T.R.
EGE UNIVERSITY
Graduate School of Applied and Natural Science

ISOLATION OF HESPERIDIN FROM ORANGE PEELS AND SYNTHESIS OF ITS SEMI-SYNTHETIC DERIVATIVES

MSc THESIS

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Department of Chemistry

İzmir
2019

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Organic Chemistry Programme

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Beyza KOÇAK tarafından Yüksek Lisans tezi olarak sunulan "***Isolation of Hesperidin from Orange Peels and Synthesis of Its Semi-Synthetic Derivatives (Portakal Kabuklarından Hesperidinin İzolasyonu ve Yarı Sentetik Türevlerinin Sentezi)***" başlıklı bu çalışma EÜ Lisansüstü Eğitim ve Öğretim Yönetmeliği ile EÜ Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 10.12.2019 tarihinde yapılan tez savunma sınavında aday oybirliği/~~oyçokluğu~~ ile başarılı bulunmuştur.

Jüri Üyeleri: **İmza**

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Üye: Doç. Dr. Tamer KARAYILDIRIM ……………………

EGE ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ

ETİK KURALLARA UYGUNLUK BEYANI

E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliğinin ilgili hükümleri uyarınca Yükseklisans Tezi olarak sunduğum "***Isolatin of Hesperidin from Orange Peels and Synthesis of Its Semi-Synthetic Derivatives (Portakal Kabuklarından Hesperedinin İzolasyonu ve Yarı Sentetik Türevlerinin Sentezi)***" başlıklı bu tezin kendi çalışmam olduğunu, sunduğum tüm sonuç, doküman, bilgi ve belgeleri bizzat ve bu tez çalışması kapsamında elde ettiğimi, bu tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara atıf yaptığımı ve bunları kaynaklar listesinde usulüne uygun olarak verdiğimi, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını, bu tezin herhangi bir bölümünü bu üniversite veya diğer bir üniversitede başka bir tez çalışması içinde sunmadığımı, bu tezin planlanmasından yazımına kadar bütün safhalarda bilimsel etik kurallarına uygun olarak davrandığımı ve aksinin ortaya çıkması durumunda her türlü yasal sonucu kabul edeceğimi beyan ederim.

10.12.2019

Beyza KOÇAK

Beyza

PREAMBLE

In this thesis, it is desired to isolate and synthesize semi-synthetic derivatives of hesperedin from orange peels.

I would like to thank my dear Professor Prof. Dr. Özgen ALANKUŞ, who shaped her work in the light of her scientific background with her knowledge and experiences, who benefited from her vast knowledge and experience in her planning, research, implementation and formation. And I would like to thank TÜBİTAK (Project number: 116Z906) for financial support.

İzmir

10.12.2019

Beyza KOÇAK

ÖZET

PORTAKAL KABUKLARINDAN HESPERİDİNİN İZOLASYONU VE YARI SENTETİK TÜREVLERİNİN SENTEZİ

Beyza KOÇAK

Yüksek Lisans Tezi, Kimya Anabilim Dalı

Tez Danışmanı: Prof. Dr. Özgen ALANKUŞ

Aralık 2019, 50 sayfa

Birçok çiçeğin petallerinde renk verici madde olarak bulunan flavonoidler, yapısal çeşitlilikleri, biyolojik ve ekolojik önemleri, hastalıkları iyileştirici etkileri ve antikanser aktiviteleri ile farklı disiplinlerdeki araştırmacıların dikkatini çekmektedir. On beş karbonlu flavonoid iskeletine hidroksil, metoksi, metil, izoprenil ve benzil gibi farklı grupların bağlanması, teorik olarak çok fazla sayıda farklı flavonoid yapısını mümkün kılmaktadır. Flavonoidlerin en çok bilinenleri yaban mersininde bulunan antosiyanidinler, turungillerde bulunan hesperidin, siyah çayda bulunan flavan-3-ollerdir. Turunçgillerde (Rutaceae) fazla miktarda bulunan hesperidin ve aglikonu hesperetin, anti kanser, anti-enflamatuvar, antioksidan ve anti-tümör gibi çok geniş yelpazede biyofarmasötik aktivite göstermektedir. Narenciye üretiminde ön sıralarda yer alan ülkemiz, hesperidince zengin olan portakal kabuklarını başlıca Almanya, Fransa olmak üzere ihraç etmekte ve bunlardan elde edilen işlenmiş değerli kimyasalları- hesperidin, hesperetin ve bunları türevleri- ise ithal etmektedir. İthal edilen bu hesperidin, hesperetin ve türevleri Daflon® ve Vendios® gibi ülkemiz ilaç piyasasında dolaşım düzenleyici olarak satılan ilaçların etken maddesi olarak kullanılmaktadır.

Bu tez çalışmasında, ülkemizde yetişen portakallardan elde edilen atık kabuklar kullanılarak farklı çözgen sistemleri ile ekstrakte edilmiş ve elde edilen hesperedince zengin alkol ekstresinden asidik çöktürme ile hesperidinin eldesi gerçekleştirilmiştir. Hesperidinin farklı şartlardaki hidrolizi ile hesperidinin aglikonu olan hesperetin elde edilmiş, hesperidin ve hesperetinden çıkılarak ayrılma tepkimesi ile alfa beta doymamış türevleri olan diosmin ve diosmetinin sentezi yapılmıştır. Elde edilen bu türevlerin Wittig tepkimesi, kısmi ve tam metillenmiş yarı-sentetik türevlerinin sentezlenmesi araştırılmıştır. Sentezlenen ve elde edilen tüm bileşikler saflandırıldıktan sonra yapı tayinleri ^{1}H-NMR, ^{13}C-NMR ve FT-IR gibi spektroskopik teknikler kullanılarak yapılmıştır.

Anahtar Kelimeler: Portakal Kabuğu, Hesperidin, Hesperetin, Yarı sentez

ABSTRACT

ISOLATION OF HESPERIDIN FROM ORANGE PEELS AND SYNTHESIS OF ITS SEMI-SYNTHETIC DERIVATIVES

KOÇAK, Beyza

MSC Thesis in Chemistry
Supervisor: Prof. Dr. Özgen ALANKUŞ
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The flavonoids found in the petals of many flowers are attracting the attention of researchers in different disciplines with their structural diversity, biological and ecological importance, healing effects and anticancer activities. The binding of different groups such as hydroxyl, methoxy, methyl, isoprenil and benzyl to the fifteen carbon flavonoid skeleton makes theoretically possible for a large number of different flavonoid structures. The most well-known flavonoids are anthocyanidins found in blueberries, hesperidin in turgans, flavan-3-ols in black tea. Hesperidine and aglyconin, which are found in large amounts in citrus fruits (Rutaceae), show a wide range of biopharmaceutical activity such as anti-cancer, anti-inflammatory, antioxidant and anti-tumor. Our country, which is at the forefront of citrus production, exports the orange shells rich in hesperidin mainly in Germany, France and imports processed precious chemicals-hesperidin, hesperetin and their derivatives. This imported hesperidin, hesperetin and its derivatives are used as active ingredients of drugs sold as circulation regulators in our country's pharmaceutical market such as Daflon® and Vendios®.

In this thesis, the extraction of hesperidine was obtained as a result of the extraction of orange peels from orange grown in our country under different conditions.After finding the optimum extraction conditions, hesperidin reach extract was purified by acidic precipitation in order to obtain hesperidin. Hesperetin, that is an aglycone of hesperidin was obtained after acidic hydrolysis of hesperidin in different conditions such as acid type, temperature, duration, etc. once hesperetin was obtained both hesperidin and hesperetin were converted into their alpha beta unsaturated derivatives, diosmin and diosmetin respectively, by elimination reaction. Then, semi-synthetic derivatives of these compounds i.e. Wittig reaction, fully and partly methylation were performed. All the synthesized and obtained compounds were purified and then their structural identifications were performed by spectroscopic techniques such as ^{1}H- NMR, ^{13}C-NMR and FT-IR.

Keywords: Orange Peel, Hesperidin, Hesperetin, Semi-Synthesis

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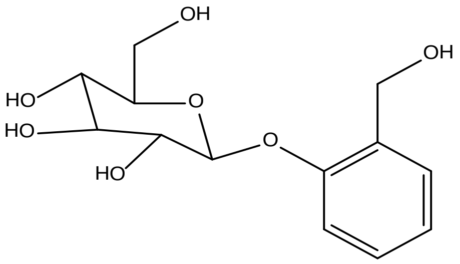
1. INTRODUCTION

1.1. Natural Products

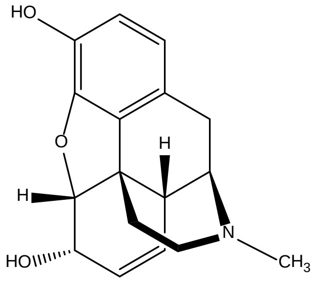
Natural products are complex chemical compounds and substances that are produced by living organisms in nature. In the old ages, people have begun to benefit from nature in order to improve diseases and have survived to the present day.

