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Aerial parts	*n*-Hexane, CH_2Cl_2, and MeOH	(Elmasri et al., 2014)
			-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	MeOH	(Oganesyan et al., 1991)
			-	Leaves	MeOH	(Pacifico et al., 2012)
	602	Poliumoside B	-	Aerial parts	MeOH	(De Marino et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
	603	Samioside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
	604	Teucreoside	-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	H_20	(Tepe et al., 2011)
	605	Teupolioside	-	Not specified	MeOH	(Oganesyan et al., 1991)
	606	Verbascoside	ssp. *gnaphalodes*	Aerial parts	MeOH	(Boghrati et al., 2016)
			-	Aerial parts	MeOH, hexane, EtOAc	(Goulas et al., 2012)
			-	Aerial parts	MeOH	(Mitreski et al., 2014)
			-	Not specified	MeOH	(Oganesyan et al., 1991)
			-	Not specified	H_20	(Tepe et al., 2011)
			-	Stems and leaves	EtOH	(Venditti et al., 2017)
Saponin glycosides	607	Poliusaposide A	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015a)
	608	Poliusaposide B	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015a)
	609	Poliusaposide C	-	Aerial parts	CH_2Cl_2–MeOH	(Elmasri et al., 2015a)
Sterols	610	Campesterol	-	Seeds	Hexane	(Hachicha et al., 2009)
	611	Clerosterol	-	Seeds	Hexane	(Hachicha et al., 2009)
	612	Obtusifoliol (methylsterol)	-	Seeds	Hexane	(Hachicha et al., 2009)
	613	Sitosterol	-	Seeds	Hexane	(Hachicha et al., 2009)
	614	Stigmasterol	-	Seeds	Hexane	(Hachicha et al., 2009)
Triterpenic alcohols	615	24-Methylenecycloartanol	-	Seeds	Hexane	(Hachicha et al., 2009)
	616	A′,Neogammacer-22(29)-en-3-ol	-	Seeds	Hexane	(Hachicha et al., 2009)
	617	Fern-7-en-3β-ol	-	Seeds	Hexane	(Hachicha et al., 2009)
	618	Lanosterol	-	Seeds	Hexane	(Hachicha et al., 2009)
	619	β-Amyrine	-	Seeds	Hexane	(Hachicha et al., 2009)

[1] The components are listed in alphabetical order.
[2] ssp : subspecies
[3] var: variety

According to Scognamiglio et al. (2012), *T. polium* is a plant that can be safely consumed as it has a negligible side effect. There are other additional studies in the literature that support this claim. Al-Asmari et al. (2014) argued that *T. polium* has a protective effect on cultured hepatocytes due to its potent antioxidant and anti-inflammatory compounds and may only cause mild toxicity at high doses. According to Kiyani et al. (2011), the hydroalcoholic extract (1:1) obtained from this plant did not show any toxicity and induce hepatotoxicity. Kulevanova et al. (2006) also claims that *T. polium* is hepatoprotective. According to these researchers, the EtOAc extract from *T. polium* significantly eliminated CCl_4-induced liver damage in rats.

Table 6. Toxic effect of *T. polium* on kidney and liver on experimental animals.

Plant part	Extract	Test subject	Dose	Duration	Method of application	Result	Reference
Reports on kidney							
Not specified	Water extract	Spragu-Dawly rats	1.0, 2.0, 3.0, and 4.0 g/kg	14 days	Not specified	The extract has been reported to cause some changes in the renal extracellular matrix. For this reason, it has been suggested that more studies are needed to be used carefully and to determine complications.	(Talaei Khozani et al., 2005)
Aerial parts	Hydroalcoholic extract	Wistar rats	50, 100, 150, 200 mg/kg	28 days	Intraperitoneally	Due to the increase in *T. polium* dose, various kidney injuries such as degeneration, destruction and vacuolization have been reported in the kidney.	(Baradaran et al., 2013; Rafieian-Kopaei et al., 2014)
Aerial parts	Hydroalcoholic extract	Wistar rats	3, 10, 30, 100, and 200 mg/kg	7 days	Intraperitoneally	Hydroalcoholic extract at 200 mg/kg caused damage to kidney tissue.	(Ghasemi et al., 2019a)
Aerial parts	Decoction	Wistar rats	5g/L	7 days	By gavage	Treatment with *T. polium* resulted in the reversal of oxidative damage and biochemical changes induced by CCl_4.	(Rahmouni et al., 2019)
Not specified	Not specified	Sprague-Dawley rats	100, 300, 600 mg/kg	45 days	By gavage	It has been reported that ALT and AST levels increased significantly in female rats receiving *T. polium* at a dose of 300 mg/kg.	(Rasekh et al., 2004)
Reports on liver							
Aerial parts	EtOAc extract	N-Mary rats	0.5 g/kg	8 weeks	By gavage	Extract treatment provided improvement in liver steatosis, ballooning degeneration and inflammation in rats with NASH.	(Aghazadeh and Yazdanparast, 2010)
Leaves	EtOAc extract	N-Mary rats	0.5 g leaves powder/kg	8 weeks	Intragastric administration	Lipoprotein profiles of NASH animals treated with the extract were significantly improved. Serine ALP, AST and ALT activities decreased, while SOD, GPx, and GSH activities were increased.	(Amini et al., 2009)
Not specified	Crude extract	N-Mary rats	0.5 g/kg	8 weeks	Orally	Grade 1 hepatosteatosis, lobular inflammation and ballooning degeneration were reduced in NASH animals receiving crude extract.	(Amini and Yazdanparast, 2011)
Aerial parts	EtOAc extract	N-Mary rats	0.5 g leaves powder/kg	3 weeks	Orally	Treatment with *T. polium* extract reduced the severity of NASH symptoms. It also reduced the hepatic TNF-α and TGF-β gene expression, caspase-3 level, phosphorylated form of JNK, and high MDA level. On the other hand, the extract increased the SOD and GPx activities, phosphorylated level of ERK1/2 and hepatic GSH level.	(Amini et al., 2011)
Not specified	Not specified	Not specified	Not specified	Not	Not specified	The extract has been	(Mimidis et al., 2009)

Plant part	Extract	Test subject	Dose	Duration	Method of application	Result	Reference
				specified		reported to cause severe acute cholestasis.	
Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	The extract has been reported to cause severe acute cholestasis.	(Polymeros et al., 2002)
Aerial parts	Polyphenolic extract (butanolic fraction)	Wistar rats	300 mg/kg	10 days	Orally	It has been reported that the application of polyphenolic extract obtained from *T. polium* has a hepatoprotective effect. The extract has also been suggested to increase mitochondrial bioenergetics and suppress liver CYP2E1, GSTpi and TNF-α mRNA levels. These mechanisms are thought to contribute to the hepatoprotective effect.	(Baali et al., 2016)
Whole plant	80% aqueous-ethanol extract	ICR mice	125, 250 and 500 mg/kg	5 days	Orally	Doses of 250 and 500 mg/kg of *T. polium* extract have been reported to have a significant hepatoprotective effect.	(Forouzandeh et al., 2013)
Aerial parts	Hydroalcoholic extract	Wistar rats	3, 10, 30, 100, and 200 mg/kg	7 days	Intraperitoneally	200 mg/kg of *T. polium* extract has been reported to increase ALT, AST and bilirubin levels and cause tissue damage to the liver.	(Ghasemi et al., 2019a)
Not specified	Hydroalcoholic extract	Mice (not specified)	125, 250, 500mg/kg	5 days	Not specified	The extract showed hepatoprotective effect at all doses administered. However, the most effective dose values have been reported to be 250 and 500 mg/kg.	(Kalantari et al., 2012)
Aerial parts	EtOH extract	Hepatocyte culture	8.16 µg/mL of *T. polium* extract	3 hours	Not specified	EtOH extract from *T. polium* has been reported to have an inhibitory effect on the mutagenicity induced by MNNG. It has been stated that the extract does not show any toxic effects such as necrosis or apoptosis.	(Khader et al., 2010)
Not specified	Aqueous extract	Hepatocytes from Fischer rats	62.7 µg/mL of *T. polium* extract	3 hours	Not specified	The extract has been reported to significantly reduce apoptosis and necrotic cell number in combination with MNNG.	(Khader et al., 2007)
Leaves	Decoction	Sprague-Dawley rats	200mg/kg	28 weeks	Intraperitoneal	In this study, where the effectiveness of *T. polium* extract against hepatocellular carcinoma was examined, serum biochemical markers including ALT, AST, AFP, GGT, ALP, HCY, TNF-γ, α2MG and CbG returned to normal after 28 weeks of treatment. Total antioxidant capacity was significantly increased, liver lesion score decreased, and glucocorticoid activity was significantly intensified.	(Movahedi et al., 2014)
Aerial parts	Decoction	Wistar rats	200 mg/kg	7 days	By gavage	Treatment with a 200 mg/kg dose of *T. polium* extract provided protection against oxidative damage and biochemical changes induced by CCl_4.	(Rahmouni et al., 2019)
Not specified	Total extract	Sprague-Dawley rats	100, 300, or 600 mg/kg	45 days	By gavage	Significant increases in ALT and AST levels were detected in female rats	(Rasekh et al., 2004)

Plant part	Extract	Test subject	Dose	Duration	Method of application	Result	Reference
						administered 300 mg/kg of *T. polium* extract. In addition, it has been reported that weights of the livers of female rats administered 600 mg/kg extract increased significantly.	

In addition to the literature data presented above, there are those who claim that *T. polium* has toxic effect on both kidney and liver. Although nearly half of the researchers suggest that this plant has a toxic effect on the liver, it is clear that in the vast majority of studies, *T. polium* has a toxic effect on the kidneys. According to Alzweiri et al. (2011), the infusion prepared from *T. polium* leads to jaundice. The same research group argued that *T. polium* had a negative impact on human health due to its anorexic effect. It has also been claimed that consuming tea prepared from the aerial parts of this plant causes low birth in pregnant women (Mosaddegh et al., 2012). In addition to the results of *in vivo* studies, case reports were also presented in Table 7. Although consensus has not been reached in the in vivo studies regarding the toxic effect of *T. polium* on the liver, all clinical findings prove that this plant has a negative effect on liver function. It is stated in all case reports that *T. polium* toxicity is observed in everyone who consumes the tea of this herb regularly, regardless of age restriction. In the majority of cases, in addition to jaundice, serum ALT, AST, total bilirubin and direct bilirubin levels increased and a significant decrease in prothrombin level was observed. In patients undergoing liver biopsy, as a result of histological examination, hepatitis findings with moderate or severe necroinflammatory activity were observed (Savvidou et al., 2007). In almost all case reports, it was found that liver enzyme levels returned to normal after stopping *T. polium* intake. Vasileiadou et al. (2003) further suggested that after continuous or intermittent use of the plant, liver damage may occur and acute or chronic hepatitis with or without cholestasis may develop. The researchers also stated that people should not consider the use of plants without being officially informed about their possible negative effects.

Table 7. Toxic effect of *T. polium* on kidney and liver as the case reports.

Case reports	
Report	Reference
Three patients, 31, 33 and 37 years of age, admitted to the clinic due to the two years of persistent jaundice and elevated liver enzymes. It was determined that the patients used *T. polium* from forty days to three months. Two of the patients continued using *T. polium* during their previous pregnancy periods and admitted to another clinic due to similar complaints. Liver enzyme levels returned to normal in approximately three months after the stopping of *T. polium* use.	(Dag et al., 2014a)
T. polium was found to be the main cause of liver damage in seven of ten hepatotoxic patients admitted to the clinic.	(Dag et al., 2014b)
It was determined that a patient who admitted to the clinic used *T. polium* tea for ten days in August 1992 and additional ten days in December 1992 for the treatment of hypercholesterolemia. Jaundice appeared five days after the end of the second treatment period. In biochemical tests, a significant increase in ALT and AST levels and a decrease in prothrombin level were detected.	(Mattei et al., 1995)
An increase in ALT, AST, total bilirubin and direct bilirubin levels of a patient who consumed *T. polium* tea for six months for the treatment of hyperlipidemia was reported.	(Mazokopakis et al., 2004)
Five cases have been reported regarding the consumption of *T. polium* in the form of tea causing intrahepatic cholestasis.	(Mazokopakis et al., 2007)
Two Greek patients who used *T. polium* extract for two-three months to treat high cholesterol levels admitted to the clinic with high aminotransferase levels. Jaundice developed in one of the patients. As a result of histological examination of liver biopsies, moderate or severe necroinflammatory findings were detected. Discontinuation of herbal medicine treatment led to the normalization of liver enzymes in both patients.	(Savvidou et al., 2007)
Twin sisters of two months admitted to the emergency with vomiting complaints. It was understood that *T. polium* was given in the form of tea for the treatment of infantile colic by their families. The consumption of the tea in question was recommended by the neighbors. The babies were hospitalized due to possible side effects of *T. polium* consumption. ALT and AST levels were found to be high.	(Sezer and Bozaykut, 2012)
A 70-year-old farmer applied to the clinic for liver disease. The patient stated that he consumed approximately one-two liters of *T. polium* tea a day. As a result of biochemical analysis, the patient's ALT, AST and bilirubin levels were found to be high.	(Starakis et al., 2006)
Between 2000-2002, five patients were hospitalized. It was learned that three of these patients used *T. polium* for the treatment of diabetes and two of them for hyperlipidemia. Three of the patients used the plant occasionally and two of them used regularly every day for one month. Liver biopsy revealed acute hepatitis in two patients and chronic hepatitis with low grade cholestasis in the other two patients.	(Vasileiadou et al., 2003)

According to Rafieian-Kopaei et al. (2014), it has been reported that the pro-oxidant activity of some antioxidants may cause toxicity through oxidative stress. According to Chitturi and Farrell (2008), which claims that *T. polium* is the first plant to be proven to cause acute liver failure, some diterpenoid-derived reactive metabolites are the main components responsible for hepatotoxicity. Today, *T. polium* toxicity is thought to be caused mainly by neo-clerodane diterpenoids (Venditti et al., 2017).

6. Conclusions

In this review, the traditional use, phytochemistry and toxic effects of *T. polium* on kidney and liver were documented. It was understood that the plant has been used frequently by many people in many parts of the world since ancient times in the treatment of certain diseases. However, literature data showed that *T. polium* has toxic effect on kidney tissue. Moreover, in some of the studies on the liver and in all clinical reports, *T. polium* has also been proven to have toxic effect on the liver. Although it is difficult to change the traditional consumption habits of the people, it has been concluded that more attention should be paid to the use of the plant. As evaluated in detail in the sections above, the plant species in question has been frequently used by humans in the treatment of various diseases (especially gastrointestinal system disorders) since ancient times. It is thought that awareness of the possible harms of this plant should be created in people by providing sufficient information. As clearly stated by Rafieian-Kopaei et al. (2014), more clinical studies are required to better understand the effects *of T. polium* on the liver. In particular, the effects of the plant on ALT, AST, bilirubin, and prothrombin levels should be documented in more detail, and the histological changes on the liver tissue should be followed as a result of the use of the plant. It was also concluded that that regular consumption of *T. polium* should be avoided for long periods of time.

