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Thymus praecox subsp. *grossheimii*'nin Fenolik Bileşikleri ve Sağlığı Artırıcı Etkileri

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Anahtar Kelimeler
Thymus praecox subsp. *grosheimii*
Tıbbi bitki
HPLC-DAD
Doğal ürün
Fenolik

ÖZ

Lamiaceae familyasına ait *Thymus* cinsi mide, ateş, grip gibi çeşitli hastalıkların tedavisinde yıllardır geleneksel tıpta kullanılmaktadır. Bu çalışmanın amacı *Thymus praecox* subsp. *grossheimii* ekstraktındaki fenolik içerik ve miktarını belirlemektir. *Thymus praecox* subsp. *grossheimii* (Ronninger) Tokat, Türkiye'den toplandı ve gölgede kurutuldu. Metanol ekstraktlarının kantitatif analizi diyot dizi algılamalı Yüksek performanslı sıvı kromatografisi (HPLC-DAD) ile gerçekleştirildi. Kumarin ana bileşik (18,3 mg/g ekstrakt) olarak tespit edilmiştir. *Thymus praecox* subsp. *grossheimii'*nin metanol ekstraktında neohesperidin (7,10 mg/g ekstrakt), rutin (2,36 mg/g ekstrakt), resveratrol (2,24 mg/g ekstrakt) kateşin (1,20 mg/g ekstrakt), vanilik asit (0,27 mg/g ekstrakt), gallik asit (0.23 mg/g ekstrakt), kuersetin (0,21 mg/g ekstrakt), *t*-sinamik asit (0,16 mg/g ekstrakt), *o*-kumarik asit (0,11 mg/g ekstrakt) bulundu. Bu bitkinin tıbbi önemi belirlenen biyoaktif bileşiklerin içeriğinden kaynaklanıyor olabilir.

Phenolic Compounds and Health-Promoting Effects of *Thymus praecox* subsp. *grossheimii*

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ABSTRACT

Thymus genus belonging to the Lamiaceae family have been used in traditional medicine for years in the treatment of various illness such as stomach, fever, and flu. The purpose of this study is to assess the phenolic content and quantity in *Thymus praecox* subsp. *grossheimii* extract. *Thymus praecox* subsp. *grossheimii* (Ronniger) was collected from Tokat, Turkey and dried at shade. Quantitative analysis of the methanol extracts was executed by High-performance liquid chromatography with diode-array detection (HPLC-DAD). The results showed, coumarin was found as a major compound (18.3 mg/g extract). Neohesperidin (7.10 mg/g extract), rutin (2.36 mg/g extract), resveratrol (2.24 mg/g extract), catechin (1.20 mg/g extract), vanillic acid (0.27 mg/g extract), gallic acid (0.23 mg/g extract), quercetin (0.21 mg/g extract), *t*-cinnamic acid (0.16 mg/g extract), *o*-coumaric acid (0.11 mg/g extract) were found in methanol extract of *Thymus praecox* subsp. *grossheimii*. The medicinal importance of this plant may be due to the determined bioactive compound contents.

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Introduction

Most of the products used as medicine since ancient times are obtained from herbal sources. Some of the natural compounds are molecules synthesized by plants as secondary metabolism products and play a protective role against microorganisms, insecticides, herbicides and free radicals (Bourgaud et al., 2001; Pagare et al., 2015). Herbal secondary metabolites are frequently used in the fields of medicine, chemistry, food, cosmetics and agriculture. Plants are literally living organic chemistry factories, and the discipline of phytochemistry has become popular thanks to the multi-purpose compounds they produce (Kennedy and Wightman, 2011; Jain et al., 2019).