And so the plants formed the cornerstones of traditional medicine. The first records were found in Mesopotamia on clay tablets. Approximately 1000 varieties of plant-related disease treatments are described. Records of the use of plant species belonging to many different civilizations describing the use of treatment emerged. The most well-known are the Egyptian Ebers Papyrus, the Materia Medica of the Chinese, and the Ayurveda of the Indians (Newman et al., 1999).

Although the use of plants in the treatment of diseases began thousands of years ago, the discovery of modern drugs and plant isolation began only in the 19th century. In the early 1800s, Morphine was recorded as the first commercially available pure substance. Isolated from the *Salix alba* plant in the late 1800s, the salicin was produced by the first semi-synthetic derivative of the aspirin drug, by Bayer (Veeresham, 2014).



Salicin



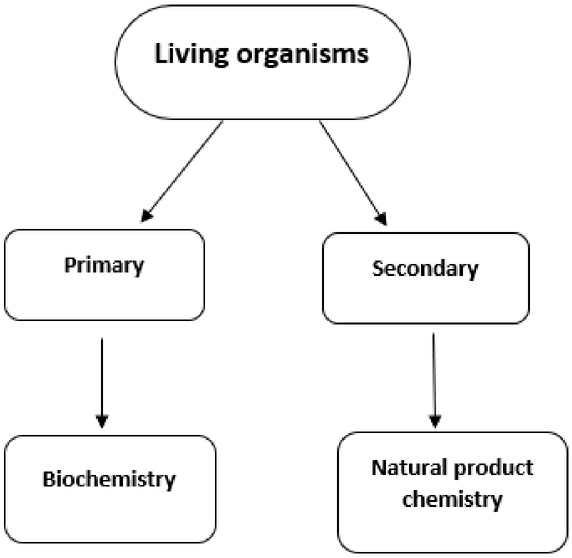
Morphine

After these developments in the 1800s, the number of natural compounds isolated from many plants for various diseases has increased and semi-synthetic derivatives have come to the fore (Veeresham, 2014).

The discovery of penicillin by Fleming in the 1920s and the commercialization of synthetic derivatives of penicillin in the 1940s, there have been significant developments in drug research (Siddiqui et al., 2014).

In the 1960s, paclitaxel isolated from yew (*Taxus brevifolia*) was introduced as Taxol, a chemotherapeutic drug, 30 years later. However, as the method of isolation was not sufficient during the treatment process, paclitaxel synthesis was performed from 10-deacetylbaccatin III isolated from *Taxus baccata* (Erdemoğlu et al., 2000).

Natural products can be examined in four main groups: fats, carbohydrates, nucleic acids and proteins. They are important for life functions and are known as primary metabolites (Scheme 1.1). Secondary metabolites are produced from primary metabolites by biosynthetic means and are defined as by-products.



Scheme 1.1. Natural Product Chemistry

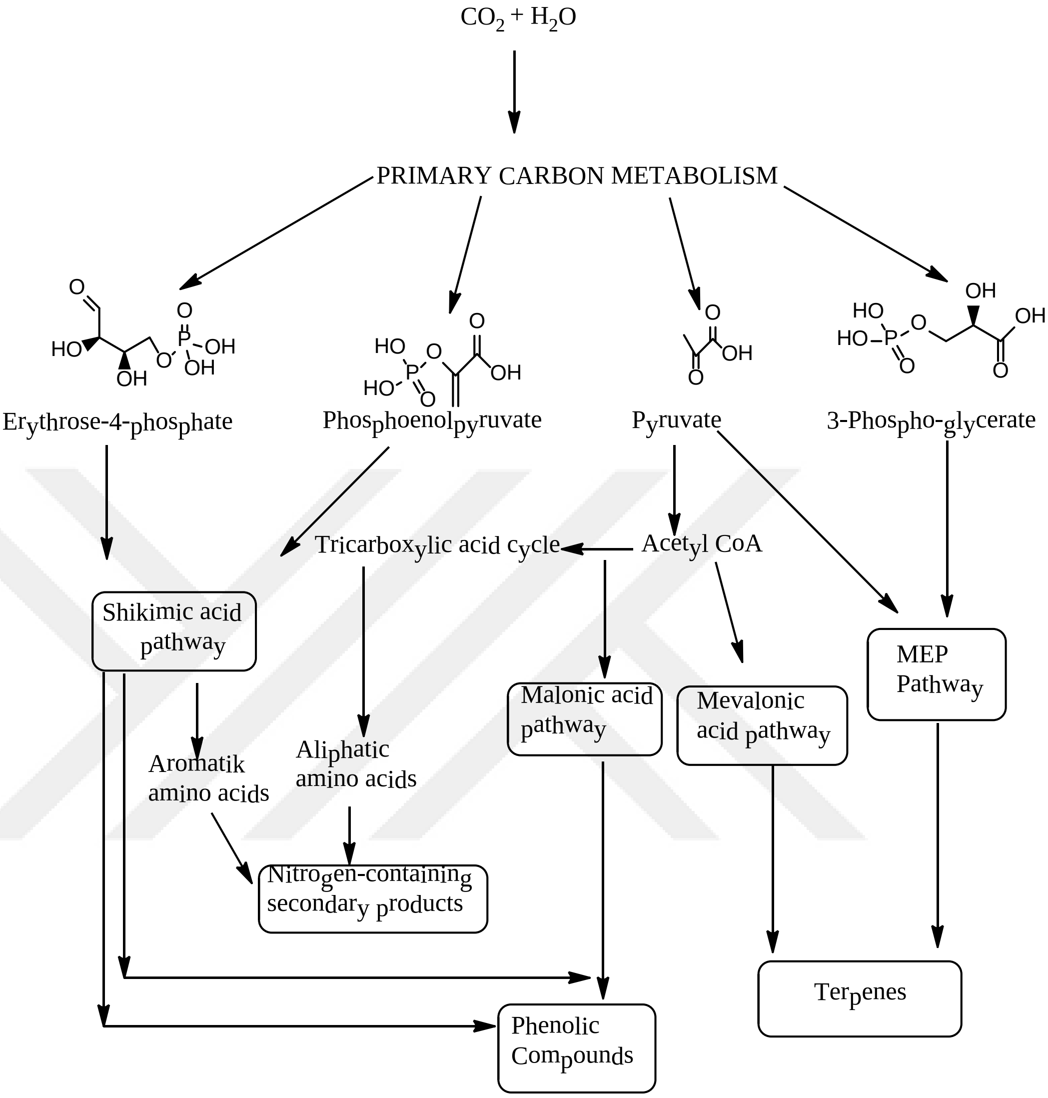
They play roles such as pollination in plants, defense against microorganisms and insects, adaptation to environmental conditions.

1.2. SECONDARY METABOLITES

Secondary metabolites are not related to the basic vital functions of the plant, but they are as important as the primary metabolites associated with the plant's essential vital functions.

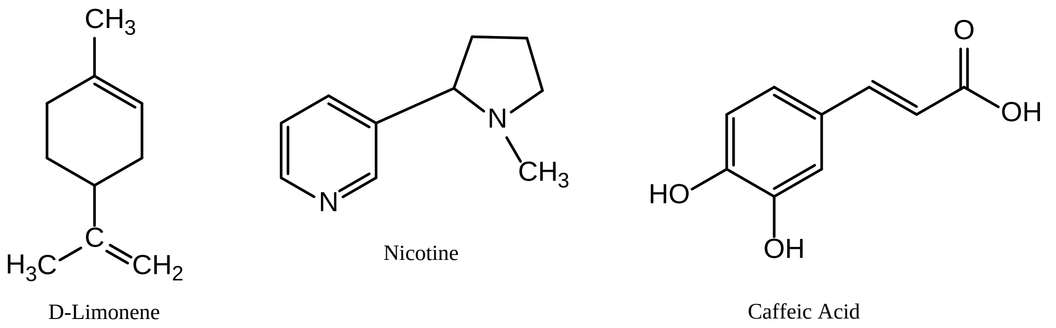
According to the researches carried out on secondary metabolites, it was determined that these metabolites have a large number of structural variations and they are produced by the plants in small numbers (Baytop, 1999).