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Conflict of Interest

The authors confirm that there are no known conflicts of interest.

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Arzuhan Sihoglu Tepe: Conceptualization, Investigation, Methodolygy, Writing, Review & Editing.
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RESEARCH ARTICLE

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Investigation of *in vitro* antimicrobial activities of some hydroxybenzoic and hydroxycinnamic acids commonly found in medicinal and aromatic plants

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ABSTRACT

Since hospital-acquired microorganisms are developing more and more resistance to antibiotics used today, researchers are turning to new searches in the treatment of infectious diseases. Unfortunately, unconscious use of antibiotics is another important reason why microorganisms develop resistance to infectious diseases. The aim of this study was to test the antimicrobial activity of some hydroxybenzoic and hydroxycinnamic acids on various gram-positive and gram-negative bacteria and a yeast strain (*C. albicans*). Agar well diffusion and minimum inhibitory concentration (MIC) tests were applied to determine the antimicrobial activities of phenolic acids. Considering the activity findings of phytochemicals on all test microorganisms, they were ranked in terms of their activities with a statistical method called the relative inhibitory capacity index (RICI) (a method that was first introduced in the literature by the current study). RICI analysis showed that the most effective phenolic acids for all test microorganisms were sinapic acid and 4-hydroxybenzoic acid. The RICI coefficients of these compounds were 1.02 and 0.99, respectively. Sinapic acid exhibited a zone of inhibition of 9.00-27.00 mm and an MIC of 18.00-72.00 mg/ml on microorganisms. Inhibition zone and MIC value ranges of 4-hydroxybenzoic acid were determined as 9.00-16.00 mm and 36.00-72.00 mg/ml, respectively. RICI analyzes confirmed that 2-phenylbutyric acid and phloroglucinol carboxylic acid did not show any antimicrobial activity. It is thought that sinapic acid and 4-hydroxybenzoic acid can be used as alternative antimicrobial agents against multi drug resistant microorganisms.

1. Introduction

Hospital-acquired infections are often extremely resistant to antibiotics (Liu et al., 2020). Antibiotic-resistant microorganisms cause the death of hundreds of thousands of people worldwide every year. If a strategy to effectively combat microorganisms resistant to antibiotics is not implemented, it is estimated that the microorganisms showing resistance to the current antibiotics will cause the death of more than half of the patients who die of cancer by 2050 (Ventola, 2015). In order to combat this problem, researchers are trying to discover new and more effective compounds by focusing on the antimicrobial activities of phytochemicals.

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Plants have been used effectively in the treatment of various diseases for centuries. It has been shown that some plant species and foodstuffs containing plant-based compounds (for example, honey) accelerate wound healing and reduce infections in wounds due to the antimicrobial phytochemicals they contain (Efem, 1988; Molan and Betts, 2004). Researchers think that phenolic acids are mainly responsible for the antimicrobial activities of plants or plant-derived foods (Wahdan, 1998). In some studies, it has been proven that phenolic acids successfully eliminate multi-drug resistant microorganisms (Merkl et al., 2010; Nascimento et al., 2000; Wahdan, 1998).

Phenolic acids are low molecular weight compounds that contain a carboxylic acid group in their structure (Monroe et al., 2018). These compounds are secondary metabolites that the plant produces to defend itself against microbial pathogens. Phenolic compounds have lethal effects on microorganisms through different mechanisms. These mechanisms include destabilization of the bacterial membrane, change in plasma permeability, inhibition of enzymes

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produced by microorganisms, disruption of microbial metabolism (for example, protein synthesis), and deprivation of the substrates required for the growth of microorganisms (Dietrich and Nikfardjam, 2017). In a study by Borges et al. (2013), it was reported that phenolic acids affect the polarity by changing the surface electron acceptors of bacteria, and accordingly, they show stronger antimicrobial effects in gram-negative bacterial strains than gram-positive ones.

Phenolic acids show promising potential in curing infections caused by antibiotic-resistant microorganisms and accelerating wound healing. Researchers focus on the use of phenolic acids to combat both hospital-acquired infections (e.g. *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, etc.) and pathogens responsible for food spoilage. However, the effectiveness of many phenolic acids on microorganisms has not yet been fully elucidated. Some researchers have published conflicting data on the antimicrobial activities of phenolic acids (Chatterjee et al., 2015; Merkl et al., 2010).

The aim of this study is to investigate the in vitro antimicrobial activities of some hydroxybenzoic and hydroxycinnamic acids commonly found in medicinal and aromatic plants. In antimicrobial activity studies carried out to date, the activity potentials of total extracts, also called crude extract, have been evaluated rather than individual phytochemicals. There are a limited number of studies evaluating the antimicrobial activities of phytochemicals individually. On the other hand, speculative discourses on this subject still continue. It is thought that elucidating the antimicrobial activities of at least some phenolic acids with this study may be beneficial in terms of ending the speculations on this subject. The antimicrobial activities of these components were evaluated both qualitatively and quantitatively. In the qualitative part of the study, first of all, the activity potentials of standard phytochemicals were determined by the agar well diffusion method and the obtained findings were quantitatively confirmed by the minimum inhibitory concentration (MIC) assay.

2. Materials and methods

2.1. Hydroxybenzoic and hydroxycinnamic acids used in the study

In this study, the antimicrobial activities of the following phytochemicals were investigated: gallic acid (1), protocatechuic acid ethyl ester (2), vanillic acid (3), 4-hydroxybenzoic acid (4), ferulic acid (5), caffeic acid (6), *p*-coumaric acid (7), sinapic acid (8), *trans*-cinnamic acid (9), *(-)*-quinic acid (10), rosmarinic acid (11), *o*-coumaric acid (12), valeric acid (13), 2-phenylbutyric acid (14), *(±)*-jasmonic acid (15), methyl paraben (16), propyl paraben (17), phloroglucinol carboxylic acid (2,4,6-trihydroxybenzoic acid) (18) and gallic acid trimethyl ether (3,4,5-trimethoxybenzoic acid) (19) (Figure 1). All the compounds were purchased from Sigma-Aldrich (St. Louis, Missouri, ABD).

2.2. Test microorganisms

In this study *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* NCTC 5046, *Candida albicans* ATCC 10231, *Salmonella typhi* NCTC 9394, *Pseudomonas aeuroginosa* ATCC 27853, *Shigella boydii* NCTC 9359, *Shigella dysanteriae* NCTC 9762, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Proteus vulgaris* RSHM 96022, and *Corynebacterium diphteriae* RSHM 633 strains were used. Bacteria were incubated overnight in Mueller Hinton Agar (MHA) at 37 °C. *C. albicans* was incubated at 30 °C in Sabouraud Dextrose Agar (SDA) medium. Two different methods were used in activity tests. Each test was repeated three times.

2.3. Agar well diffusion method

Antimicrobial activities of hydroxybenzoic and hydroxycinnamic acids were determined by the agar well diffusion method. According to this method, standard bacterial strains were cultured at 37 °C overnight in Mueller Hinton Agar medium and *C. albicans* was cultured at 30 °C overnight in Sabouraud Dextrose Agar medium, and then suspensions conforming to the Mc Farland 0.5 chart were prepared in isotonic sodium chloride (NaCl) solution (108 cfu/µl for bacteria, 106 cfu/µl for *C. albicans*). Then, wells with a diameter of 6.0 mm were opened with a sterile glass tube and spread cultivation was carried out from each bacterial strain suspension with a swab stick into the agar medium. 25.0 µl of 10 mg/ml solutions of phytochemicals were transferred to the wells. Inhibition zone diameters observed in petri plates after 24 hours of incubation at 37 °C were recorded in mm (Sokmen et al., 1999). At the end of the incubation, the diameters of the inhibition zones formed around the wells loaded with the extract were measured from the bottom surface of the petri plates with the help of a ruler. Standard antibiotic discs Gentamicin and Nystatin were used as positive control agents.

2.4. Determination of the minimum inhibitory concentration (MIC)

The liquid microdilution method recommended by the NCCLS (National Committee for Clinical Laboratory Standards) was used to determine the MIC values of phytochemicals (Sokmen et al., 1999). Mueller Hinton Broth (MHB) for bacteria and Sabouraud Dextrose Broth (SDB) for *C. albicans* were used. Bacterial strains were incubated overnight in MHA at 37 °C. After incubation, 50 µl of medium and 50 µl of microorganisms were placed in each well. In the dilution process of phytochemicals, serial dilutions were carried out starting from 72.00 mg/ml and making the final concentration 4.50 mg/ml. It was then made up with dH_2O so that the total volume was 172.00 µl. In addition, growth control (MHB + microorganism) and sterility control (MHB + phytochemical) tests were applied. Microtiter plates on which the tests were made were incubated for 24 hours at 37 °C for bacteria and 48 hours at 30 °C for *C. albicans* under normal atmospheric conditions. In order to determine the susceptibility of test microorganisms, MIC values of Gentamycin for bacteria and Nystatin for *C. albicans* were also determined in parallel with the experiments. Bacterial growth was detected by re-culturing each well, as well as observing a white precipitate at the bottom of the wells in microtiter plates.

2.5. Determination of relative inhibition capacity index (RICI)

In this study, RICI was applied to statistically rank the activity potentials of phytochemicals using the zone diameter values obtained from the antimicrobial activity analysis. The aforementioned analysis was brought to the literature for the first time by this study by modifying the relative binding capacity index (RBCI) and relative antioxidant capacity index (RACI) methods performed by Istifli et al. (2020) and Sarikurkcu and Zengin (2020), respectively. Using RICI, it is possible to compare statistically relevant data with different scientific meanings. Since the inhibition zones of phytochemicals are different for each microorganism, phytochemicals can only be ranked in terms of their potential at this parameter if they are performed in the light of their inhibition zones only to one microorganism. However, sequencing based on only one of these microorganisms cannot represent the full activity potential of these molecules. The most common method used to calculate the interaction between each phytochemical and microorganism is the "central bias" in which components are ranked according to the mean value of each component.

If the values (inhibition zone) in each data set are converted into standard scores, it is possible to compare them with each other. Arithmetic mean and standard deviation values were calculated for each phytochemical by using the inhibition zones of the molecules. Raw standard scores were obtained by subtracting the inhibition zones of each phytochemical for each microorganism from this arithmetic mean and then dividing by the standard deviation value (see equation given below) (Sharma, 1996). The RICI values of each phytochemical were then calculated by taking the average of these standard scores obtained separately for each microorganism target.

$$\text{Standard score} = (x - \mu)/\sigma$$

where ‘x’ is the raw data, ‘μ’ is the mean, and ‘σ’ is the standard deviation.

3. Results and discussion

In order to determine the antimicrobial activities of hydroxybenzoic and hydroxycinnamic acids, agar well diffusion method, which is a qualitative test system, was first applied (Tables 1, 2 and 3). Then, the MIC test was performed to determine the concentration range in which phytochemicals inhibit the growth of microorganisms (Tables 4 and 5).

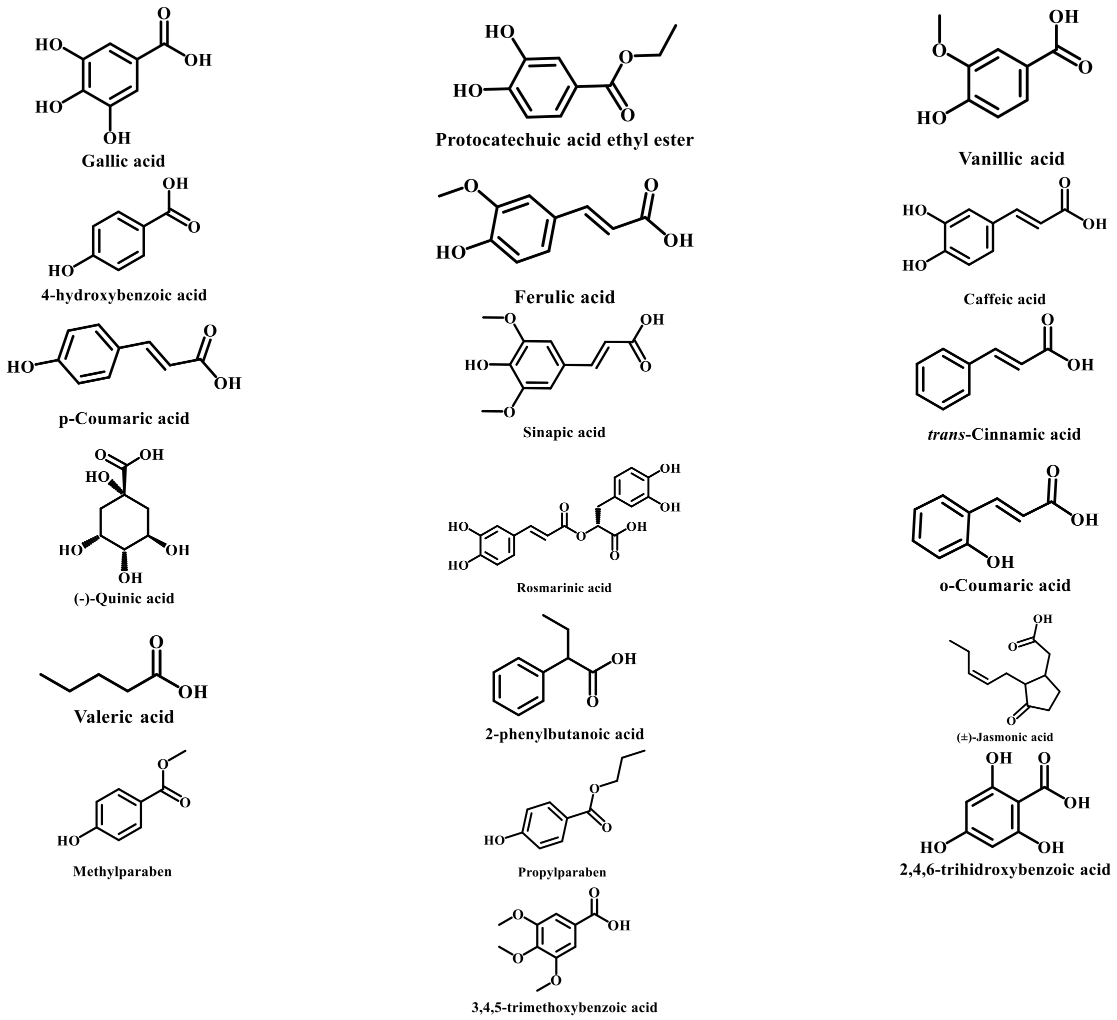

Figure 1. Chemical structures of compounds

Considering the data obtained as a result of the agar well diffusion method, it was determined that all phytochemicals except 2-phenylbutyric acid and phloroglucinol carboxylic acid exhibited various levels of antimicrobial activity on test microorganisms. Gallic acid, vanillic acid, 4-hydroxybenzoic acid, sinapic acid, *trans*-cinnamic acid, rosmarinic acid, *o*-coumaric acid and *(±)*-jasmonic acid were effective on all microorganisms, while protocatechuic acid ethyl ester, ferulic acid, caffeic acid, *p*-coumaric acid, *(-)*-quinic acid, valeric acid, methyl paraben, propyl paraben and gallic acid trimethyl ether did not show any effect on some microorganisms.