Plants have the ability to synthesize a large number of aromatic and aliphatic substances, most of which are phenolic compounds or substituted with oxygen. Phenolic compounds are the main components of plants and they are aromatic compounds containing hydroxyl groups (-OH) directly attached to the six-membered aromatic ring (benzene) and giving properties such as colour, smell and taste (Friedman and Jurgens, 2015). Phenolic compounds as food components are important for human health because they show antimicrobial and antioxidative effects and cause enzyme inhibition (Silva et al., 2018). The common feature of phenolic acids, a subclass of phenolic compounds, is that they contain a phenol group with a carboxylic acid function. Phenolic acids, commonly found in the plant crown, also have antioxidant properties (Kiokias et al., 2020). Flavonoids are secondary metabolites that are common in almost every plant species from the highest plants to simple fungi and exhibit important biological activities. The diversity of their structures results from substitution patterns such as hydroxylation, methoxylation or glycosylation (Wang et al., 2020). In recent years, flavonoids have been used in the fields including medicine, agriculture, food, textile, leather, metallurgy due to their antioxidant properties and their ability to dye materials. In addition, some flavonoids are preferred as additives in the cosmetic industry as they are protective against UV rays (Julkunen-Tiitto at al., 2015; Roy et al., 2022).

High performance liquid chromatography (HPLC), a popular chromatographic separation technique, is based on the idea that analytes soluble in the liquid phase depart the column at different periods depending on their interests in the column material. HPLC is a suitable technique for the separation of compounds that are not suitable for other chromatographic techniques and is widely used in medicine, pharmacology, biotechnology, biochemistry analysis (Zohra et al., 2019; Manousi and Samanidoui, 2020). Phenolic compounds such as rutin, hesperidin, ferulic acid and p-coumaric acid were detected in HPLC analysis of *Lamium purpureum* methanol extract (Yalcin at al., 2007). The phytochemical compounds of *Asparagus* *officinalis* are caffeic acid, quercetin, apigenin, ferulic acid and campherol (Zhang et al., 2019). Routine, fisetin, quercetin, myricetin, kaemferol, galangin, isorhamnetin are important flavonols in onions, leeks, broccoli and blueberries (Kopustinskiene et al., 2020).

Thyme (*Thymus* spp.), which is one of the plants of the Lamiaceae family, and its extracts are used in traditional medicine, in the treatment of colds, bronchitis and whooping cough, as a mouthwash against tonsillitis and laryngitis (Fecka and Turek, 2008). *Thymus* spp. essential oils (EOs) can also be used as wound healing ointment and syrup for the treatment of respiratory ailments. Thymol and carvacrol are the most notable phenolic terpenes in terms of both value and amount (Ceylan and Ugur, 2015). Thyme extracts with biological and pharmacological activities contain rich compounds including flavonoids and polyphenols. Luteonine, apigenin, scutellarein, quercetin, camferol, caffeic acid and rosmarinic acid are metabolites found in many thyme (Bubenchikova et al., 2014; Maher et al., 2015; Dalai et al., 2022).

The aim of this study is to elucidate the phenolic components in the extract of *Thymus praecox* subsp. *grossheimii* by HPLC-DAD.

Material and Methods

Thymus praecox subsp. *grossheimii* was collected in Tokat, July 2023 and dried at shade. Dr. Ozgur Eminagaoglu performed the botanical identification, and the specimen was deposited in the Herbarium. (ART 5361). The plant leaves (50 g) were extracted with methanol (250 mL x 3). The mixture was filtered with Whatman filter paper. Then, the solvent was removed by a rotary evaporator to yield the crude extract (Karan, 2018). HPLC-DAD chromatography (Agilent 1260 infinite) was used to separate the bioactive substances for the quantitative analysis, with an ACE Generix, 4.6 mm x 250 mm, 5 m column. For compound detection, a Diode-Array Detection (DAD) detector was utilized, with the injection volume of 10 L and the flow rate was set to 0.8 mL/min. The gradient system was modified to contain A: 0.1% phosphoric acid in water and B: acetonitrile. The gradient program was modified as follows: 0 minute, 80% A; 0-5 min, 75% A; 6-10 min, 65% A; 11-20 min, 60% A; 21-30 min, 55% A; 31-35 min, 50% A; 36-45 min, 45% A; 46-50 min (Erenler et al., 2022).