Secondary metabolites are produced by biosynthetic pathways from primary metabolites and are therefore more complex than their structures. (Scheme 1.2).



Scheme 1.2. Secondary metabolites are derived from Primary metabolites

Secondary metabolites produced in plants, species and genus are more valuable products than primary metabolites. The secondary metabolites are composed of three main groups, phenolics, terpenes and alkaloids.



D-Limonene Nicotine Caffeic Acid

Example of terpenes, alkaloids and phenolics

1.3. Phenolic Compounds

Phenolic compounds are one of the largest groups of phytochemicals and have been proven to be at least as important as primary metabolites (Bravo, 1998). According to research, phenolic compounds play an important role in the growth and growth of plants, protection against threats or protection against oxidative stress caused by free radicals.

These compounds are derivatives of metabolic pathways such as pentose phosphate and shikimate. Phenolic compounds found naturally in nature are composed of an aromatic ring having one or more hydroxyl groups in their structure. They classify the phenol numbers as simple phenols and polyphenols (Table 1.1). Phenolic compounds are divided into simple phenols, coumarins, tannins, flavonoids and phenolicacids (Randhir et al., 2004).

Table 1.1. Major classes of phenolics

Skeleton	**Class**	**Examples**
C_6	Simple phenols	Hydroquinone
C_6-C_1	Phenolic acids	Salicyclic acid
C_6-C_2	Acetophenones	3-Acetyl-6-methoxybenzaldehyde
C_6-C_3	Hydrocinnamic acids, Phenylpropenes, Coumarins, Isocoumarins	Caffeic acid, Eugenol, Umbelliferone, Bergenin
C_6-C_4	Napthaquinones	Juglone
C_6-C_1-C_6	Xanthones	Mangiferin

C_6-C_2-C_6	Stilbenes, Anthraquninones	Lunularic acid, Emodin
C_6-C_3-C_6	Flavonoids, isoflavonoids	Quercetin, Genistein
$(C_6$-$C_3)_2$	Lignans	Pinoresinol
$(C_6$-C_3-$C_6)_2$	Biflavonoids	Amentoflavone
$(C_6$-$C_3)_n$	Lignins	Polymers
$(C_6)_n$	Catechol melanins	
$(C_6$-C_3-$C_6)_n$	Flavolans (tannins)	Proanthocyanidins

Phenolics, which can be found everywhere in plants, have a great importance in terms of human nutrition due to their antioxidant properties (Balasundram et al., 2006). The antioxidant activity of phenolic compounds varies according to the position and number of hydroxyl groups in the aromatic ring. It has many physiological effects such as anti-allergenic, anti-microbial and anti-thrombotic, anti-inflammatory, anti-carcinogenic as well as antioxidant effects (Samman et al., 1998).

1.4. FLAVONOIDS

Flavonoids are the largest group of phenolic compounds with the C6-C3-C6 framework that can be formed in each part of the plants (Figure 1.1). First, flavonids found in green algae 5000 years ago have become complicated by the evolution of plants (Swain et al., 1975).

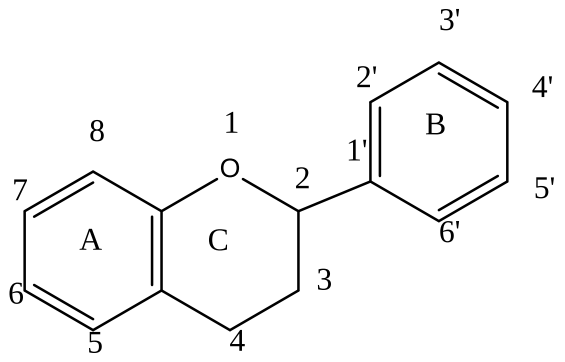


Figure 1.1. Basic Flavonoid structure

Flavonoids are found in the leaves, flowers and fruit parts of the plants, giving blue, red and bright colors, and they are also found in seeds, nuts, red wine, tea and in lowlevels (Kuhnau, 1976**).** Phenols are generally found in the form of glycosides, which can be easily dissolved in water (Harborne, 1998).

In plants, glycosylated flavonoids are very important not only for plants but also for humans and animals (Brouillard, 1988). As a result of the research, it is known that there are more than 9000 flavonoids today (Hernández et al., 2009). Flavonoids, considered the natural source of antimicrobial drugs, have been shown to show many biological activities. They are antiviral, antibacterial, antiprotozoal and anti-inflammatory (Sharma et al., 2006).

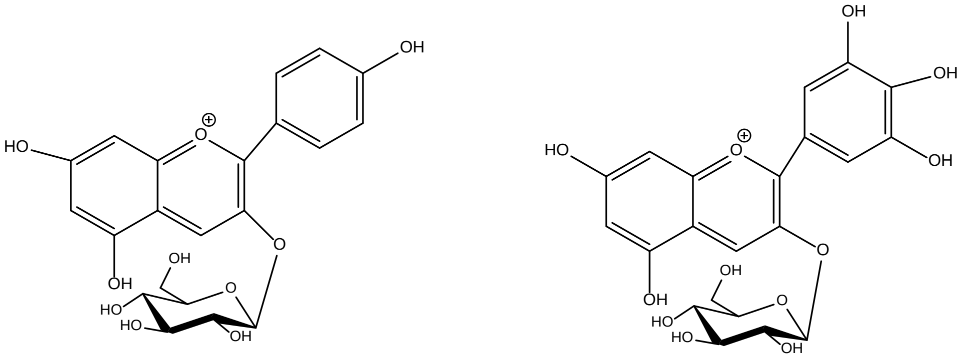
Flavonoids have the benefit of eliminating reactive oxygen species and enhancing the body’s antioxidant activity (Nako et al., 2011). Flavonids are the most well-known and most frequently studied subjects; naringin, hesperidin and naringenin. According to Toumi et al., Hesperidin has the ability to cleanse DPPH free radicals and provides renewal in pancreatic B cells (Toumi et al., 2009).

1.4.1. Classification of flavonoids

Flavonoids can be studied under five groups as flavones, flavonols, flavanones, isoflavones and anthocyanins (Halfon, 2005).

1.4.2. Flavones and Flavonols

The most abundant varieties of flavonoids flavones and flavonols are generally formed in the form of glycosides (Figure 1.2). The main sources of flavonols are fruits and vegetables such as apples, broccoli. A lot of positive effects of flavonols on health are known. With the increase in the intake of flavonols, the risk of cardio risk reduction is determined as a result of the studies (Hollman et al., 1998).



Pelargonidin 3-glucoside

Myrtillin

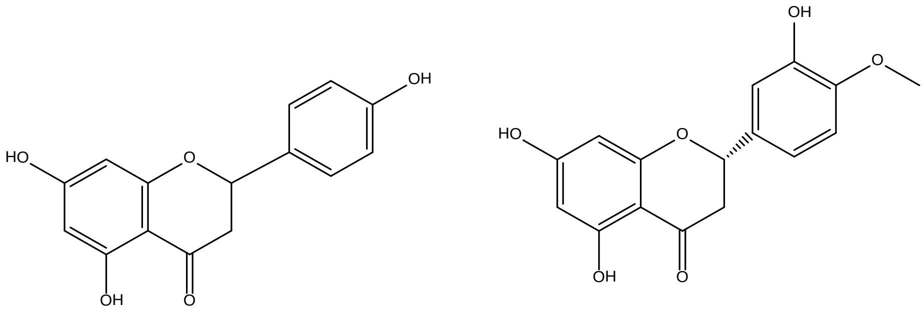
Figure 1.2. Basic Flavones and Flavonols structure

There is an extra hydroxyl group in the c-3 position of flavone which is very similar in structure to flavonols. The most well-known examples of flavones are found in carrots, onions, cabbage and peppers; luteolin (Lin et al., 2008) and found in tea, oranges, daisies; apigenin may be administered (Patel et al., 2007).

1.4.3. Flavanones

Flavanones are seen in around 42 plant families, mainly Compositae, Leguminosae and Rutaceae. The most common examples of the pomegranate and grapefruit found in orange and lemon are hesperetin aglycone and grapefruit naringenin flavanon (Figure 1.3).

Flavanones shows various biological activities such as anti-inflammatory, cardiovascular, antiviral effects, anticancer. However, there has not been much work on the anti-cancer feature (Trzeciakiewicz et al., 2010).