When the resistance and/or sensitivity of test microorganisms against phytochemicals were evaluated, the most sensitive ones were determined as *S. boydii*, *S. dysanteriae* and *S. aureus*. Most of the phytochemicals showed stronger activity on the microorganisms in question than gentamycin, which was used as a positive control agent.

The data obtained from the RICI analysis, in which the activities of phenolic acids against all test microorganisms were evaluated as a whole, are given in Figure 2. The data in the figure in question showed that the most effective phenolic acids for all test microorganisms were sinapic acid and 4-hydroxybenzoic acid. The RICI coefficients of these compounds were 1.02 and 0.99, respectively. Sinapic acid exhibited an inhibition zone of 9.00-27.00 mm and a MIC value of 18.00-72.00 mg/ml on the microorganisms. Inhibition zone and MIC value ranges of 4-hydroxybenzoic acid were determined as 9.00-16.00 mm and 36.00-72.00 mg/ml, respectively. RICI analyzes confirmed that 2-phenylbutyric acid and phloroglucinol carboxylic acid failed to show any antimicrobial activity. The RICI coefficient of both phenolic acids was determined as -1.48.

Table 1. Antimicrobial activities of compounds 1-6 obtained as a result of the agar well diffusion assay [1]

Microorganisms	Compounds						Controls	
	1	2	3	4	5	6	Gentamycin[2]	Nystatin[2]
S. typhi	9.00 ± 0.12^{bc}	8.00 ± 0.20^{ab}	11.00 ± 0.73^{de}	12.00 ± 0.14^{e}	-	-	10.00 ± 0.45^{cd}	n.t. [3]
P. aeruginosa	6.00 ± 0.63^{a}	-	8.00 ± 0.23^{abc}	9.00 ± 0.06^{bc}	-	-	20.00 ± 1.06^{d}	n.t.
S. boydii	13.00 ± 0.42^{bcd}	12.00 ± 0.79^{bc}	15.00 ± 1.40^{def}	16.00 ± 0.76^{efg}	16.00 ± 0.22^{efg}	13.00 ± 0.46^{bcd}	12.60 ± 0.20^{abcd}	n.t.
S. dysanteriae	7.00 ± 0.26^{ab}	6.00 ± 0.06^{a}	9.00 ± 0.22^{ab}	10.00 ± 0.40^{bc}	25.00 ± 1.14^{ijk}	23.00 ± 2.08^{ghi}	13.50 ± 0. 00^{cd}	n.t.
B. subtilis	13.00 ± 1.10^{ef}	12.00 ± 1.20^{de}	15.00 ± 1.16^{fg}	16.00 ± 0.48^{g}	6.00 ± 0.26^{a}	12.00 ± 0.22^{de}	29.00 ± 1.15^{h}	n.t.
K. pneumoniae	7.00 ± 0.20^{ab}	6.00 ± 0.26^{a}	9.00 ± 0.08^{cd}	10.00 ± 0.06^{d}	-	-	20.00 ± 0.70^{e}	n.t.
S. aureus	18.00 ± 1.40^{cde}	17.00 ± 1.14^{cd}	20.00 ± 1.54^{def}	21.00 ± 0.80^{efg}	25.00 ± 1.22^{hij}	11.00 ± 0.06^{a}	23.00 ± 0.76^{fgh}	n.t.
E. coli	8.00 ± 0.74^{ab}	7.00 ± 0.32^{a}	10.00 ± 0.82^{cd}	11.00 ± 0.42^{d}	-	-	16.00 ± 0.96^{e}	n.t.
P. vulgaris	7.00 ± 0.22^{ab}	6.00 ± 0.18^{a}	9.00 ± 0.14^{bc}	10.00 ± 0.14^{c}	6.00 ± 0.06^{a}	-	22.00 ± 1.40^{d}	n.t.
C. diphteriae	7.00 ± 0.14^{ab}	6.00 ± 0.14^{a}	9.00 ± 0.26^{cd}	10.00 ± 0.22^{d}	7.00 ± 0.40^{ab}	10.00 ± 0.43^{d}	23.00 ± 0.10^{f}	n.t.
C. albicans	12.00 ± 0.74^{d}	11.00 ± 0.84^{cd}	14.00 ± 0.40^{e}	15.00 ± 0.26^{e}	10.00 ± 0.08^{bc}	11.00 ± 0.07^{cd}	n.t.	n.t.

[1] The measured inhibition zone diameter includes the well diameter of 6.0 mm. Values indicated by the same superscripts within the same row are not different from the honestly significant difference after Tukey's post hoc test at 5% significance level.
[2] The concentration of antibiotics is 30 μg/disc.
[3] n.t.: Not tested

In studies carried out by some researchers on both gram-negative and gram-positive bacterial species, it has been reported that sinapic acid has an inhibitory activity of 97-99% on microorganisms (Nowak et al., 1992). In another study conducted by Engels et al. (2012), it was determined that this compound had a lethal effect only on sinapic acid-resistant microorganisms without harming the lactic acid bacteria in the environment. There are also reports that sinapic acid or some of its derivatives exhibit antifungal activity (Kelly et al., 2008).

Table 2. Antimicrobial activities of compounds 7-12 obtained as a result of the agar well diffusion assay [1]

Microorganisms	Compounds						Controls	
	7	8	9	10	11	12	Gentamycin[2]	Nystatin[2]
S. typhi	-	10.00 ± 0.24^{cd}	7.00 ± 0.12^{a}	-	8.00 ± 0.76^{ab}	7.00 ± 0.10^{a}	10.00 ± 0.45^{cd}	n.t. [3]
P. aeruginosa	-	10.00 ± 0.63^{c}	7.00 ± 0.24^{ab}	-	8.00 ± 0.96^{abc}	7.00 ± 0.04^{ab}	20.00 ± 1.06^{d}	n.t.
S. boydii	15.00 ± 1.08^{def}	17.00 ± 0.82^{fgh}	18.00 ± 0.16^{gh}	14.00 ± 0.44^{cde}	19.00 ± 0.40^{h}	16.00 ± 0.69^{efg}	12.60 ± 0.20^{abcd}	n.t.
S. dysanteriae	24.00 ± 0.73^{hij}	27.00 ± 1.60^{jk}	27.00 ± 0.49^{jk}	18.00 ± 0.32^{ef}	28.00 ± 2.15^{k}	26.00 ± 0.67^{ijk}	13.50 ± 0. 00^{cd}	n.t.
B. subtilis	-	16.00 ± 0.89^{g}	8.00 ± 0.06^{ab}	13.00 ± 0.15^{ef}	9.00 ± 0.16^{bc}	15.00 ± 0.07^{fg}	29.00 ± 1.15^{h}	n.t.
K. pneumoniae	-	9.00 ± 0.06^{cd}	7.00 ± 0.14^{ab}	-	7.00 ± 0.10^{ab}	8.00 ± 0.50^{bc}	20.00 ± 0.70^{e}	n.t.
S. aureus	24.00 ± 1.42^{ghi}	17.00 ± 0.14^{cd}	27.00 ± 1.26^{ij}	13.00 ± 0.45^{ab}	28.00 ± 1.06^{j}	16.00 ± 0.15^{bc}	23.00 ± 0.76^{fgh}	n.t.
E. coli	-	9.00 ± 0.49^{bc}	7.00 ± 0.04^{a}	-	7.00 ± 0.12^{a}	7.00 ± 0.06^{a}	16.00 ± 0.96^{e}	n.t.
P. vulgaris	-	9.00 ± 0.12^{bc}	8.00 ± 0.26^{abc}	-	8.00 ± 0.90^{abc}	7.00 ± 0.12^{ab}	22.00 ± 1.40^{d}	n.t.
C. diphteriae	6.00 ± 0.76^{a}	15.00 ± 0.24^{e}	9.00 ± 0.84^{cd}	10.00 ± 0.20^{d}	9.00 ± 0.76^{cd}	14.00 ± 0.60^{e}	23.00 ± 0.10^{f}	n.t.
C. albicans	-	10.00 ± 0.16^{bc}	7.00 ± 0.46^{a}	9.00 ± 0.10^{b}	11.00 ± 0.06^{cd}	9.00 ± 0.54^{b}	n.t.	25.00 ± 0.90

[1] The measured inhibition zone diameter includes the well diameter of 6.0 mm. Values indicated by the same superscripts within the same row are not different from the honestly significant difference after Tukey's post hoc test at 5% significance level.
[2] The concentration of antibiotics is 30 μg/disc.
[3] n.t.: Not tested

Table 4. Antimicrobial activities of compounds 1-10 obtained as a result of MIC assay (mg/ml)

Microorganisms	Compounds									
	1	2	3	4	5	6	7	8	9	10
S. typhi	72.00	72.00	72.00	72.00	> 72.00	> 72.00	> 72.00	72.00	72.00	> 72.00
P. aeruginosa	72.00	> 72.00	72.00	72.00	> 72.00	> 72.00	> 72.00	72.00	72.00	> 72.00
S. boydii	72.00	72.00	36.00	36.00	72.00	72.00	36.00	36.00	36.00	72.00
S. dysanteriae	72.00	72.00	72.00	72.00	36.00	36.00	36.00	18.00	18.00	36.00
B. subtilis	72.00	72.00	72.00	36.00	72.00	72.00	> 72.00	36.00	72.00	72.00
K. pneumoniae	72.00	72.00	72.00	72.00	> 72.00	> 72.00	> 72.00	72.00	72.00	> 72.00
S. aureus	36.00	36.00	36.00	36.00	36.00	72.00	36.00	36.00	18.00	72.00
E. coli	72.00	72.00	72.00	72.00	> 72.00	> 72.00	> 72.00	72.00	72.00	> 72.00
P. vulgaris	72.00	72.00	72.00	72.00	72.00	> 72.00	> 72.00	72.00	72.00	> 72.00
C. diphteriae	72.00	72.00	72.00	72.00	72.00	72.00	72.00	36.00	72.00	72.00
C. albicans	72.00	72.00	36.00	36.00	72.00	72.00	> 72.00	72.00	72.00	72.00

[1] The concentration of antibiotics is 30 ?g/disc.
[2] n.t.: Not tested

There are also some reports in the literature that 4-hydroxybenzoic acid has antimicrobial activity. In a study by Cho et al. (1998), this compound was reported to be effective on many gram-negative and gram-positive microorganisms tested.

In addition to the literature data given above, there are some data in the literature regarding the antimicrobial activities of some of the hydroxycinnamic and hydroxybenzoic acids analyzed in the present study. In a study carried out by Merkl et al. (2010), antimicrobial activities of some phenolic acids and their derivatives were reported. The data obtained from the aforementioned study support those in the current study.

Table 5. Antimicrobial activities of compounds 11-19 obtained as a result of MIC assay (mg/ml)

Microorganisms	Compounds								
	11	12	13	14	15	16	17	18	19
S. typhi	72.00	72.00	72.00	> 72.00	72.00	72.00	> 72.00	> 72.00	> 72.00
P. aeruginosa	72.00	> 72.00	72.00	> 72.00	72.00	> 72.00	> 72.00	> 72.00	> 72.00
S. boydii	36.00	36.00	36.00	> 72.00	36.00	72.00	72.00	> 72.00	72.00
S. dysanteriae	18.00	18.00	36.00	> 72.00	36.00	> 72.00	72.00	> 72.00	36.00
B. subtilis	72.00	72.00	72.00	> 72.00	72.00	72.00	72.00	> 72.00	72.00
K. pneumoniae	72.00	72.00	72.00	> 72.00	72.00	> 72.00	> 72.00	> 72.00	> 72.00
S. aureus	18.00	36.00	36.00	> 72.00	18.00	72.00	36.00	> 72.00	36.00
E. coli	72.00	72.00	72.00	> 72.00	72.00	72.00	> 72.00	> 72.00	> 72.00
P. vulgaris	72.00	72.00	72.00	> 72.00	> 72.00	> 72.00	> 72.00	> 72.00	> 72.00
C. diphteriae	72.00	72.00	> 72.00	> 72.00	> 72.00	> 72.00	> 72.00	> 72.00	> 72.00
C. albicans	72.00	72.00	72.00	> 72.00	72.00	72.00	> 72.00	> 72.00	> 72.00

[1] The concentration of antibiotics is 30 μg/disc.
[2] n.t.: Not tested

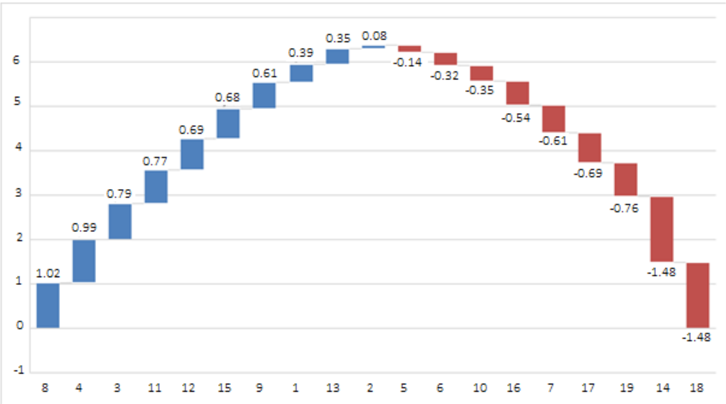

Figure 2. Sorting phytochemicals from strongest to weakest according to the RICI values

4. Conclusions

In this study, antimicrobial activities of some hydroxybenzoic and hydroxycinnamic acids on gram-negative and gram-positive bacteria and *C. albicans* were analyzed. According to the results obtained, it has been concluded that sinapic acid and 4-hydroxybenzoic acid can be used as alternative antimicrobial agents today, where hospital-acquired infectious agents make treatment with existing antimicrobial drugs difficult.

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Conflict of interest

The author confirms that there are no known conflicts of interest.

CRediT authorship contribution statement

Aslihan Gurbuzer: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing, Review & Editing

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RESEARCH ARTICLE

OPEN ACCESS

Investigation of the anti-parasitic effect of the water extract of *Thymbra spicata* on *Acanthamoeba castellani* (L.) trophozoites and cysts

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ABSTRACT

This study aimed to determine the anti-parasitic activity of the water extract obtained from *Thymbra spicata* (L.). The plant material was extracted with methanol in a Soxhlet apparatus. The extract was then fractionated with water and chloroform. The water phase was frozen and freeze-dried. Afterward, this extract was applied on *A. castellani* trophozoites and cysts at various concentrations, and the viability rates were determined by counting under the microscope. At the end of the experimental process, it was determined that there was a strong correlation between the increasing extract concentration and the anti-parasitic effect. *T. spicata* extract was not effective enough to neutralize all cysts at any of the concentrations examined. *T. spicata* extract, at concentrations of 16.0 and 32.0 mg/ml, removed all trophozoites in the medium from the 24th h of the experiment. Based on this result, it was determined that the plant species evaluated here could be used to treat *A. castellani* infections. It will be possible to achieve a good effect on the cyst forms of the parasite species by testing higher extract concentrations, which cannot be evaluated during the experimental process. In addition, with advanced analyzes, it will be possible to reveal the chemical substance responsible for the activity in the plant species in question and to perform further analyzes on this substance.