Results and Discussion

In this study, quantitative analysis of phenolic acids and flavonoids was performed by HPLC-DAD. Coumarin (18.3 mg/g extract) and neohesperidin (7.10 mg/g extract) were found as major compounds. The other compounds were determined as rutin (2.36 mg/g extract), resveratrol (2.24 mg/g extract), catechin (1.20 mg/g extract), vanillic acid (0.27 mg/g extract), gallic acid (0.23 mg/g extract), quercetin (0.21 mg/g extract), *t*-cinnamic acid (0.16 mg/g extract) and *o*-coumaric acid (0.11 mg/g extract), respectively (Table 1).

Table 1. Quantitative analysis of compounds in *Thymus praecox* subsp. *grossheimii*

Compounds	Quantities (mg/g extract)
Ascorbic acid	nd
Gallic acid	0.23
Protocatechuic acid	nd
Catechin	1.20
Hydroxy benzoic acid	nd
Vanillic acid	0.27
Gentisic acid	nd
p-Coumaric acid	nd
Rutin	2.36
Ferulic acid	nd
Naringin	nd
o-Coumaric acid	0.11
Neohesperidin	7.10
Coumaric	18.30
Resveratrol	2.24
Quercetin	0.21
t-Cinnamic acid	0.16
Hesperidin	nd
Alizarin	nd
Flavone	nd

nd: not detected

Standard references with pharmacological activities were selected to determine the phenolic content of the plant by HPLC-DAD. Important medicinal compounds were detected in *Thymus praecox* and their chemical structures were given in Figure 1. The chemical compounds contained in herbal medicines are non-toxic and reliable natural treatment sources. Plant extracts are known to be used for the treatment of febrile diseases, sleep disorders, wounds, diarrhea, reproductive problems, respiratory and circulatory disorders. In addition to these properties, it has been proven by studies that they have antiviral, anticancer, antioxidant and antimicrobial effects (Palombo 2011; Parham et al., 2020). In recent years, it has been determined that phenolic compounds have anticancer properties by affecting more than one mechanism. Various phytochemicals (resveratrol, phenolic acids and flavonoids) inhibit the growth of cancer by inducing apoptosis, and some phytochemicals (resveratrol, quercetin) stop the cycle at a specific moment of the cell cycle in cancer cells. Curcumin, resveratrol, epigallocatechin gallate phytochemicals are known to inhibit the proliferation of cancer cells by inhibiting signaling pathways (Weng and Yen, 2012; Tan et al., 2014).