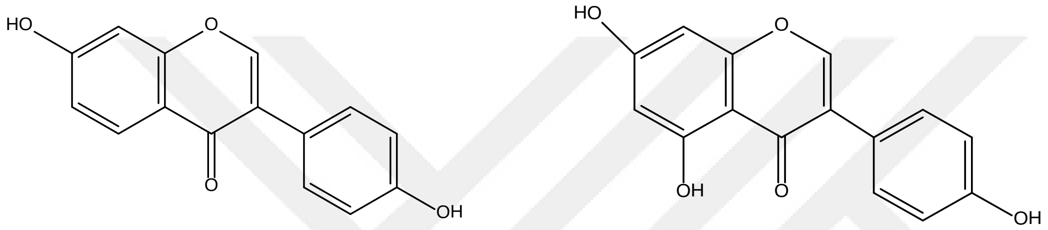
Naringenin

Hesperetin

Figure 1.3. Aglycone of Flavanones

1.4.4. Isoflavones

Isoflavones with polyphenolic structure are known for their antioxidant effects (Tsao, 2010). Soy (Glycine max L.) is one of the main sources of isoflavones. The species of genus genus Clover (*Trifolium pratense* L.) and Genista are other plant sources of isoflavones. Isoflavones in soybeans are found in the form of glycosides, that is, sugar-bound to the structure. As a result of digestion of soybean, glycosides are released and remain aglycone. Examples of known isoflavone aglycones are shown in Figure 1.4. Antioxidant properties as well as anticancer, antimicrobial and anti-inflammatory properties have emerged as a result of research (Crozier et al., 2009).



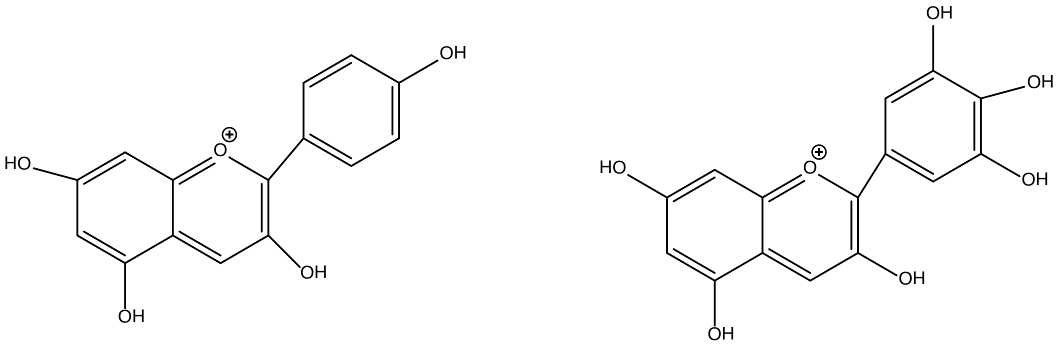
Daidzein

Genistein

Figure 1.4 Aglycone of Isoflavonoids.

1.4.5. Anthocyanins

Anthocyanins, also known as flavylium, are found in plants as purple, red and blue color pigment. The anthocyanins have two conjugated planar rings in their structure and have been isolated from the plants up to about 500 different structures (Kendrcik, 2012). Besides antioxidant properties, they are used as coloring in food and dyes. The most common examples are cyanidin, also known as magenta pigment (Casals et al., 2003), pelargonidine, known as red color pigment (Katsumoto et al., 2007), and delphinidine as purple color pigment (Barczak et al., 2005) (Figure 1.5).



Pelargonidine

Delphinidine

Figure 1.5. Anthocyanins

1.4.6. Flavonoids Biosytnhesis

Flavonoid compounds are synthesized in plants by two separate biosynthesis. The ring A in the simple flavonoid skeleton is the polyketide derivative while the B ring is the shikimate derivative (Haflon, 2005). All known 9,000 flavonoid skeletons have the same biosynthetic pathway, so they share the same basic skeleton. Much work has been done to elucidate the biosynthetic pathways of flavonoids. The compounds involved in flavonoid synthesis were first isolated from Maize (*Zea mays*), snapdragon (*Antirrhinum majus*), and petunia (*Petunia hybrida*) (Holton et al., 1993; Mol et al., 1998). Most plant phenolics are formed by the shikimic acid pathway, and this pathway is the link between primary and secondary metabolism in high plants (Macheroux et al., 1999). Aromatic amino acids such as tryptophan, tyrosine and phenylalanine are synthesized by the shikimic pathway consisting of seven enzymatic reactions. Although the "Shikimic Pathway" is found in bacteria, fungi and plants, it does not occur in humans and animals.

1.4.7. Biological and Pharmacological Properties of Flavanoids

Flavonoids have remarkable biological activities. Their biological activity is related to the number and location of the hydroxyl group in their structure or the location of other functional groups (Crozier et al., 2009). In addition to the presence of many biological activities, antitoxidant activities have attracted the most interest from researchers. According to the definition of Halliwell and Gutteridge, compounds that inhibit the damage of cellular components by the reaction of free radicals are called antioxidants. Recent research has shown that flavonoids are more potent antioxidants than vitamin C and E. Recent research has shown that flavonoids are more potent antioxidants than vitamin C and E (Dai et al., 2010).

Flavones and catechins are the most effective flavonoids to protect the body against reactive oxygen species (ROS) (Kim et al., 1993). Shariffer et al. reported that lipid peroxidation of the antioxidant activity of the methanol extract of the *Teucrium polium* plant were potent inhibitors of beta-carotene oxidation (Sharififar et al., 2009). Ghasemzadeh et al. reported that they had high levels of flavonoid as a result of their studies on Halia Bara and therefore have high antioxidant activity (Ghasemzadeh et al., 2010). *Chrozophora tinctoria* (L.) A. Juss. The anti-inflammatory activity of two new acylated flavonoids isolated from the plant was determined by the measurement of IL-1, IL-6, TNF-a and PGE2 levels from the upper phase of human blood cells induced by phytohaemagglutinin (PHA) (Hossam et al., 2015) Gibellini, L., et al. reported that kaempferol 3-O-β-D-galactoside inhibited the production of TNF-α and NOS and thus showed significant anti-inflammatory effect (Gibellini et al., 2011). The *Santalam album* plant, which is widely used for skin treatment, has also been reported to have anti-microbial and anti-inflammatory effects (Herbmed et al., 2016). The anti-microbial effects of flavonoids are based on quite old times. And still studies are carried out on these effects.

For example, it has been reported that Propolis was used in ancient Greece by Hippocrates for the treatment of wounds and ulcers (Fearnley et al., 2001). *Scutellaria baicalensis* plant has been used in China due to its anti-microbial effect in periodontal treatments (Verzera et al. (2003), *Citrus bergamia* Risso plant oil has been used in the pharmaceutical industry because of the anti-microbial and anti-septic activity of the oil obtained from the peels (Verzera et al., 2003). Green tea is made up of 30% polyphenols and therefore shows a variety of activities. It is the result of studies (Toda et al. 1989) and showing anti-microbial properties against the bacteria causing caries (Rasheed and Haider 1998).

1.5. RUTACEAE FAMILY

The Rutaceae family is a large family of many fruit-bearing and medicinal plants, consisting of about 158 genera and 1730 species (Koblovská et al., 2008). The Rutaceae family is found in warm and tropical areas, especially in Africa and Australia (Heywood, 1978). Most of the species in the family are trees and bushes. Aromatic fragrant leaves are a characteristic feature of Rutaceae. The Rutaceae family, commonly known as the Rue or the citrus family, is closely related to the families of Sapindaceae, Simaroubaceae and Meliaceae. Rutaceae is considered to be composed of six sub-families whose sub- families cannot be solved. These families are Aurantioideae, Dictyolomatoideae, Flindersioideae, Rutoidea, Spathelioideae, Toddalioideae. Rutaceae family represents the population in Turkey taxa and *Ruth* L., *Haplophyll* A. Juss., *Dictamnus* L., *Citrus* L., *Poncirus* shelf., From the resultant is represented by five genera (Güner et al., 2000). The Auranthioideae, known as the *Citrus* subfamily and contains genera, is the most economically important taxa (Groppo et al., 2012).

Studies conducted in Turkey has emerged as a result of new species belonging to the family Rutaceae. For example; Ulukus et al. (2018) defined a new species of Haplophyllum species in Ermenek region of Karaman province (Ulukuş et al., 2018). In other studies, they found the *Haplophyllum sahinii* new species found in the Central Anatolian province of Konya (Ulukuş et al., 2017). Rutaceae family, which is used in various fields such as traditional medicine, perfumery and gastronomy, has been shown to have various biological activities. For example; *Z. Limonella* shows the anti-septic and anti-inflammatory effect of the oil obtained from the fruits of the genus (Nadkami, 1982). It has been shown that citrus oils such as limonene and linalool obtained from fruit crust of plants show antimicrobial properties (Jayant et al., 2014). Suarez et al. (2008) demonstrated the antibacterial and antimalarial activity of secondary metabolites from Peltostigma guatemalense, a new species of the Ruatecea family (Enrique et al., 2009).