1. Introduction

Plants have been used as medicine for thousands of years (Samuelsson, 2004). Medications were initially used in herbal teas, poultices, powders, and other herbal formulations (Balick and Cox, 2020; Samuelsson, 2004). Some specific plants used and the treatment methods against various diseases were transferred from language to language and brought to the next generations. If we look at the recent past, it is seen that the use of plants as medicine has come up to the isolation of the active substances. The first active substance obtained from plants was morphine, which was was followed by cocaine, codeine, digitoxin, and quinine, which are isolated from the poppy plant in the early 19th century (Kinghorn, 2001; Sam-uelsson, 2004). In the following years, this development still in use today (Butler, 2004; Newman et al., 2000; Samuelsson, 2004). The isolation and identification of compounds with pharmacological activity from medicinal plants continue today.

Acanthamoeba is a parasite that causes painful and often progressive cases of keratitis (Jones, 1986). This organism is also the causative agent of the disease, also known as granular amoebic encephalitis (GAE), which causes severe immune system deficiencies. It is possible to collect the genus Acanthamoeba under three main morphological groups. Most of the pathogenic species are in the morphological group II. On the other hand, there are reports that some members of group III are also responsible for diseases (Pussard, 1977).

Unfortunately, there is no practical method that can be used in the treatment of *Acanthamoeba* infections today. In only a minority of

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cases, patients respond positively to treatment. *Acanthamoeba* keratitis is treated with a combination of cationic antiseptics such as olyhexamethylene biguanide, which inhibits membrane function, aromatic diamines, and propamidine isethionate (Brolene), which inhibits DNA synthesis. This treatment is given to the patient every hour for three days, and the dose can be increased up to 6 times a day (Duguid et al., 1997; Pussard, 1977). However, almost half of the patients show improvement, while others develop resistance to propamidine (Ficker et al., 1990). Another substance used as an alternative to this substance is chlorhexidine, which is used together with propamidine isethionate (Hay et al., 1994). Chlorhexidine is one of the substances successfully applied alone today (Kosrirukvongs et al., 1999). However, all these treatment methods need to be applied for a few days and hourly. Therefore, in the majority of cases, patients must be in stable conditions.

Most of the chemical agents used to treat parasitic infections today are highly toxic to humans and cause undesirable side effects. For this reason, intense efforts are being made to find alternative methods in the treatment of such infections, where serious problems and sometimes deaths occur. Herbal medicines are currently used to treat parasitic infections caused by agents other than *Acanthamoeba (Arrieta et al., 2001; Fernández et al., 2005; Kayser et al., 2003)*.

In light of the information obtained within the current study, if it is determined that the related plant species has anti-parasitic activity, it aims to recommend this information to industrial areas such as pharmacology, which need new active molecules used in product development studies. This study aimed to investigate the anti-parasitic activity of the water extract obtained from *Thymbra spicata*.

2. Materials and methods

2.1. Plant material and extraction processes

The aerial parts of the plant material were dried in a room where there is no direct sunlight and airflow. Following the literature studies, the extraction processes of all plants were carried out according to the following methods.

A 100 g sample was ground by using a blender. The sample was then extracted with methanol in a Soxhlet apparatus for 6 h. The extract was then evaporated to remove the methanol. The medium to be used in anti-parasitic activity studies is an aqueous medium. Therefore, substances that can dissolve in this medium are also polar.

For this reason, the extract, which became viscous, was shaken by adding water and chloroform to separate polar and nonpolar substances from each other. While chloroform remained in the lower part, the water remained in the upper part so that nonpolar substances in chloroform and polar substances in water were dissolved and separated from each other. The water phase was separated, frozen, and lyophilized into powder. The extract was stored in a refrigerator at +4 ^{o}C until testing (Sokmen et al., 1999).

2.2. Determination of anti-parasitic activity

In the determination of anti-parasitic activity, the method used by Tepe et al. (2011) was followed.

2.2.1. Trophozoites

A. castellani was incubated on non-nutritive agar plates and covered with *Escherichia coli* at 26 ^{o}C. Trophozoites in the exponential growth stage (72-96th h) were isolated from the plates using a sterile cell scraper. The trophozoites in the plates were washed twice with sterile Page's salt [(PS) (0.12 g NaCl, 0.004 g $MgSO_4.7H_2O$, 0.004 g $CaCl_2.2H_2O$, 0.142 g Na_2HPO_4, and 0.136 g KH_2PO_4 per 1 liter distilled water]. Afterward, centrifugation increased its concentration (1500g, 5 min) (Garcia, 2001). Viable trophozoites were counted using a hemocytometer.

2.2.2. Cysts

In this part of the study, three-week cultures of *A. castellani* were used. Cysts were isolated using sterile Page's salt, and the final concentration was adjusted to 10x10^5 cysts/ml. The viability of the cysts was determined by the trypan blue method.

2.2.3. Anti-parasitic activity

In this study, 1.5 ml microcentrifuge tubes were used. 200 µl of calibrated trophozoite and/or cyst solutions were mixed with the same volume of test solutions (1, 2, 4, 8, 16, and 32 mg/ml) in microcentrifuge tubes. Then, the tubes were incubated at 26 ^{o}C in a bacterial incubator (Electro-Mag) for periods of 1, 3, 6, 8, 24, 48, and 72 h. The exact process was applied to control tubes containing only sterile distilled water and trophozoite/cyst.

2.2.4. Determination of the effects of the extract on the trophozoite stage

After incubation at 26 ^{o}C, 25 µl of parasite solution was mixed with the same volume of 0.05% trypan blue and taken into the counting chamber. This mixture was left to stabilize for 3 min at room temperature, and live and dead trophozoites were counted separately in the hemocytometer. Approximately one hundred *A. castellani* trophozoites were counted each time, and this process was repeated 3 times.

2.2.5. Determination of the effects of the extract on the stage of the cyst

After each incubation period, 25 µl of parasite solution was mixed with the same volume of 0.05% trypan blue as stated above and transferred to the counting chamber. This mixture was left to stabilize for 3 min at room temperature, and live and dead cysts were counted separately in the hemocytometer. Approximately one hundred *A. castellani* cysts were counted each time, and this procedure was repeated 3 times. In addition, cultures without live cysts were plated with *E. coli* on non-nutritive agar plates, and the results were confirmed. Parasite growth was observed daily for 14 days using a light microscope (Nikon, Eclipse E 200) at 26 ^{o}C.

2.2.6. Statistical analysis

All tests were carried out in triplicate. To determine the degree of statistical difference, using SPSS v. 22.0, Tukey's test was used.

3. Results and discussion

The extract obtained from *T. spicata* was tested by following the protocol detailed in the method section to determine its anti-parasitic activity. The anti-parasitic effect was determined by testing the lethal effect on trophozoites and cysts periodically between 1-72nd h of increasing extract concentrations. The results were given in Tables 1 and 2. At the end of the experimental process, it was determined that there was a strong correlation between the increased extract concentration and the anti-parasitic effect. The water extract of the *T. spicata* was not effective enough to neutralize all cysts at any of the concentrations tried. As can be seen from Table 1, *T. spicata* extract eliminated all trophozoites in the environment from the 24th hour of the experiment at concentrations of 16.0 and 32.0 mg/ml (Figure 1).

Table 1. Effect of extract from *T. spicata* on *A. castellani* trophozoites[1]

Dose (mg/ml)	Duration						
	1 h	3 h	6 h	8 h	24 h	48 h	72 h
32.0	72.0 ± 2.6^{a}	59.3 ± 1.2^{a}	42.7 ± 0.6^{a}	24.0 ± 1.0^{a}	0	0	0
16.0	82.3 ± 1.5^{b}	70.7 ± 2.5^{b}	59.7 ± 1.5^{b}	44.0 ± 2.0^{b}	0	0	0
8.0	87.3 ± 2.5^{bc}	81.7 ± 2.1^{c}	73.3 ± 2.1^{c}	62.3 ± 2.1^{c}	47.3 ± 2.5^{a}	34.0 ± 1.7^{a}	21.0 ± 1.0^{a}
4.0	91.3 ± 1.2^{d}	86.7 ± 2.1^{c}	82.3 ± 1.2^{d}	72.7 ± 3.1^{d}	60.3 ± 1.5^{b}	46.0 ± 1.7^{b}	36.7 ± 1.5^{b}
2.0	93.0 ± 1.0^{d}	90.7 ± 1.2^{d}	86.3 ± 1.2^{d}	77.7 ± 2.5^{d}	65.0 ± 1.0^{b}	57.3 ± 1.5^{c}	42.7 ± 2.9^{c}
1.0	96.7 ± 1.2^{d}	93.3 ± 1.5^{d}	90.7 ± 1.2^{d}	84.0 ± 1.7^{de}	77.7 ± 2.5^{c}	71.1 ± 1.0^{d}	64.3 ± 2.5^{d}
Control	97.3 ± 0.6^{d}	94.7 ± 0.6^{d}	94.7 ± 1.2^{de}	94.0 ± 1.7^{e}	93.3 ± 1.5^{d}	92.3 ± 1.5^{e}	91.0 ± 1.7^{e}

[1]The values indicated by the same superscripts within the same column are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level.

There is no study in the literature on the effect of *T. spicata* on *A. castellani* trophozoites and cysts. Therefore, the data presented in the present study is the first record for literature. However, there are some literature data regarding the effect of some closely related species on the parasite in terms of taxonomical point of view. It has been reported that *Satureja cuneifolia* (1.0-32.0 mg/ml), which has similar phytochemical content to *T. spicata*, exhibited a time and dose-dependent activity on *A. castellani* torphozoites and cysts. In the study, as mentioned earlier, the methanol extract of *S. cuneifolia* killed all trophozoites at a concentration of 32.0 mg/ml within 24 h of the experiment (Malatyali et al., 2012).

Table 2. Effect of extract from *T. spicata* on *A. castellani* cysts[1]

Dose (mg/ml)	Duration						
	1 h	3 h	6 h	8 h	24 h	48 h	72 h
32.0	86.0 ± 0.0^{a}	79.7 ± 2.5^{a}	78.3 ± 1.5^{a}	63.3 ± 2.9^{a}	51.3 ± 1.2^{a}	42.3 ± 2.5^{a}	28.3 ± 2.9^{a}
16.0	89.3 ± 1.5^{a}	87.7 ± 0.6^{b}	84.0 ± 2.0^{b}	73.3 ± 1.5^{b}	65.7 ± 1.2^{b}	60.0 ± 2.0^{b}	46.3 ± 1.5^{b}
8.0	91.0 ± 1.0^{b}	89.0 ± 1.0^{b}	88.7 ± 2.1^{c}	84.3 ± 1.2^{c}	82.3 ± 0.6^{c}	75.3 ± 1.5^{c}	67.7 ± 2.5^{c}
4.0	93.7 ± 0.6^{b}	92.3 ± 0.6^{c}	90.0 ± 2.0^{c}	87.0 ± 2.0^{d}	86.3 ± 0.6^{cd}	83.7 ± 1.5^{d}	81.3 ± 1.2^{d}
2.0	94.3 ± 1.2^{b}	93.0 ± 1.0^{c}	90.3 ± 0.6^{c}	89.0 ± 1.0^{d}	88.0 ± 0.5^{d}	85.7 ± 2.3^{d}	84.3 ± 1.2^{d}
1.0	94.7 ± 0.6^{b}	93.7 ± 1.5^{c}	93.0 ± 2.6^{d}	92.3 ± 0.6^{e}	91.0 ± 1.0^{de}	90.3 ± 0.6^{e}	89.7 ± 1.5^{e}
Control	95.7 ± 0.6^{b}	95.0 ± 1.0^{cd}	94.0 ± 1.0^{d}	94.0 ± 2.0^{e}	93.3 ± 0.6^{de}	93.0 ± 1.0^{e}	92.3 ± 0.6^{e}

[1]The values indicated by the same superscripts within the same column are not different according to Tukey's honestly significant difference post hoc test at a 5% significance level.

Many researchers agree that *T. spicata* has a high carvacrol content (Hancı et al., 2003; Markovic et al., 2011; Ozel et al., 2003). This suggests that the phytochemical responsible for the activity might be carvacrol, which is in the oxygenated monoterpene. Quintanilla-Licea et al. (2014) support this idea. As mentioned earlier, the study determined that *Lippia graveolens* and *Ruta chalepensis* exhibited significant antiprotozoal activity on *Entamoeba hystolytica* (91.54% and 90.50% growth inhibition at a concentration of 150 µg/mL, respectively). As a result of bioactivity-guided fractionation, carvacrol was determined as the compound responsible for this activity.

4. Conclusions

Based on this result, it was determined that the plant species (*T. spicata*) discussed here can be used in the treatment of *A. castellani* infections. It will be possible to achieve a good effect on the cyst forms of the parasite species by testing higher extract concentrations, which cannot be evaluated during the experimental process. In addition, with advanced analyzes, it will be possible to reveal the chemical compounds responsible for the activity in the plant species in question.

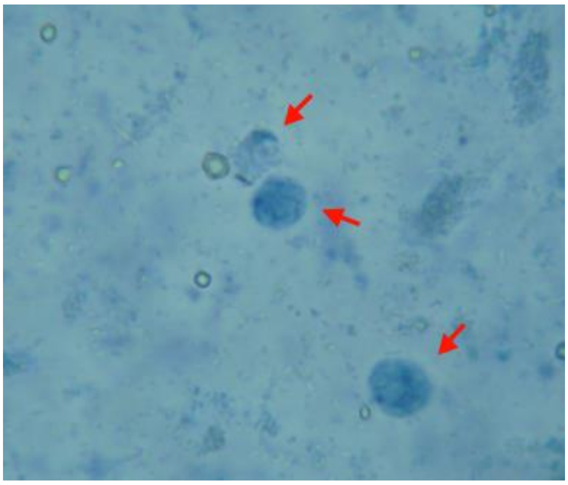

Figure 1. 24-hour image of *A. castellani* trophozoites treated with 16.0 mg/ml of*T. spicata* extract (optical microscope magnification: 40X)

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Conflict of Interest

The authors confirm that there are no known conflicts of interest.

CRediT authorship contribution statement

Baris Gulec: Conceptualization, Investigation, Methodology, Data curation.
Duhan Tore: Resources, Conceptualization, Visualization, Formal analysis, Methodology.
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Supplementary file

None.