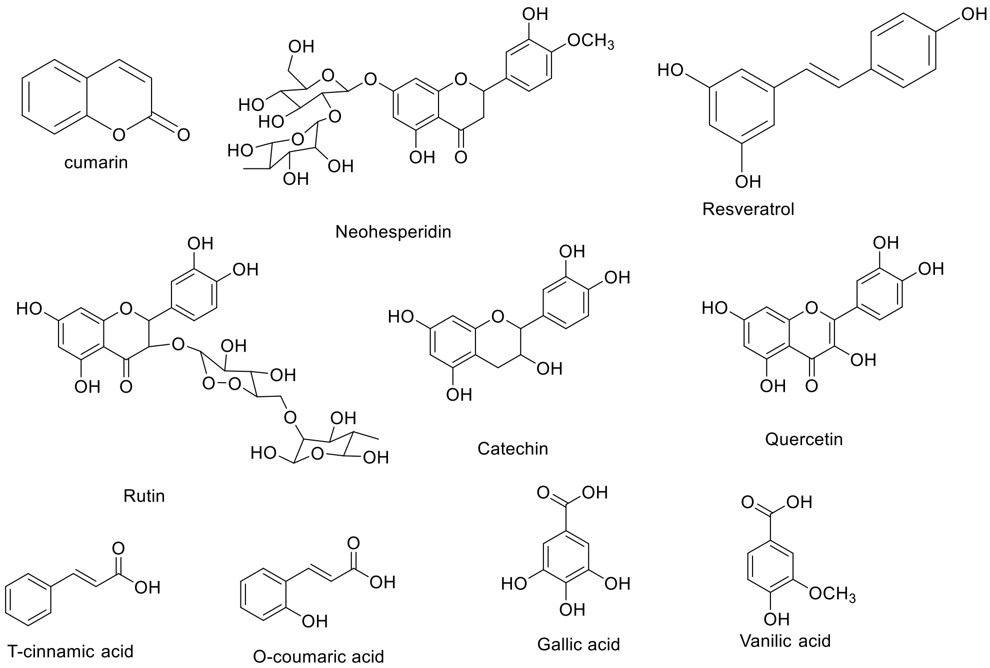

Figure 1. Chemical structures of medically important phenolic compounds

Coumarin and its derivatives exhibit very important pharmacological activities such as anticoagulant, anticancer, antiHIV, antibacterial, antifungal, antioxidant, antiinflammatory, antihypersive, antihyperglycemic. It is also one of the main components of thyme and is a flavone naturally found in many plants. (Wu et al., 2009). *Thymus algeriensis* contains the flavonone glycoside neohesperidin, which has been shown to have a wide variety of therapeutic effects in the treatment of different complicated disorders including neurodegenerative, hepato-cardiac, diabetes, obesity, infectious, allergic cancer, and inflammatory diseases. (Sobeh et al., 2020; Akhter et al., 2022). Polyphenol resveratrol, which has medical importance in recent years, has anticancer, antidiabetes, phytoestrogenic, antiaging, immuno-regulator, hepatitis, fungal disease and dermatitis (Zhang et al., 2021). In a previous study, coumarin, vanilic acid, luteolin and rosmarinic acid were revealed as the major compounds in the extracts of *T. clinics* and *T. cariensis* (Kucukaydin et al., 2021). In another study, rutin flavonoid with high antioxidant potential was detected in *T. sibthorii* and *T. vulgaris* (Dilberovic et al., 2010). The flavonoid catechin, which has antiepileptic, antiparkinson and anti Alzheimer's effects, has also been determined in *T. moroderi* (Díaz-García et al., 2015). Vanillic acid is a natural phenolic acid with good antimicrobial, antioxidant, anticancer, antidiabetic, anti-inflammatory effects and presents in *T.vulgaris* (Bistgani et al., 2019).

There are many thyme species belonging to the Lamiaceae family, which are known as a rich source of phenolics and flavonoids. In HPLC studies with thyme plants, it was determined that the extracts generally contained valuable secondary metabolites such as caffeic acid, vanillic acid, rosmarinic acid, luteolin, apigenin, hesperidin, eriodictyol, rutin, naringenin and isosacuraetin. (Pereira et al., 2023). Although the content and amount of compounds in plants are primarily related to the genetic structure of the plant species, they also vary depending on environmental factors (climate, altitude, etc.) and growing conditions (Mikulic-Petkovsek et al., 2015; Asensio et al., 2020). In this study, the phenolic contents of *Thymus praecox* subsp. *grossheimii* were compared with other thyme species.

Conclusion

Thymus genus contains a variety of secondary metabolites that have been linked to human health. *Thymus praecox* subsp. *grossheimii* extract contains metabolites that exhibit significant pharmacological activities. Especially, coumarin is the major compound in this plant. Coumarin and its derivatives are associated with many drugs as anticancer, antimicrobial, antituberculosis, anti-HIV and anti-inflammatory agents and provide therapeutic benefits. In future studies, researchers should focus on the targeted bioactive compounds for the pharmaceutical industries and isolate them purely.

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Author contributions

Author solely responsible for the entire work.

Conflict of interest

The author declares no conflict of interest.

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