1.5.1. *Citrus* Genus

The *Citrus* genus, a member of the Rutaceae family, was first mentioned in ancient times. The oldest known source is Sanskrit literature dating back to 800 BC (Scora, 1975) As a result of epidemiological studies, the consumption of citrus species as a food has been found to have a reducing effect on chronic diseases such as Alzeheimer's disease, cardiovascular diseases and diabetes (Liu, 2003, Temple,2000, Willett,2002).

There are numbers of studied that focused on the biological activity of this species such as anti-tumor, anti-fungal and anti-inflammatory effects. In addition, its most known activity is anti-oxidant activity (Middleton and Kandaswami, 1994, Olson, 1988, Yehoshua et al., 1995).

1.5.2. Traditional Medicinal Uses of Rutaceae Family

Many different species of Rutaceae have been used in traditional medicine for various purposes.The fruit of Toddalia asiatica is used for malaria, and the roots of indigestion have been used in the treatment of lung diseases (Orwa et al., 2008). Arbab et al. Conducted studies showing the anti-inflammatory, anti-viral, antioxidant activity of different species of the genus Clausena (Arbab et al., 2013). Zanthoxylum bungeanum Maxim, which is used as a traditional herbal medicine in China, is used in the treatment of diseases such as digestive disorders, dental pain treatments, stomach pain, diarrhea (Zhang et al., 2017). *Citrus aurantium* used in the treatment of various diseases such as colds, skin spots, rheumatism, epilepsy, digestive problems, Paul et al. In the study conducted by (Paul et al., 1995). *C. macroptera* belonging to the citrus genus known as sat kara is known to have anti-aging effect and it is used in diseases such as diabetes and cancer (Fusco et al., 2007). Ruta gravelons, which has been used since ancient times, is a traditional herbal remedy used as a sedative or for stomach disorders (Gachathi et al., 2017).

2. MATERIALS AND METHODS

2.1. General

The 1D NMR spectra were measured in DMSO and $CDCl_3$ at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR on a Varian AS-400 spectrometer.

FT-IR spectra were recorded on Perkin Elmer Spectrum Two Series and UATR Accessory.

Open column chromatography was carried out using Silica Gel 60 F254 (Merck 7734) as a sorbent. TLC analyses were carried out on Silica gel 60 F254 (Merck 5554). Compounds were detected by under the UV light (254 and 336 nm) for UV active components. They were also heated 120 C^o for 2-3 minuntes after spraying by 20% H_2SO_4/water spraying reagent for spots and bands become visible

HPLC analysis were carried out on a Thermo Finnigan Surveyor HPLC with a UV-detector (280 nm), a Cosmosil C18 250:4.6:5 µm.

2.2. Plant Material

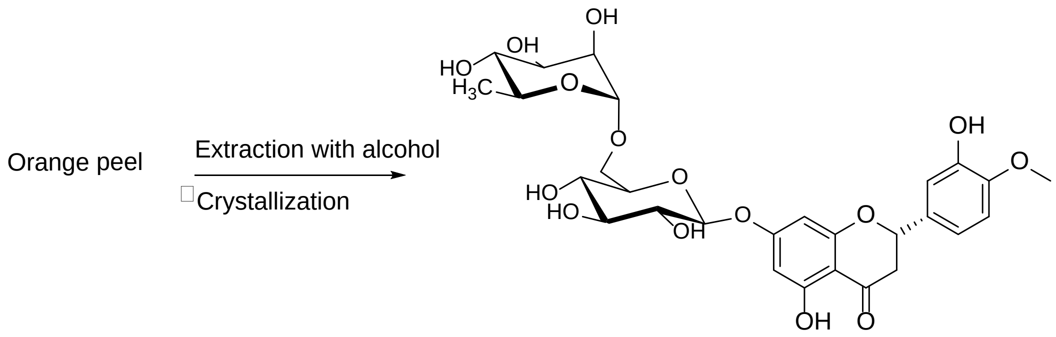
Orange peels were supplied from orange juice manufacturer Dimes-İzmir then dried in the shade and ground.

2.3. Extraction Conditions

Orange peels were extracted with different parameters such as temperature, solvent type, solvent ratio and time. Based on the experimental results and HPLC analysis, optimum extraction conditions were selected.

2.4. Isolation and Purification of Hesperidin

The air-dried and powdered orange peel was extracted with ethanol: water (80:20) solvent system at 40 °C. After filtration and evaporation procedures, the extract was dissolved in alcohol, and then acetic acid (6%) was added. After 2 hours, hesperidin was filtered as light yellow precipitate and was obtained in 3.2% yield. (Berger and Sicker, 2009, Lahmer et al., 2015)

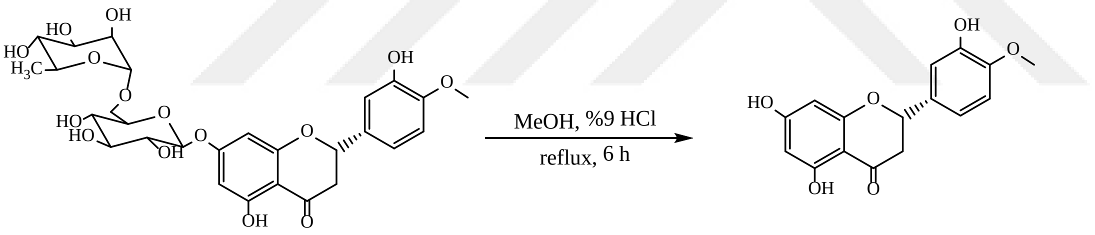


Hesperidin

2.5. Synthesis of Hesperetin

Hesperetin was obtained from hydrolysis of hesperidin in different conditions such as acid type, solvent systems and time. Based on the experimental results, optimum hydrolysis conditions were selected.

Hesperidin is solved in methanol, and then hydrochloric acid (9%) is added while stirring. After 6 hours, cooling in ice bath, hesperetin precipitate (Lahmer et al., 2015; Seitz and Wingard, 1978).



Hesperidin

Hesperetin

Hesperidin (0.500 g, 0.82 mmol) was dissolved in 50 ml of MeOH and 9% HCl was added to boil under reflux for 6 h. It was neutralized with 5% NaOH solution and dried under vacuum. The reaction mixture extracted with water (50 mL) and AcOEt (2x50 mL). The organic layers were collected. The solvent was removed by evaporation under reduced pressure. Dissolve the crude product in minimum of acetone, and the resulting solution was added to a vigorously stirred mixture of water (200 mL) and acetic acid (3 mL). In an ice bath, precipitated hesperetin was washed and cooled with water. Afforded Hesperetin (0.140 g, 0.46 mmol). Yield: 56%.

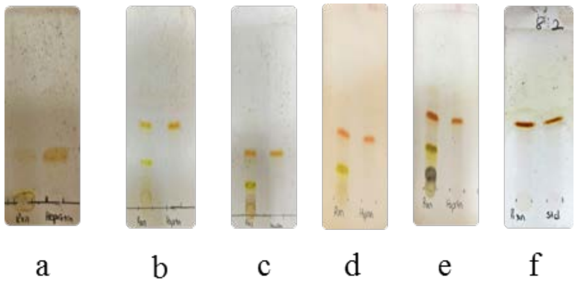
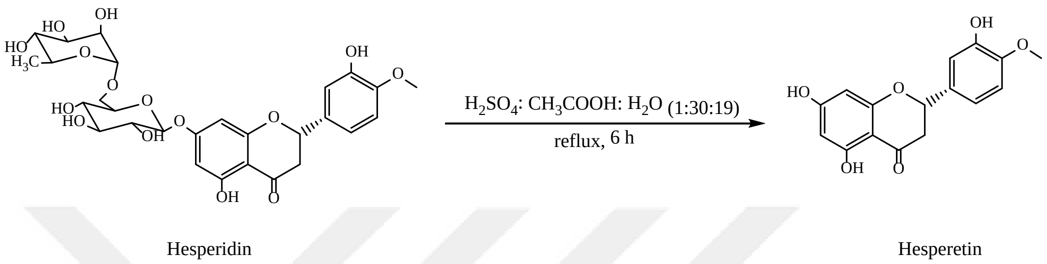


Figure 2.1. Hesperetin on TLC plate (a: 2h, b: 4h, c: 6h, d: 8h, e: 10h, f:end of precipitation)