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RESEARCH ARTICLE OPEN ACCESS

Chemical composition and antioxidant activity of the essential oil and various extracts of *Inula graveolens* (L.) Desf.

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ABSTRACT

In this study, chemical composition and *in vitro* antioxidant activity potential of the essential oil and various extracts of *Inula graveolens* (L.) Desf. were evaluated. While identifying the phytochemical composition of the essential oil and extract, GC-MS analyses were used. Chromatographic analysis of the essential oil resulted in identifying twenty compounds representing 99.5% of the total oil. Main constituents of the oil were determined as bornyl acetate (68.5%), borneol (7.7%), camphene (4.6%), *epi*-α-cadinol (4.0%) and eicosane (3.2%), respectively. Antioxidant activity was determined using four complementary test systems named β-carotene/linoleic acid, DPPH free radical scavenging, reducing power, and chelating effect. A strong correlation between the antioxidant activity and phenolic acid contents of the samples was determined. The methanol extract was the most active one in all tested systems. The weakest activity was exhibited by chloroform extract. While methanol extract showed 88.34%, 91.38, and 63.43 activities in β-carotene bleaching, DPPH radical scavenging, and chelating effect tests, respectively, the absorbance value in reducing power assay was measured as 0.273 nm.

1. Introduction

Essential oils obtained from medicinal and aromatic plants have been used by humankind since ancient times in the composition of various medicines. Essential oils are mixtures of terpenoids obtained by water or steam distillation, usually from the aerial parts of plants. With many studies so far, essential oils have been shown to have antimicrobial, antioxidant, anti-inflammatory, analgesic, insecticidal, etc., properties (Abu-Shanab et al., 2005). Essential oils isolated from some plants are used in cancer treatment due to their antiproliferative properties, while others are trendy in the food and perfumery industry (Kelen and Tepe, 2008). The antimicrobial properties of essential oils are of great importance for both producers and consumers, especially in the food industry. Because some essential oils are used as preservatives in canned foods and extend their shelf life (Celiktas et al., 2007). In addition, essential oils are one of the indispensable elements of aromatherapy due to the aromatic phytochemicals they contain (Lee et al., 2012).

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Researchers have conducted numerous studies on the antimicrobial properties of essential oils to combat infections caused by opportunistic and persistent pathogens. Some terpenoids in these oils have found use in the pharmaceutical industry due to their strong bactericidal and fungicidal properties (Tepe et al., 2007). In the coming decades, essential oils are expected to be one of the more frequently used resources in treating infectious diseases (Rios and Recio, 2005).

In addition to their antimicrobial properties, essential oils are also an excellent source of antioxidant compounds. These compounds help to increase the unsaturated fatty acids in animal tissues. In mammals, essential oils also play a hepatoprotective role due to their antioxidant properties. Essential oils can scavenge many reactive radicals such as superoxide, hydrogen peroxide, free hydroxyl radicals, and singlet oxygen due to the antioxidant terpenoids they contain. In this way, essential oils clear the body from reactive radicals (Pérez Gutierrez et al., 2006).

Inula is one of the leading genuses of the Asteraceae family and is generally distributed in Asia, Europe, and Africa. Approximately 90 species represent it. *Inula*, a paraphyletic genus from the

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taxonomical point of view, can grow from a few centimeters to over 3 m in height. Their capitulas are usually straight. There is information that it is used for landscaping purposes in parks and gardens. Smaller species are generally grown in rocky areas, while larger ones are grown in the borders of parks and gardens. Considering the historical records, it is known that the name Inula goes back to ancient Rome (Blanc et al., 2004).

It is known that humans have used some Inula species since ancient times for various reasons from an ethnopharmacological point of view. *Inula* species have been most commonly referred to as an appetizer, expectorant, anti-inflammatory, and diuretic. In addition, there is literature information on the use of Inula species in the treatment of urogenital system diseases such as urinary tract inflammation, cystitis, nephritis, and uremia (Afifi et al., 2015).

This study aimed to reveal the chemical composition and antioxidant activity of the various extracts of *Inula graveolens* L. (Desf.) [Synonyms: *Dittrichia graveolens* (L.) Greuter, *Jacobea graveolens* (L.) Merino, *Cupularia graveolens* (L.) Gren. & Godr.].

2. Materials and methods

2.1 Plant material and extract preparation

Aerial parts of *I. graveolens* were collected from Gaziantep-Karatas highway (steppe land), Gaziantep- Turkey, on 26.09.2020. The plant sample was deposited at the Herbarium of Cumhuriyet University Biology Department (CUFH Voucher No: AA 6753). Details of the extract preparation process were given in the supplementary file.

2.2. Isolation of the essential oil

Details of the essential oil isolation were given in the supplementary file.

2.3. GC-MS analysis conditions

Details of the GC-MS analysis were given in the supplementary file.

2.4. Total antioxidant activity by β-Carotene–linoleic acid method

Details of the β-carotene–linoleic acid method were given in the supplementary file.

2.5. Scavenging effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH)

Details of the DPPH radical scavenging assay were given in the supplementary file.

2.6. Reducing power

Details of the reducing power assay were given in the supplementary file.

2.7. Chelating effects on ferrous ions

Details of the chelating effect assay on ferrous ions assay were given in the supplementary file.

3. Results and discussion

3.1. Chemical composition of the essential oil

The chemical composition of the essential oil of *I. graveolens* was presented in Table 1. The results revealed that 99.5% of the total oil was identified. Main constituents of the oil were determined as bornyl acetate (68.5%), borneol (7.7%), camphene (4.6%), *epi*-α-cadinol (4.0%) and eicosane (3.2%), respectively.

Table 1. Chemical composition of the essential oil of *I. graveolens*[1]

No	Compounds	Area (%)	Exp. RI	Ident. LRI
1	α-Pinene	0.2	934	939
2	Camphene	4.6	950	954
3	β-Pinene	0.6	974	979
4	Dehydro-1.8-cineole	1.5	986	991
5	Limonene	0.7	1025	1029
6	*Neo*-3-Thujanol	0.8	1150	1154
7	Borneol	7.7	1165	1169
8	Bornyl acetate	68.5	1280	1289
9	β-Caryophyllene	1.8	1410	1419
10	Prenyl benzoate (-3-Methyl-2-butenyl-benzoate)	0.4	1421	MS [1]
11	Drima-7,9(11)-diene	0.5	1470	1473
12	γ-Muurolene	0.4	1479	1480
13	Muurola-4(14),5-diene	0.3	1487	1494
14	β-Atlantol	1.6	1600	1608
15	*Epi*-α-Cadinol	4.0	1640	1640
16	*(E)*-Bisabol-11-ol	0.8	1665	1668
17	Eicosane	3.2	2000	2000
18	Tricosane	0.7	2300	2300
19	Tetracosane	1.0	2400	2400
20	Pentacosane	0.5	2500	2500
21	Total	99.5		
22	α-Pinene	0.2	934	939
23	Camphene	4.6	950	954
24	β-Pinene	0.6	974	979
25	Dehydro-1.8-cineole	1.5	986	991
26	Limonene	0.7	1025	1029
	Neo-3-Thujanol	0.8	1150	1154

[1] MS: 68(100), 105(80), 77(40), 51(15)

The essential oil composition of this plant has previously been reported several times (Blanc et al., 2004; Dinis et al., 1994). According to Dinis et al. (1994), the main constituents of the oils obtained from 20 different plant samples collected from the various locations and at different growing stages were mainly determined as bornyl acetate and borneol (7.6%) typically. Some other samples within the same study showed slight differences in terms of their major compounds. In addition to these major compounds, tau-cadinol was also determined within the oil of these atypical samples. According to another study carried out by Blanc et al. (2004), major compounds of the essential oil of I. graveolens from Lebanese origin were determined as bornyl acetate (70.6-72.3%), *t*-cadinol (1.4-13.4%), borneol (2.7-12.4%) and caryophyllene oxide (1.9-2.3%), respectively. *I. graveolens* is also known as *Dittrichia graveolens* in the literature. The essential oil composition of *D. graveolens* was also studied (Ghosn et al., 2006; Petropoulou et al., 2004). In both studies, bornyl acetate was determined as the major compound. Additionally, *epi*-α-cadinol (30.2%) was defined as the main constituent (Ghosn et al., 2006). In the latter, borneol (60.7%) and β-caryophyllene were identified among the major compounds (Petropoulou et al., 2004).

Compared to the literature data given above, the essential oil of I. graveolens commonly consists of borneol, bornyl acetate, and cadinol derivatives. As can be seen from the results presented in Table 1, the main compounds identified in the present study are highly inconsistent with those published before.

3.3. Antioxidant activity

It is possible to determine the antioxidant activities of plant extracts or essential oils, or individual phytochemicals with more than one test system. For antioxidant activity results to be consistent, researchers are expected to use at least two different test systems together. In each antioxidant test system, test samples may exhibit different activity profiles. These differences can be mainly affected by the solvent used for the extraction, the experimental medium temperature, the chemicals used, etc.

Lipid peroxidation in organisms is one of the most important causes of cellular damage. The main factor causing lipid peroxidation is free radicals. Free radicals can cross-link membrane lipids. Therefore, lipid oxidation causes severe damage to cell membranes, the main components of which are largely lipids (Lanzetta et al., 1991). Oxidation of lipids can cause many diseases, especially ischemia, as it increases the sensitivity of phospholipids to phospholipase (Esterbauer et al., 1991; Sevanian et al., 1981) and increases membrane calcium permeability (Weglicki et al., 1984).

A strong correlation between the antioxidant activity and phenolic acid contents of the samples was determined. In β-carotene/linoleic acid test system, the most potent activity was exhibited by methanol extract, of which inhibition value is 88.34% (Table 2). This value is greater than that of synthetic antioxidant BHA (86.48%). This activity is followed by acetone extract. On the other hand, essential oil and ethanol extract showed almost the same activity profile. Chloroform extract showed the weakest activity in this test system.

Table 2. Antioxidant of the essential oil and different solvent extracts of Inula graveolens in β-carotene/linoleic acid and DPPH test systems[1]

Antioxidant activity in β-carotene/linoleic acid test system			
Samples	Inhibition (%)		
Essential oil	55.40 ± 0.12		
Chloroform extract	42.14 ± 0.70		
Acetone extract	71.26 ± 0.19		
Acetonitrile extract	45.20 ± 0.42		
Ethanol extract	56.19 ± 0.38		
Methanol extract	88.34 ± 0.57		
BHA	86.48 ± 1.93		
BHT	92.14 ± 0.15		
DPPH free radical scavenging capacity (%)			
Samples	0.2 mg/ml	0.4 mg/ml	0.8 mg/ml
Essential oil	14.85 ± 0.02	26.50 ± 0.24	56.19 ± 0.46
Chloroform extract	6.85 ± 1.25	12.52 ± 0.50	26.92 ± 0.41
Acetone extract	18.27 ± 0.34	33.40 ± 0.21	71.80 ± 0.24
Acetonitrile extract	8.56 ± 0.25	15.65 ± 0.56	33.65 ± 0.53
Ethanol extract	18.11 ± 0.17	32.32 ± 0.50	68.53 ± 0.63
Methanol extract	24.15 ± 0.40	43.10 ± 1.22	91.38 ± 0.24
BHA	92.83 ± 0.84	nt [2]	nt
BHT	81.41 ± 0.00	nt	nt

[1] Values expressed are means ± S.D. of three parallel measurements
[2] nt: Not Tested

Plant extracts, essential oils, or phytochemicals can terminate oxidant chain reactions by reacting with peroxyl radicals. Many researchers agree that natural antioxidants derived from plant sources effectively terminate free radical reactions (Bagchi et al., 1997). In addition, it is known that L-tryptophan (one of the precursors of secondary metabolites) reacts with phenolic aldehydes in foods to form phenolic tetrahydro-β-carboline alkaloids. This compound can effectively scavenge 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (Shimada et al., 1992).

As presented in Table 2, all samples showed a concentration-dependent activity in DPPH free radical scavenging assay. In this test system, methanol extract showed the most substantial radical scavenging effect. The scavenging value exhibited by this extract was measured as 91.38% at 0.8 mg/mL concentration. This is followed by acetone and ethanol extracts. The radical scavenging capacity of the essential oil was determined as 56.19% at 0.8 mg/mL. In this test system, the weakest activity was exhibited by chloroform extract at all concentration values.

Molecules with chelating ability reduce the redox potential of biochemical reactions. As a result, they contribute to the stabilization of the oxidized forms of metal ions. Therefore, secondary metabolites with chelating properties can act as good antioxidants. Chain oxidation reactions initiated by metal ions are important causes of oxidation-related spoilage in foods (Herraiz et al., 2003). Due to these properties, metal ions are also associated with cancer and arthritis cases (Gordon, 1990). Ferrous ions are one of the most effective pro-oxidants and are widely available in foods (Halliwell et al., 1995).

In the present study, the chelating ability of the extracts and essential oil toward ferrous ions was also investigated. Table 3 also shows the chelating effects of the samples obtained from *I. graveolens*. In this test system, EDTA was also used as a standard on ferrous ions. According to the data presented in the table, the most potent chelating agent was determined as methanol extract (63.43%). On the other hand, chloroform extract's metal chelating ability was found weak compared with the other test materials. The essential oil also showed a weak activity profile. The chelating effect of EDTA was determined as 99.74%.

Table 3. Chelating effect and reducing power of the essential oil and different solvent extracts of Inula graveolens[1]

Chelating effect			
Samples	(%)		
Essential oil	27.35 ± 0.52		
Chloroform extract	18.79 ± 0.85		
Acetone extract	36.68 ± 0.32		
Acetonitrile extract	22.30 ± 0.09		
Ethanol extract	47.57 ± 0.81		
Methanol extract	63.43 ± 0.75		
EDTA	99.74 ± 0.15		
Reducing power (absorbance at 700 nm)			
Samples	0.2 mg/ml	0.4 mg/ml	1.0 mg/ml
Essential oil	0.045 ± 0.006	0.100 ± 0.008	0.171 ± 0.003
Chloroform extract	0.024 ± 0.005	0.053 ± 0.003	0.091 ± 0.002
Acetone extract	0.037 ± 0.012	0.082 ± 0.005	0.140 ± 0.002
Acetonitrile extract	0.025 ± 0.009	0.057 ± 0.004	0.098 ± 0.006
Ethanol extract	0.054 ± 0.006	0.120 ± 0.002	0.204 ± 0.001
Methanol extract	0.072 ± 0.004	0.160 ± 0.006	0.273 ± 0.008
BHA	2.303 ± 0.064	nt [2]	nt
BHT	1.258 ± 0.121	nt	nt

[1] Values expressed are means ± S.D. of three parallel measurements
[2] nt: Not Tested

Reducing molecules generally donate a hydrogen atom to free radicals, reducing their tendency to react. Therefore, the reaction chain that causes oxidative damage can be broken by reducing molecules (Bagchi et al., 1997; Yamaguchi et al., 1998). The basis of the reducing power test applied in the present study is based on the principle that the Fe^{3+}/ferricyanide complex turns into Fe^{2+} in the presence of antioxidant compounds (Hatano et al., 1988).

Table 3 also shows the reducing power of the samples. The activity increased with concentration. The reducing power of methanol extract was determined as 0.273 nm at 1.0 mg/mL. The reducing power of BHA and BHT were measured as 2.303 and 1.258 nm, respectively, at 0.2 mg/mL.

4. Conclusions

In this study, antioxidant activities of extracts and essential oil obtained from the aerial parts of *I. graveolens* with solvents of different polarities were investigated. In the tests, the essential oil showed moderate antioxidant activity, while the activity of the methanol extract was comparable to the positive control. Therefore, it was concluded that the methanol extract of *I. graveolens* could be considered as one of the new and alternative sources of antioxidant compounds. However, further chromatographic analyzes are needed to determine the bioactive compounds responsible for the activity in the methanol extract.

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None.

Conflict of Interest

The author confirms that there are no known conflicts of interest.

CRediT authorship contribution statement

H. Askin AKPULAT: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing, Review & Editing.
Saliha Seyma SAHINLER: Conceptualization, Data curation, Formal analysis, Resources, Software, Visualization.

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Supplementary file

The supplementary file accompanying this article is available at https://dergipark.org.tr/en/download/journal-file/23546.

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RESEARCH ARTICLE

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Assessment of apigenin-7-glucoside and luteolin-7-glucoside as multi-targeted agents against Alzheimer's disease: a molecular docking study

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ABSTRACT

Although the incidence of Alzheimer's disease (AD) is increasing in society, unfortunately, no definite progress has been made in treating this disease yet. In this study, the potential of apigenin-7-glucoside (A7G) and luteolin-7-glucoside (L7G) to be used as multi-targeted agents in AD was investigated by molecular docking calculations against the acetylcholinesterase (AChE), butyrylcholinesterase (BChE), amyloid precursor protein (APP) and 42-residue beta-amyloid peptide (Aβ). A7G and L7G exhibited very high binding affinity (-9.42 and -9.60 kcal/mol for A7G; -9.30 and -9.90 kcal/mol for L7G) to AChE and BChE, respectively, while the affinities of these two flavonoid glycosides towards APP and Aβ peptide (-6.10 and -6.0 kcal/mol for A7G; -6.30 and -6.10 kcal/mol for L7G) were moderately strong. Compared to rivastigmine, A7G and L7G exhibited a highly significant binding affinity, even stronger than rivastigmine, for AChE and BChE. Although A7G showed a more drug-like physicochemical character than L7G, both ligands were within the normal range for ADMET and did not show high affinity for cellular proteins, according to the results of SwissTarget analysis. According to the STITCH interaction analysis, both ligands had the potential to inhibit enzymes predominantly in the inflammatory pathway (ADIPOQ, NOS1, NOS2 and NOS3). As a result, A7G and L7G exhibit multi-targeted agent properties in AD. Our results should also be verified by experimental enzyme inhibition studies, which may be performed simultaneously on AChE, BChE, APP, and Aβ peptides.

1. Introduction

Dementia is a clinical syndrome that manifests itself with a series of symptoms and signs as memory, language disorders, psychological and psychiatric abnormalities, and damage to some activities in daily life (Burns and Iliffe, 2009). The most common form of dementia in the elderly population is Alzheimer's disease (AD), and the frequency of this disease increases with age (Ferri et al., 2005).

According to the cholinergic hypothesis, the oldest hypothesis about AD, the disease occurs due to decreased synthesis of the neurotransmitter acetylcholine (Francis et al., 1999).

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The two main enzymes responsible for this reduced acetylcholine in the brain are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In healthy brain tissue, AChE and BChE function together and coordinate cholinergic neurotransmission via hydrolysis of ACh (Li et al., 2000). However, while BChE activity increases in AD patients, AChE activity remains unchanged or decreases (Greig et al., 2002). In addition to these known cholinergic functions of both enzymes, their non-cholinergic functions determined in the pathology of AD have started to emerge in recent years. In addition to its role in cholinergic synapses, it has been reported that AChE accelerates the formation of amyloid-beta-peptide (Aβ) and may play a role in the amyloid deposition in the brain of AD patients (Álvarez Rojas et al., 1996). In addition, biochemical studies have shown that AChE induces amyloid fibril formation and forms highly toxic AChE-Aβ complexes (Carvajal and Inestrosa, 2011; Dinamarca et al., 2010; Inestrosa et al., 2005). In addition, similar to AChE, BChE has also been reported to be associated with Aβ subpopulations and may play a role in plaque maturation observed in AD disease

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(Darvesh et al., 2012). Thus, due to their altered roles in AD disease, both enzymes exhibit functional therapeutic target properties, and inhibition of these enzymes may be advantageous in ameliorating cholinergic function and blocking Aβ fibril formation in affected individuals.

Another hypothesis valid in the etiology of AD and has been put forward more recently is the amyloid hypothesis, which postulates that the extracellular Aβ peptide deposits in the brain tissue are the leading cause of the disease (Hardy and Allsop, 1991). The source of these neurotoxic Aβ peptides is the amyloid precursor protein (APP) a type-1 transmembrane glycoprotein that plays a significant role in the pathogenesis of AD (Spoerri et al., 2012). APP undergoes two different types of proteolytic processing, and the proteolytic pathway associated with AD is the amyloidogenic pathway. In this pathway, APP is first cleaved from the β-site, resulting in the sAPPβ ectodomain and the membrane-bound C-terminal domain (C99) (Gabuzda et al., 1994; Seubert et al., 1993). C99 can be enzymatically processed by γ-secretase, resulting in Aβ and the intracellular APP domain (Anderson et al., 1992).
Interestingly, the amyloidogenic process of APP occurs under physiological conditions, and thus imbalances in the amyloidogenic pathway are closely associated with the pathology of AD (Haass et al., 1992; Seubert et al., 1992). It has been reported that co-mutation of His149 and His151 residues localized in the N-terminal APP copper-binding domain (CuBD), which plays a role in APP maturation, to asparagine reduces the proteolytic processing of APP and that the CuBD domain may be a potential target of novel drugs in AD treatment (Spoerri et al., 2012). In addition, direct evidence has been reported that Aβ, the distinct hallmark of AD, is an activator of gradual damaging events induced in brain tissue by the aggregation of P-Tau (Sun et al., 2015).

Flavonoids are plant-based phytochemicals present in almost all plants found on earth, and as a result, they are consumed by humans through diet. Evidence obtained in recent years suggests that various flavonoids have positive effects on dementia and AD and reports that they positively affect neurocognitive performance (Airoldi et al., 2018). In addition, recent studies showed that flavonoid glycosylation was associated with a modest increase in the inhibitory activity (affinity) of these phytochemicals on various AD-causing proteins (Ali et al., 2019; Guzzi et al., 2017). In this context, the chronic treatment of apigenin 7-glucoside (A7G) was reported to ameliorate cognitive deficits in aged and LPS-intoxicated mice (Patil et al., 2003), while luteolin 7-O-β-d-glucoside (luteolin 7-glucoside) (L7G) significantly inhibited BChE and AChE activity in silico and in vitro studies (Uddin et al., 2020).

This study aimed to evaluate the inhibitory potentials of A7G and L7G, the flavonoid glycosides, against AChE, BChE, APP, and Aβ, which have direct roles in AD pathology using a molecular docking method. There has been no definitive treatment for AD, and current therapies only act to slow the course of the symptoms. A multi-target-inhibition approach could be rational in treating multifactorial AD, and such studies are scarce in the literature. Therefore, the development of a low side effect hit molecule(s) capable of inhibiting AChE, BChE, APP, and Aβ in a combined manner would have the potential to revolutionize the treatment of AD.

2. Materials and methods

2.1. Protein and ligand preparation

Molecular docking is a frequently used computational method for calculating the affinity of small molecules to their respective receptors, predicting their most favorable binding modes, and studying their mutual interactions within specific binding pockets of these receptors. The resolved crystal structures of recombinant human acetylcholinesterase (AChE) (PDB ID: 4EY6), human butyrylcholinesterase (BChE) (PDB ID: 4BDS), amyloid precursor protein copper-binding domain (APP) (PDB ID: 2FK1), and 42-residue beta-amyloid fibril (Aβ) (PDB ID: 2MXU) were retrieved from Protein Data Bank (PDB) with resolutions of 2.40 Å, 2.10 Å, 1.60 Å, respectively. However, the resolution information of 42-residue beta-amyloid fibril (PDB ID: 2MXU) was not available. Heteroatoms such as water molecules, co-crystallized inhibitors, and non-interacting ions on all proteins and Aβ fibril were removed, and missing atoms on amino acid residues were added with the 'Repair Missing Residues' tool of the AutoDockTools interface. Protonation states of ionizable side chains were assigned using the PropKa module of Vega ZZ (Li et al., 2005).

The 3D conformers of A7G and L7G (Figure 1) simulated in this study were retrieved from the PubChem database in .sdf format. The energy minimization of both ligands was performed using the universal force field (UFF) implemented in the Avogadro software (Hanwell et al., 2012). Then, the geometrically optimized ligand files were converted to .pdbqt format using the Open Babel open-source chemistry toolbox for further use in molecular docking simulations (O'Boyle et al., 2011). Rivastigmine, which was used as a positive control of AChE and BChE, was prepared for molecular docking simulations using the same processes as the other two ligands after being separated from the complex downloaded as PBD ID: 6EUE from the Protein Data Bank.

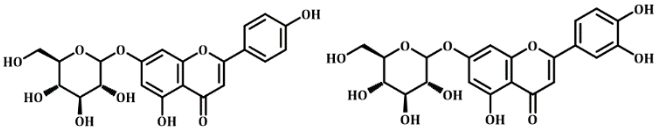


Figure 1. Chemical structures of A7G and L7G

2.2. Molecular docking

AMDock (https://github.com/Valdes-Tresanco-MS/AMDock-win) assisted molecular docking tool with AutoDock Vina was implemented in molecular docking of A7G, L7G, and rivastigmine into the defined catalytic sites of the AChE, BChE, APP, and Aβ (Trott and Olson, 2010; Valdes-Tresanco et al., 2020). The grid box coordinates were set to allow the ligands to interact with these target proteins' catalytic amino acid residues. In this context, we thought that it would be helpful to give brief information on the active regions of the APP and the Aβ: The copper-binding domain (His147, His149, and His151) of APP has been identified as the active site since this region has been reported to be important in APP folding and stability (Spoerri et al., 2012). In addition, the key residues His14, Glu22, Asp23, Gly33, Gly37, and Gly38 were selected as the active site of Aβ peptides since the side chains of these amino acids play a prominent role in the formation of amyloid fibrils (Hsu et al., 2018).

Prior to docking, the grid boxes of AChE, BChE, APP and Aβ were adjusted as follows: a) 82×56×54Å points (x: 15.71, y: 7.75, z: 49.32) for AChE; b) 70×62×54Å points (x: 139.93, y: 115.80, z: 41.74) for BChE; c) 30×30×30 Å points (x: 10.30, y: 18.90, z: 8.30) for APP; and 40×40×40Å points (x: -24.30, y: 6.21, z: 11.14) for Aβ.
In the configuration settings prepared for docking, the exhaustiveness was set as '56', and the number of separate docking

runs (number of poses) was set as '20'. In separate docking analyses, all potential binding modes (conformations) of A7G, L7G, and rivastigmine were clustered and ranked based on binding free energies (ΔG˚; kcal/mol) of the ligands' conformations which showed the lowest binding free energy against AChE, BChE, APP, and Aβ. The best docking conformations of the three ligands against protein targets calculated by AMDock were visualized and analyzed using Discovery Studio Visualizer v16.

2.3. Drug-likeness, ADMET profile, and target prediction

The determination of drug-likeness, ADMET, and target profiles of promising hit compounds in structure-based drug design (SBDD) is essential to reduce their side effects on the target organism. In this study, SwissADME, pkCSM, and SwissTargetPrediction online tools were used to investigate such effects of A7G and L7G (Daina et al., 2019a; Pires et al., 2015).

2.4. Network pharmacology analysis

The one-drug/one-target approach in drug discovery has some deficiencies in terms of safety and efficacy (Chandran et al., 2015; Istifli et al., 2021). Therefore, prior knowledge of the interactions of hit molecules with the protein network of the host organism may help to reveal possible side effects or novel therapeutic effects of these molecules. In this study, the targets-components analysis of A7G and L7G was performed by selecting the target organism as '*Homo sapiens*' through the STITCH (http://stitch.embl.de/) public database.

3. Results and discussion

The molecular docking calculation results of A7G with AChE, BChE, APP, and Aβ peptide are demonstrated in Table 1 and Figures 2 and 5. A7G formed 3 H-bonds with Tyr62, Gln63, and His201, a π-π stacked interaction with Trp59, an unfavorable acceptor-acceptor interaction with Glu233, and a small number of van der Waals interactions in the catalytic cavity of AChE (Figure 5A).