Hesperidin (0.500 g, 0.82 mmol) was dissolved in 50 mL of a mixture of H_2SO_4:CH_3COOH H_2O (1:30:19) was kept under reflux for 6 h. It was neutralized with 5% NaOH solution and dried under vacuum. The reaction mixture extracted with water (50 mL) and DCM (2x50 mL). The organic layers were collected. The solvent was removed by evaporation under reduced pressure. Afforded Hesperetin (0.034 g, 0.112 mmol).Yield: 14%

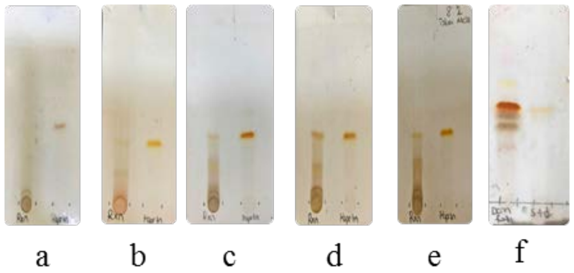
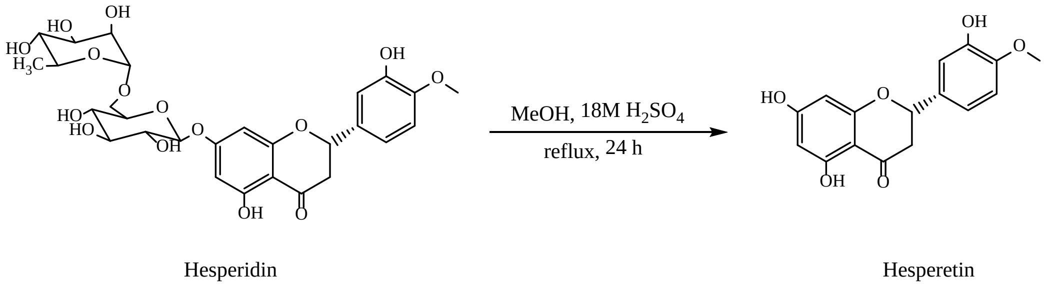


Figure 2.2. Hesperetin on TLC plate(a: 2h, b: 4h, c: 6h, d: 8h, e: 10h, f: DCM phase)



Hesperidin (1.00 g, 1.64 mmol) was dissolved in 30 mL of MeOH and 1 mL 18 M H_2SO_4 was added to boil under reflux for 24 h. It was neutralized with

10% NaOH solution and dried under vacuum. The reaction mixture extracted with water (50 mL) and AcOEt (2x50 mL). The organic layers were collected. The solvent was removed by evaporation under reduced pressure. Afforded Hesperetin (0,350g). Yield: 70%

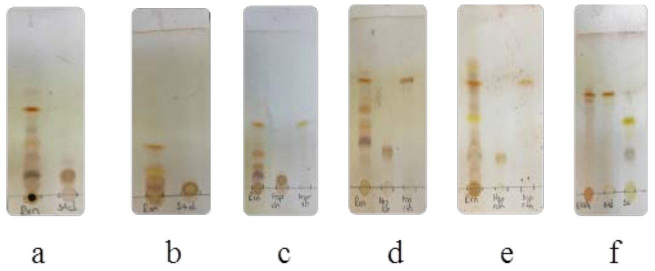
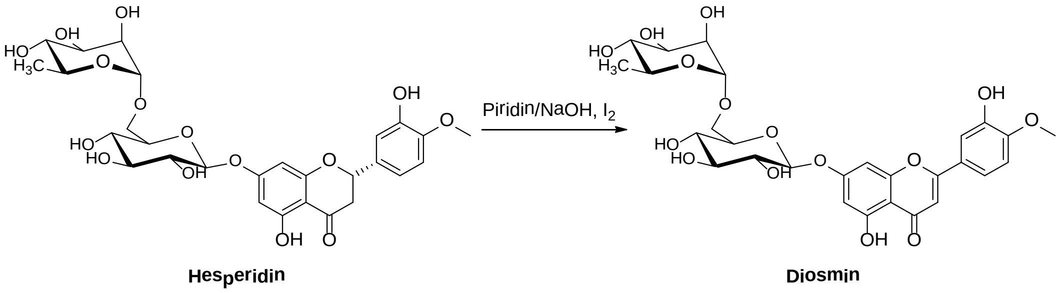


Figure 2.3. Hesperetin on TLC plate (a: 2h, b: 4h, c: 6h, d: 8h, e: 10h, f: EtOAc phase)

2.6. Synthesis of Diosmin

Hesperidin is solved in NaOH and I_2 is added. Then solution is heated up to 95-100 °C and stirred 9-10 h. After evaporation, excess I_2 is removed by adding $Na_2S_2O_3$ and 5% NaOH then pH is set 2-4, Diosmin is precipitated (Sakhardande et al., 2010; Esprimont et al.; 1998; Voigtländer et al., 1983).



100 mg hesperidin,3 mL pyridine, 9.8 mg sodium hydroxide and 45.6 mg of iodine were charged in 50 mL clean glass assembly,The resulting soluiton was heated to 95-105 C for 9-10 hours. Reaction was monitored by TLC.T he pyridine was recovered completely by distillation. Charged methanol to the resulting solid,the reaciton mass was heated to reflux and filtered at room temperature.Iodine was recovered from mother liquor solid obtained was treated with sodium thiosulfate solution and 5% aqueous NaOH solution. pH 2-4 was adjusted with cone sulfuric acid. Reaction mass was filtered to obtain crude diosmin (0.054). Yield: 54%

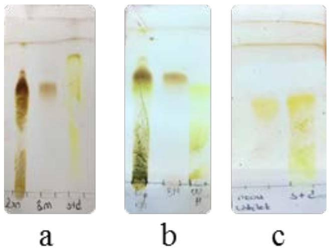
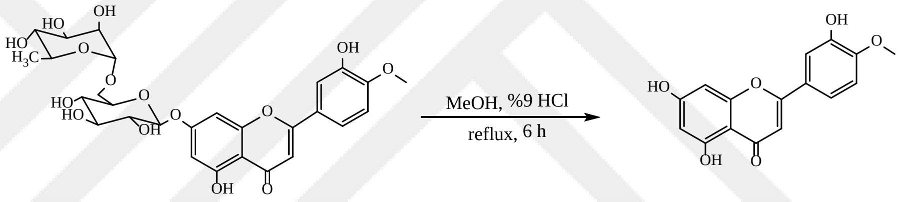


Figure 2.4. Diosmin on TLC plate(a: 2h, b: 4h, c: end of MeOH reflux)

2.7. Synthesis of Diosmetin

Diosmin is refluxed with sulphuric acid: acetic acid: water (1:30:19) solution for 12 h. After cooling the solution, pure diosmetin is obtained (Correia-Da-Silva et al., 2010; Matsuda et al., 2002).



Diosmin

Diosmetin

Diosmin (0.500 g, 0.82 mmol) was dissolved in 50 mL of MeOH and 9% HCl was added to boil under reflux for 6 h. After cooling ıt was neutralized with 5% NaOH solution. The insoluble precipitate was filter and wash with water afforded to Diosmetin (0.181g). Yield: 73%

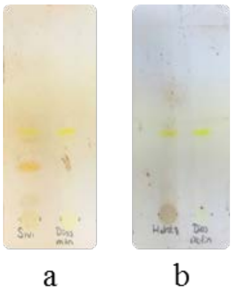
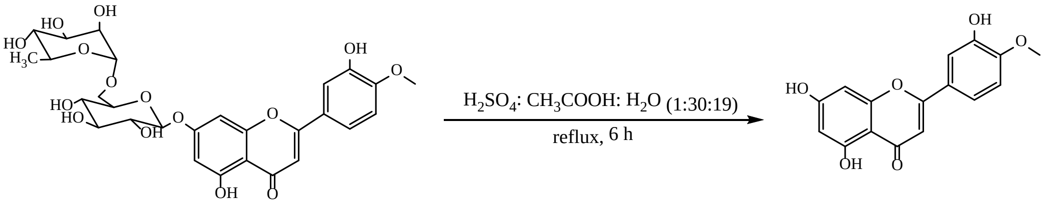


Figure 2.5. Diosmetin on TLC plate(a: 6h, b: end of precipitation)