Table 1. Free energy of binding and calculated inhibition constants of apigenin-7-glucoside, luteolin-7-glucoside and rivastigmine, a dual-inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), against Alzheimer's disease-related proteins

No	Compound	Free energy of binding (kcal/mol)				Calculated inhibition constant (µM)			
		AChE	BChE	APP[1]	Aβ[2]	AChE	BChE	APP	Aβ
1	Apigenin-7-glucoside	-9.42	-9.60	-6.10	-6.0	0.180	0.091	33.78	39.99
2	Luteolin-7-glucoside	-9.30	-9.90	-6.30	-6.10	0.152	0.050	24.10	33.78
3	Rivastigmine*	-6.50	-6.90	-	-	17.20	8.76	-	-

* The binding free energy values obtained from the docking calculations of rivastigmine with AChE and BChE were used as positive controls.
[1] Amyloid precursor protein
[2] Amyloid-beta peptide

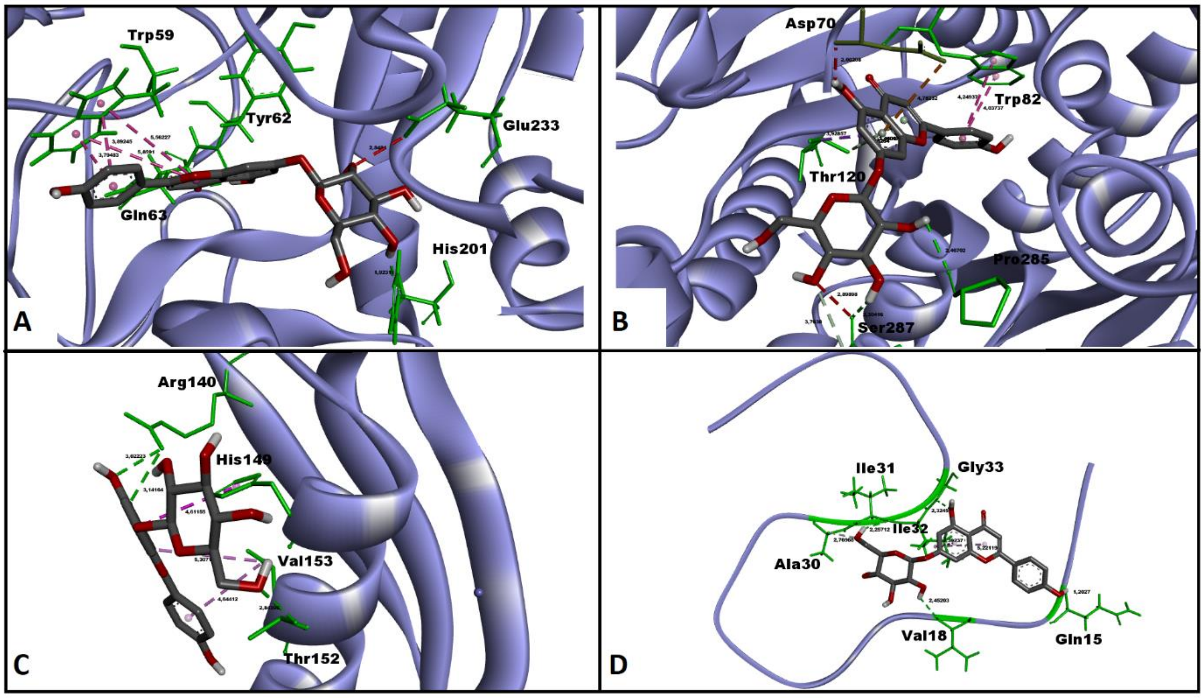


Figure 2. Figure 2. Top-ranked conformations of apigenin-7-glucoside (A7G) (A- Human AChE, B- Human BChE, C- Amyloid precursor protein [APP], D- Beta-amyloid [Aβ] peptide

The calculated free energy of binding of A7G with AChE is highly favorable (-9.42 kcal/mol; Ki=0.180 µM) (Table 1), and the top-ranked pose of A7G within the catalytic pocket of AChE is demonstrated in Figure 2A. A7G formed 3 H-bonds with Pro285, Ser287, a π-anion interaction with Asp70, two pi-donor hydrogen bonds with Thr120, a π-π stacked interaction with Trp82, and two unfavorable donor-donor and acceptor-acceptor interactions with Asp70 of BChE. In addition, A7G engaged in many van der Waals interactions in the catalytic cavity of BChE (Figure 5B). The calculated free energy of binding of A7G with BChE is highly favorable (-9.60 kcal/mol; Ki=0.091 µM) (Table 1), and the top-ranked pose of A7G within the catalytic pocket of BChE is demonstrated in Figure 2B. The interactions of A7G within the catalytic pocket of APP included 3 H-bonds with Arg140 and Thr152, a π-π T-shaped, and a π-alkyl interaction with His149 and Val153, respectively, and a small number of van der Waals interactions in the copper-binding domain of APP (Figure 5C). The calculated free energy of binding of A7G with APP is moderately strong (-6.10 kcal/mol; Ki=33.78 µM) (Table 1), and the top-ranked pose of A7G within the copper-binding domain of APP is demonstrated in Figure 2C. A7G formed 3 H-bonds with Val18, Ile31, and Gly33, two π-alkyl interactions with Ile32, and an unfavorable donor-donor interaction with Gln15 of Aβ peptide (Figure 5D). It is noteworthy that the interaction of A7G with Gly33 is significant since this residue lies within the nucleation-dependent polymerization site of the Aβ peptide (Hsu et al., 2018). The calculated free energy of binding of A7G with Aβ peptide is moderately strong (-6.0 kcal/mol; Ki=39.99 µM) (Table 1), and the top-ranked pose of A7G within the nucleation-dependent polymerization site of Aβ peptide is demonstrated in Figure 2D.

The docking calculation results of L7G with AChE, BChE, APP, and Aβ peptide are demonstrated in Table 1 and Figures 3 and 5. L7G formed 7 H-bonds with Gln63, Arg195, Glu233, His299, and Asp300, three π-π stacked interactions with Trp59 and an unfavorable donor-donor interaction with Gln63 of AChE. The interactions of L7G with AChE also included many van der Waals interactions with surrounding residues (Figure 5E). The calculated free energy of binding of L7G with AChE is powerful (-9.30 kcal/mol; Ki=0.152 µM) (Table 1), and the top-ranked pose of L7G within the catalytic cavity of AChE is demonstrated in Figure 3A. L7G formed 8 H-bonds with Asn83, Thr120, Tyr128, Glu197, Thr284, Pro285, and Ser287, a π-anion interaction with Asp70 and a π-π stacked interaction with Trp82 of BChE. The interactions of L7G with BChE included many van der Waals contacts with surrounding residues (Figure 5F).

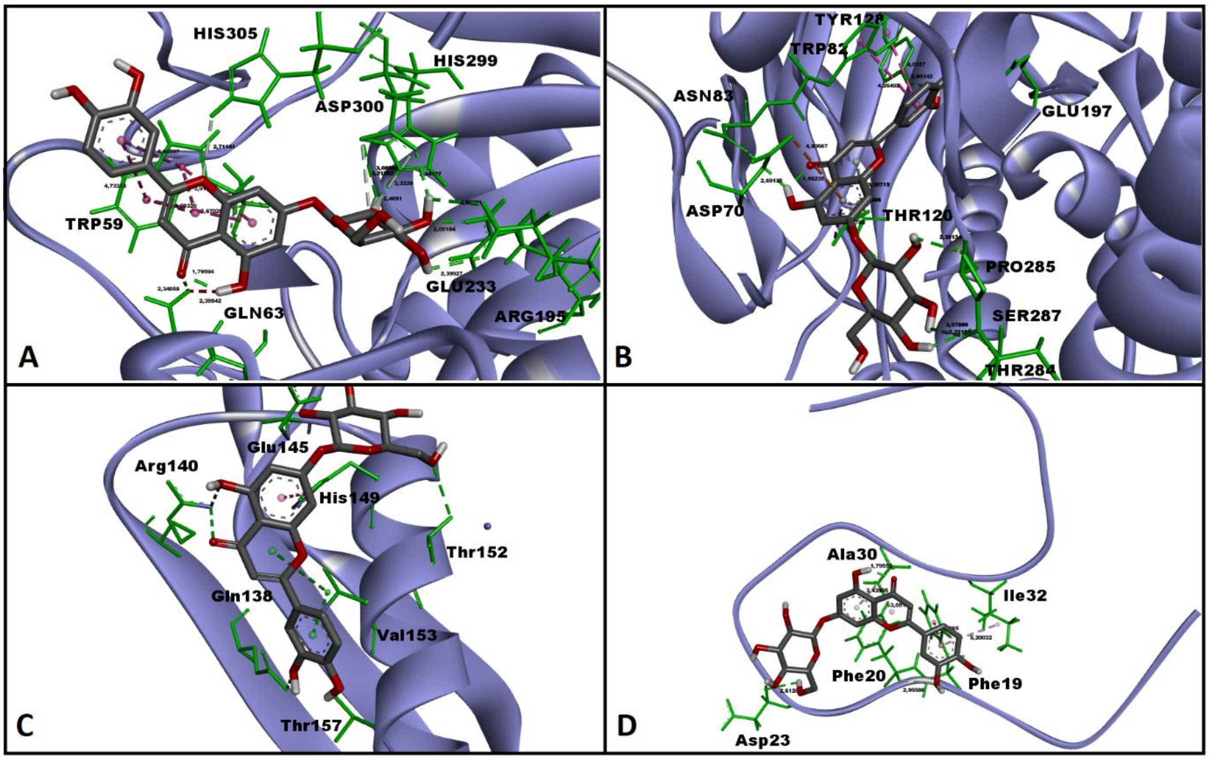


Figure 3. Top-ranked conformations of luteolin-7-glucoside (L7G) (A- Human AChE, B- Human BChE, C- Amyloid precursor protein [APP], D- Beta-amyloid [Aβ] peptide

The calculated free energy of binding of L7G with BChE is powerful (-9.90 kcal/mol; Ki=0.050 µM) (Table 1), and the top-ranked pose of L7G within the catalytic cavity of BChE is demonstrated in Figure 3B. The interactions of L7G with APP mainly included 7 H-bonds with Gln138, Arg140, Glu145, Thr152, and Thr157, a π-π T-shaped interaction with His149 and a π-alkyl interaction with Val153. However, the van der Waals contacts played a minor role in L7G's interactions with the APP (Figure 5G). The calculated free energy of binding of L7G with APP is moderately strong (-6.30 kcal/mol; Ki=24.20 µM) (Table 1), and the top-ranked pose of L7G within the copper-binding domain of APP is demonstrated in Figure 3C. Finally, L7G formed 2 H-bonds with Phe20 and Asp23, a π-π T-shaped interaction with Phe19 and three π-alkyl interactions with Ala30 and Ile32 of the nucleation-dependent polymerization site of the Aβ peptide. Van der Waals contacts also played a role in the interaction of L7G with the Aβ peptide (Figure 5H). The calculated free energy of binding of L7G with Aβ peptide is moderately strong (-6.10 kcal/mol; Ki=33.78 µM) (Table 1), and the top-ranked pose of L7G within the polymerization site of Aβ peptide is demonstrated in Figure 3D. Since Asp23 (D23) is significant for the sigmoidal aggregation kinetics of Aβ peptide, the hydrogen bond interaction between L7G and Asp23 is of utmost importance (Hsu et al., 2018).

In this study, the binding free energy and inhibition constant of rivastigmine against AChE and BChE were used as a positive control group to compare A7G and L7G interactions with these two enzymes. The docking calculation results of rivastigmine with AChE and BChE can be seen in Table 1 and Figure 5. Rivastigmine formed 3 H-bonds with Gln63, Glu233, and Asp300, two π-sigma interactions with Trp59 and Tyr62, a π-π stacked interaction with Tyr62 and two π-alkyl interactions with Leu165 and His299 of AChE. A certain number of van der Waals interactions also played a role in the rivastigmine-AChE interaction (Figure 5I). The calculated free energy of binding of rivastigmine with AChE is strong (-6.50

kcal/mol; Ki=17.20 µM) (Table 1), and the top-ranked pose of rivastigmine within the catalytic cavity of AChE is demonstrated in Figure 4A.

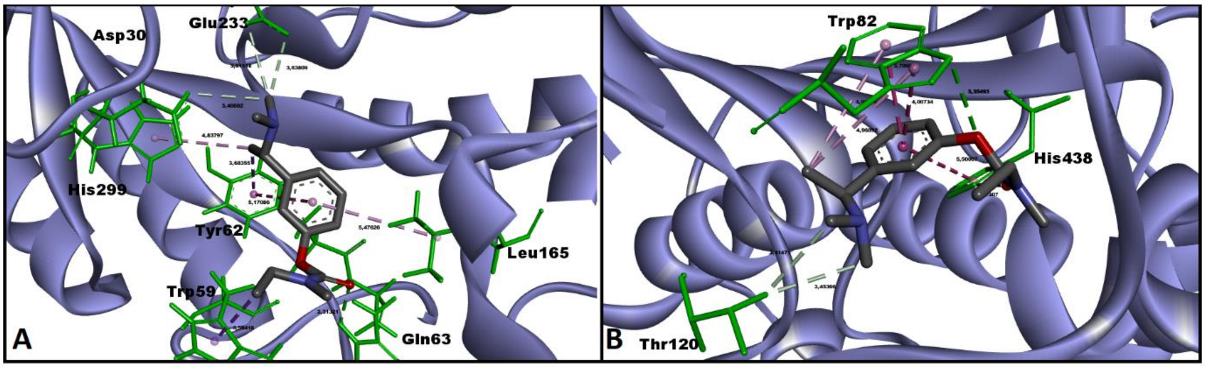
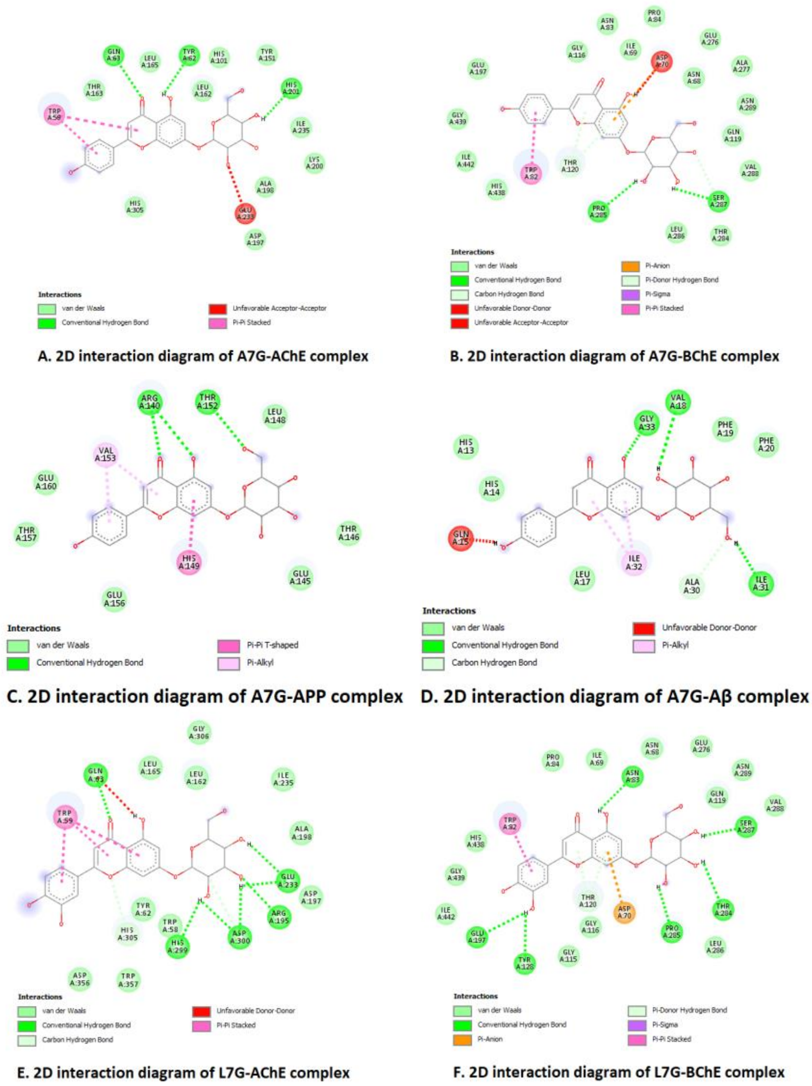


Figure 4. Top-ranked conformations of rivastigmine A- Human AChE, B- Human BChE



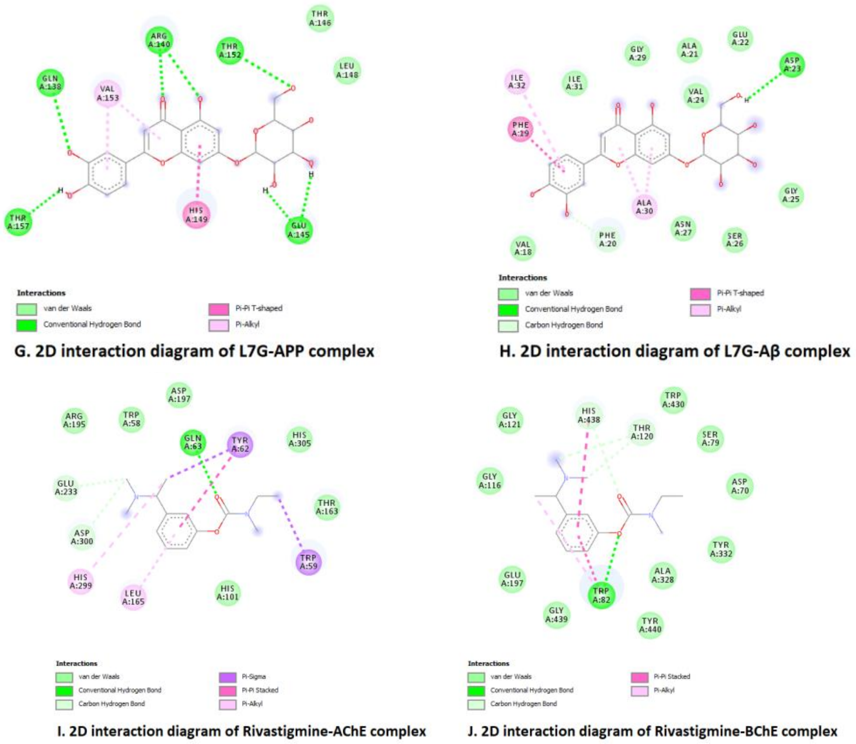


Figure 5. 2D interaction diagrams of the molecular interactions of apigenin-7-glucoside, luteolin-7-glucoside, and rivastigmine with their receptors in molecular docking simulations. A, B, C, D. Apigenin-7-glucoside; E, F, G, H. Luteolin-7-glucoside; I, J. rivastigmine

In the case of BChE, rivastigmine formed 4 H-bonds with Trp82, Thr120, and His438, two π-π stacked interactions with Trp82 and His438 and a π-alkyl interaction with Trp82. It can also be said that the effect of van der Waals contacts on the rivastigmine-BChE interaction is moderately strong (Figure 5J). The calculated free energy of binding of rivastigmine with BChE is also strong (-6.90 kcal/mol; Ki=8.76 µM) (Table 1), and the top-ranked pose of rivastigmine within the catalytic cavity of BChE is demonstrated in Figure 4B. The constant inhibition values obtained from the docking simulations of rivastigmine against AChE and BChE (17.20 µM for AChE; 8.76 µM for BChE) are in good agreement with the experimental IC_{50} values of the same ligand against both enzymes. It has been reported that the human AChE IC_{50} value of rivastigmine is 6.33 µM, and the human BChE IC_{50} value is 0.803 µM at the end of a 40-minute incubation period (Dighe et al., 2016).

Table 2. Drug-likeness properties of apigenin-7-glucoside and luteolin-7-glucoside

No	Compound	Number of rotatable bonds	TPSA[1]	Consensus Log P	Log S (ESOL[2])	Drug likeness (Lipinski's rule of five)
1	Apigenin-7-glucoside	4	170.05	0.55	-3.78	Yes; 1 violation: NH or OH>5
2	Luteolin-7-glucoside	4	190.28	0.16	-3.65	No; 2 violations: N or O > 10, NH or OH>5

[1] TPSA: Topological polar surface area ($Å^2$)
[2] ESOL: Estimated aqueous solubility [(Insoluble < -10 < Poorly < -6 < Moderately < -4 < Soluble < -2 Very < 0 < Highly), according to Delaney, J.S. (2004)].
Data source: http://www.swissadme.ch/index.php

Drug-likeness, ADMET, and intracellular target profiles of A7G and L7G are given in Tables 2, 3, and Figure 6, respectively. Except for L7G, A7G was determined to obey Lipinski's rule of five. L7G violates this rule because it has N or O > 10 and NH or OH > 5 (Table 2). ADMET data of A7G and L7G are presented in Table 3.

Table 3. ADMET profiles of apigenin-7-glucoside and luteolin-7-glucoside

No	Compound	BBB permeation[1,*]	P-gp substrate[2,*]	CYP inhibition[3,*]	AMES Toxicity[4]	Hepatotoxicity[4]	LD_{50} in rat (mol/kg)[4]
1	Apigenin-7-glucoside	No	Yes	No	No	No	2.595
2	Luteolin-7-glucoside	No	Yes	No	No	No	2.547

[1] BBB: Blood Brain Barrier
[2] P-gp: P-glycoprotein substrate
[3] CYP: Cytochrome P
[4] http://biosig.unimelb.edu.au/pkcsm/prediction
[*] http://www.swissadme.ch/index.php

According to ADMET profiles, A7G and L7G cannot cross the blood-brain barrier (BBB) and are substrates of p-glycoprotein (P-gp); however, both compounds do not show CYP inhibition, AMES toxicity, and hepatotoxicity, and their acute toxicity potency (LD_{50}) is not high in rats (Table 3). The intracellular target of A7G is depicted in Figure 6A. According to the search by the SwissTarget online tool, the intracellular targets of A7G most frequently include various enzymes, kinases, and family A G protein-coupled receptors. However, results from the same tool show that A7G is unlikely to have a positive/negative interaction on these targets (p <= 0.432) (Figure 6A). Intracellular targets of L7G, similar to A7G, most commonly include kinases, various enzymes, and family A G protein-coupled receptors. However, statistical analysis performed by the same tool indicates that L7G is less likely to have a positive/negative interaction on these targets (*p* <= 0.430) (Figure 6B).

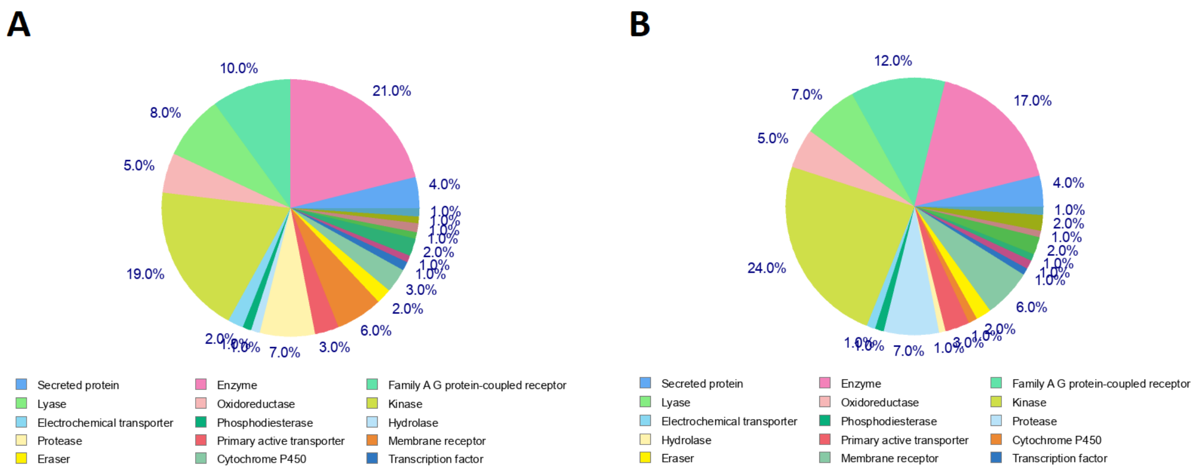


Figure 6. Intracellular target predictions of apigenin-7-glucoside and luteolin-7-glucoside

The STITCH platform was used to predict putative targets of A7G and L7G in the human proteome. Before mapping target-component interactions, the minimum required interaction score was set to a high confidence score which was ≥ 0.7. A high confidence score indicates a strong interaction between hit compounds and protein(s). According to the targets-components interaction network in Figure 7, it can be stated that A7G (cosmosiin) interacts directly with ADIPOQ and SLC2A4 proteins, while L7G directly interacts with MTRR, POR, HMOX1, NOS1, NOS2 and NOS3 proteins. Thus, the direct interactions of A7G and L7G with different proteins have been mapped (Figure 7).

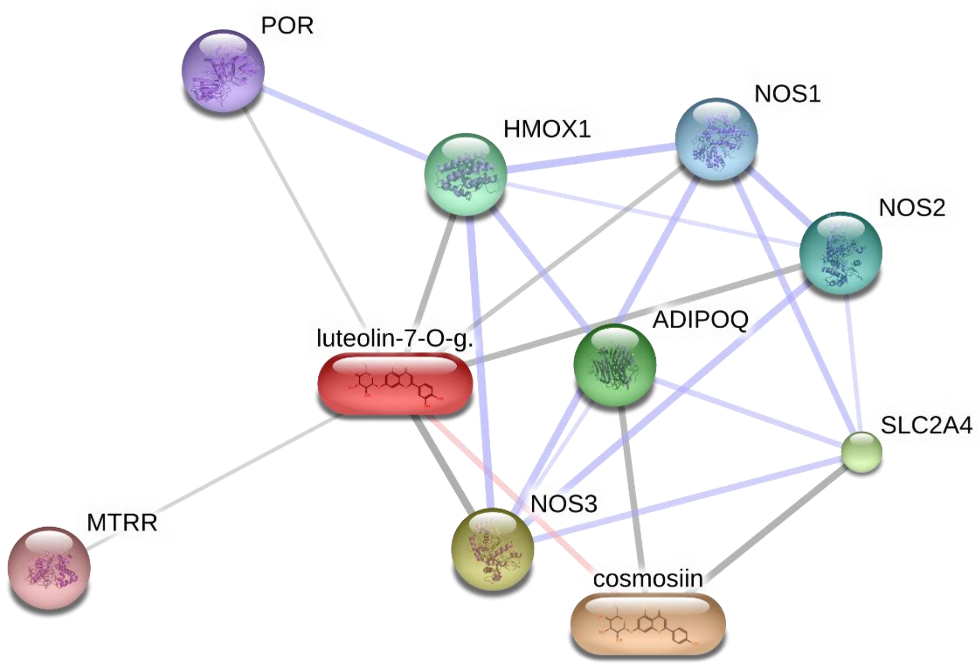


Figure 7. Targets-components analysis (chemical-protein interactions) of apigenin-7-glucoside (cosmos in) and luteolin-7-glucoside performed via the STITCH platform (http://stitch.embl.de)

So far, no drug discovery study was found targeting AChE, BChE, APP, and Aβ simultaneously involved in the etiology of AD. Therefore, this present study is the first to investigate the potential of A7G and L7G as drug candidates in the rational treatment of AD using a structure-based drug design approach. In this study, the binding affinity of A7G and L7G against AChE and BChE was found to be considerably higher than that of rivastigmine, a dual inhibitor of both enzymes (Table 1). With these properties, A7G and L7G can be recommended as natural AChE and BChE inhibitors. Consistent with the findings of this study, a chemical composition, and molecular

docking analysis study reported that the dominant flavonoid glycosides (A7G and L7G) in two *Onosma* species showed very high binding affinities for AChE and BChE (Istifli, 2021). At the molecular level, A7G exerts its ameliorative effect on cognitive impairment by inhibiting COX-2 and iNOS enzymes and may slow down the cognitive regression in AD disease (Patil et al., 2003).
Interestingly, these reported protein targets of A7G are not similar to those predicted by the STITCH server (ADIPOQ and SLC2A4) (Figure 7) and have no relation in the human proteome. Thus, A7G is likely to have more targets in the human proteome than is known in the AD-related literature. It has been shown in an experimental and molecular modeling study that L7G has the potential to inhibit AChE and BChE similar to A7G (Istifli, 2021). Since inducible nitric oxide synthase (iNOS) enzymes involved in inflammation are among the enzymes targeted by L7G in the human proteome (Figure 7), L7G may also show its positive effect in AD by blocking iNOS enzymes. The genetic ablation of iNOS protects against AD-like disease in mice supports this hypothesis (Nathan et al., 2005).

In this study, A7G and L7G showed a moderately favorable binding affinity against the APP and Aβ peptide (Table 1). No experimental or docking studies of A7G and L7G against APP and Aβ peptides were found in the literature. However, it has been reported that the non-glycosidic form of A7G, apigenin, reduces the phosphorylation of ERK1/2 and CREB proteins (Salehi et al., 2019; Zhao et al., 2013), while the non-glycosidic form of L7G, luteolin, inhibits the N-acetyl-alpha-galactosaminyltransferase (ppGalNAc-T) enzyme thereby slowing down the amyloidogenic process (Liu et al., 2017). These reported experimental results are in agreement with the results we obtained from the docking analyses. It has been reported that flavonoids are hydrolyzed and then glucuronidated in the gut (Tundis et al., 2012). Glucuronidation, in turn, results in much higher water solubility; therefore, glycosidic forms are likely to reach the central nervous system. According to the ADMET predictions (Table 3) we employed, A7G and L7G were both found unable to pass the blood-brain barrier; however, this estimation via the SwissADME web tool is based on a machine-learning algorithm, and the cross-validation accuracy of this technique has been reported to be 88% (Daina et al., 2019b). On the other hand, experimental results confirmed that A7G and L7G could, hopefully, cross the blood-brain barrier (Qin et al., 2019; Salehi et al., 2019).

4. Conclusions

This study determined that in molecular docking simulations, A7G and L7G showed high affinity against AChE, BChE, APP, and Aβ peptides. Compared with rivastigmine, A7G and L7G exhibit a highly favorable binding free energy against AChE and BChE and possess the potential of being hit molecules. Considering the drug-likeness, ADMET, and intracellular targets, A7G and L7G have the physicochemical properties that should be found in a drug (although L7G violates Lipinski's rule of 5 at two points), they do not show significant toxicity. They do not have the potential to inhibit intracellular enzymes or proteins significantly. Both compounds may have the potential to positively change the course of AD by showing an inhibitory effect on enzymes (ADIPOQ, NOS1, NOS2, and NOS3) that play essential roles in cellular inflammation processes. However, the possible interaction of L7G with POR (cytochrome P450 oxidoreductase) could be problematic and may influence the patient's response to particular drugs. Although the affinity of A7G and L7G towards APP and Aβ is not highly favorable, molecular modification may increase the affinity of both drugs for these essential AD-related proteins. Further experimental studies will elucidate the actual applicability of these two ligands in the multi-targeted treatment of AD in more detail.

Acknowledgments

None.

Conflict of Interest

The authors confirm that there are no known conflicts of interest.

CRediT authorship contribution statement

Erman Salih Istifli: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing, Review & Editing
Cengiz Sarikurkcu: Data curation, Investigation, Software, Methodology, Review & Editing

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