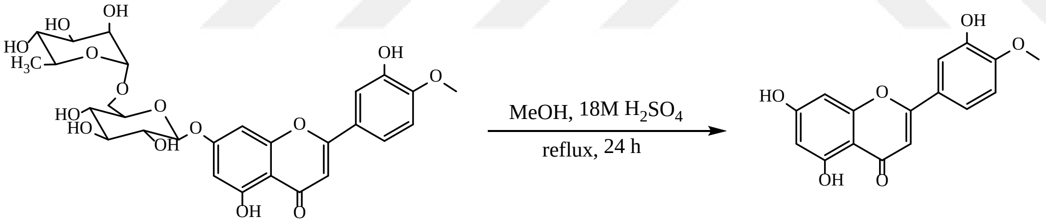


Diosmin

Diosmetin

Diosmin (0.500 g, 0.82 mmol) was dissolved in 50 mL of a mixture of H_2SO_4:CH_3COOH H_2O (1:30:19) was kept under reflux for 6 h. After cooling ıt was neutralized with 5% NaOH solution. The insoluble precipitate was filter and wash with water afforded to Diosmetin (0.190g). Yield: 76%

Figure 2.6. Diosmetin on TLC plate(a: 2h, b: 6h)



Diosmin

Diosmetin

Diosmin (0.500 g, 0.82 mmol) was dissolved in 30 mL of MeOH and 1 mL 18 M H_2SO_4 was added to boil under reflux for 24 hour. After cooling it was neutralized with 5% NaOH solution. The reaction mixture extracted with water (50 mL) and AcOEt (2x50 mL). The organic layers were collected. The solvent was removed by evaporation under reduced pressure. Afforded Diosmetin (0.038g). Yield: 15%

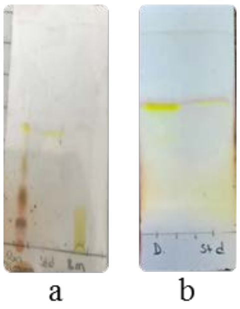
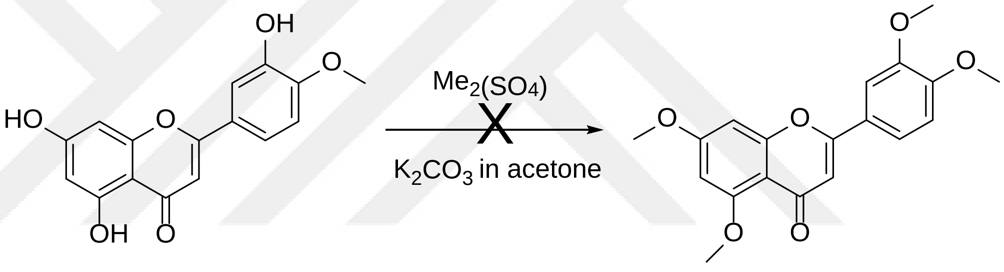


Figure 2.7. Diosmetin on TLC plate(a: 24h, b: EtOAc phase)

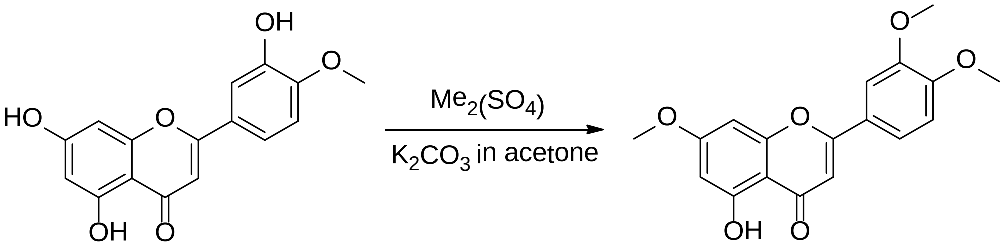
2.8. Fully Methylation of Diosmetin

K_2CO_3 is added into suspension of diosmetin in acetone then the solution is refluxed for 24 h. After cooling solution, water and CH_2Cl_2 are added then removing of organic solvent is resulted in compound **2** (0,498g). Yield: 43%



Diosmetin

1



Diosmetin

2

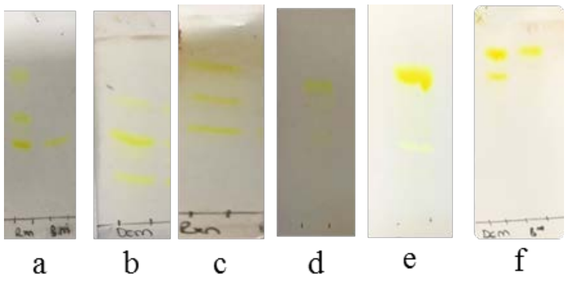
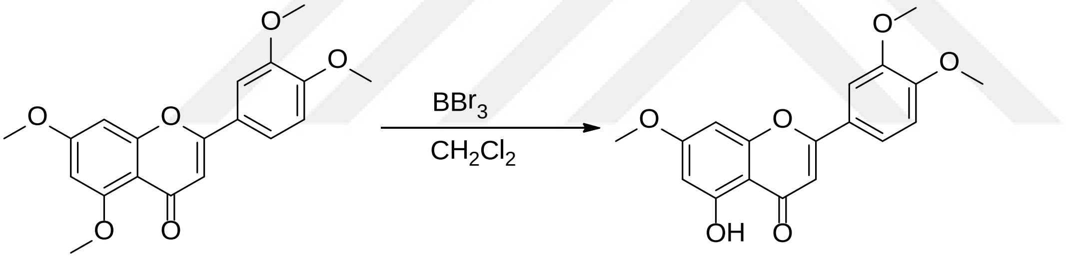


Figure 2.8. Compound **2** on TLC plate (a: 6h, b: 8h, c: 10h, d: 12h, e: 16h, f: DCM phase)

2.9. Synthesis of Compound 2

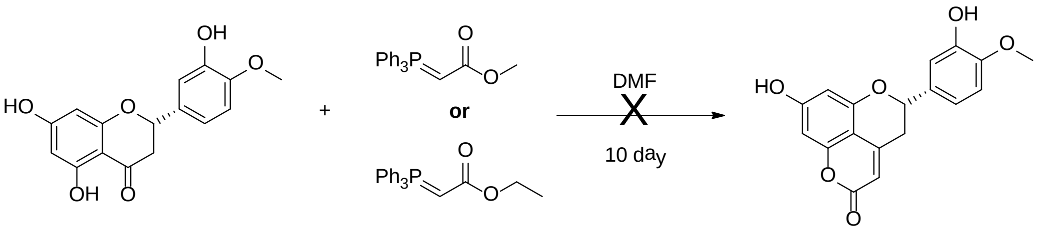
Compound **1** is solved in CH_2Cl_2 then 15 equivalent 1 M BBr_3 are added at 0 °C and stirred for 24 h. After 24 h, solution is cooled 0 °C and methanol is added and compound **2** is precipitated. The further purifications of compound **2** are made by adding water then extraction with ethyl acetate (Coowar et al., 2010).



1 **2**

2.10. Synthesis of Compound 3-5

Hesperetin, diosmetin and compound **2** are solved in DMF separately and phosphoranes that are suitable for ring formation- carbmethoxy methylene triphenylphosphorane or carbethoxy methylene triphenylphosphorane- are added at 90-100 °C. After evaporation, pure compounds are obtained from column chromatography (Bissel et al., 1999; Gallagher et al., 2009).



Hesperetin **3**

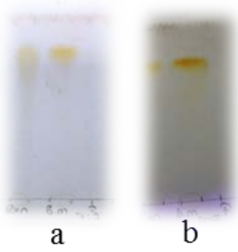


Figure 2.9. Compound **3** on TLC plate(a: first day, b: last day)

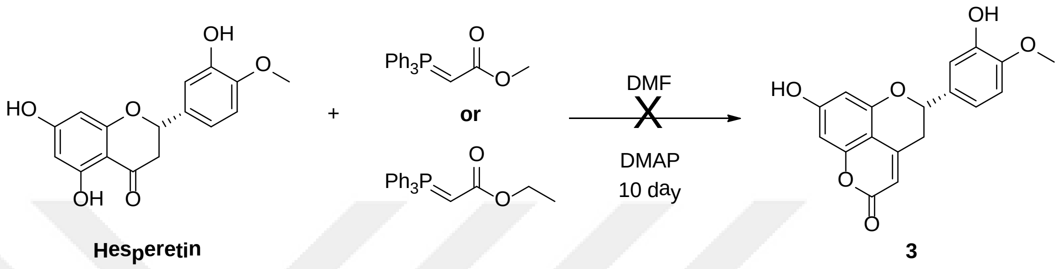
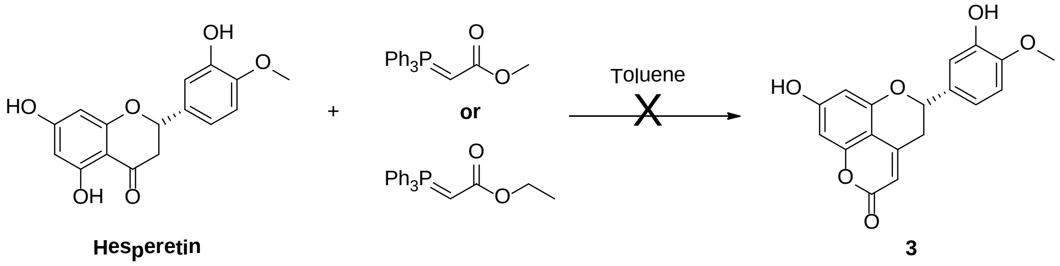


Figure 2.10. Compound **3** on TLC plate



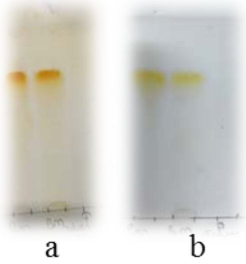


Figure 2.11. Compound **3** on TLC plate (a: first day, b: last day)

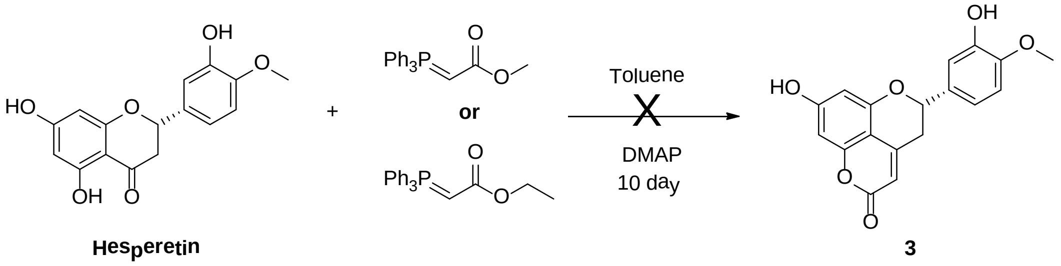


Figure 2.12. Compound 3 on TLC plate

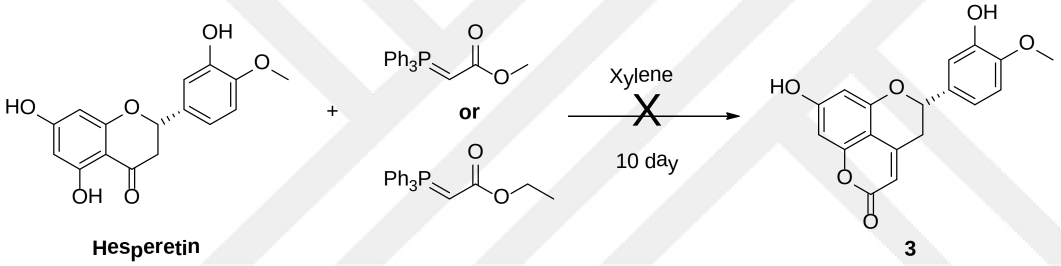
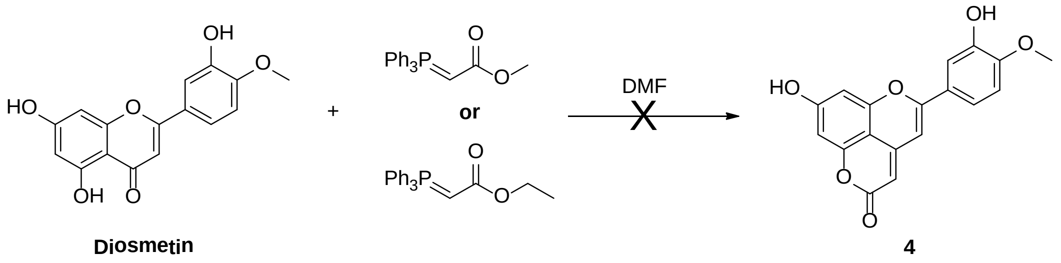
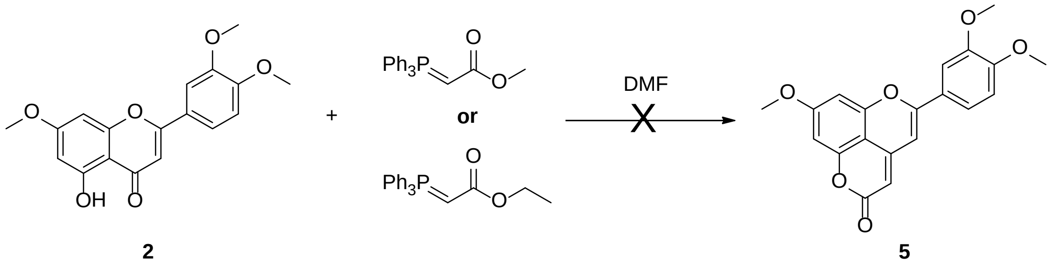


Figure 2.13. Compound **3** on TLC plate




OH
O
+
Ph3P
or
DMF
2
5

3. RESULTS AND DISCUSSION

3.1. Isolation and Structural Identification of Hesperidin

3.1.1. The extraction conditions of Orange peels

Orange peels were extracted with different parameters such as temperature, solvent type, solvent ratio and time.

Table 3.1. Extraction Yields of orange peel with different parameters

	Material	**Solvent**	**Temperature**	**Time**	**Peel:Solvent Ratio**	**Yield %**
1.	Orange peels	*n*-Hexane	r.t.	2 hours	1:5	0,35
2.	Orange peels	*n*-Hexane	r.t.	2 hours	1:10	0,19
3.	Orange peels	*n*-Hexane	r.t.	4 hours	1:5	0,28
4.	Orange peels	*n*-Hexane	r.t.	4 hours	1:10	0,27
5.	Orange peels	*n*-Hexane	r.t.	6 hours	1:5	0,38
6.	Orange peels	*n*-Hexane	r.t.	6 hours	1:10	0,37
7.	Orange peels	*n*-Hexane	40 °C	2 hours	1:5	0,31
8.	Orange peels	*n*-Hexane	40 °C	2 hours	1:10	0,36
9.	Orange peels	*n*-Hexane	40 °C	4 hours	1:5	0,39
10.	Orange peels	*n*-Hexane	40 °C	4 hours	1:10	0,49
11.	Orange peels	*n*-Hexane	40 °C	6 hours	1:5	0,41
12.	Orange peels	*n*-Hexane	40 °C	6 hours	1:10	0,51
13.	**Residue of Extr. 1**	EtOH	r.t.	2 hours	1:5	3,07
14.	**Residue of Extr. 2**	EtOH	r.t.	2 hours	1:10	1,19
15.	**Residue of Extr. 3**	EtOH	r.t.	4 hours	1:5	1,85
16.	**Residue of Extr. 4**	EtOH	r.t.	4 hours	1:10	1,91
17.	**Residue of Extr. 5**	EtOH	r.t.	6 hours	1:5	2,25
18.	**Residue of Extr. 6**	EtOH	r.t.	6 hours	1:10	2,07
19.	**Residue of Extr. 7**	EtOH	40 °C	2 hours	1:5	3,96
20.	**Residue of Extr. 8**	EtOH	40 °C	2 hours	1:10	3,77
21.	**Residue of Extr. 9**	EtOH	40 °C	4 hours	1:5	4,02
22.	**Residue of Extr. 10**	EtOH	40 °C	4 hours	1:10	5,12
23.	**Residue of Extr. 11**	EtOH	40 °C	6 hours	1:5	5,89

24.	**Residue of Extr. 12**	EtOH	40 °C	6 hours	1:10	4,82
25.	Orange peels	MeOH	r.t.	2 hours	1:5	4,32
26.	Orange peels	MeOH	r.t.	2 hours	1:10	6,38
27.	Orange peels	MeOH	r.t.	4 hours	1:5	5,38
28.	Orange peels	MeOH	r.t.	4 hours	1:10	8,01
29.	Orange peels	MeOH	r.t.	6 hours	1:5	6,03
30.	Orange peels	MeOH	r.t.	6 hours	1:10	12,04
31.	Orange peels	MeOH	40 °C	2 hours	1:5	11,23
32.	Orange peels	MeOH	40 °C	2 hours	1:10	10,04
33.	Orange peels	MeOH	40 °C	4 hours	1:5	11,19
34.	Orange peels	MeOH	40 °C	4 hours	1:10	14,80
35.	Orange peels	MeOH	40 °C	6 hours	1:5	13,01
36.	Orange peels	MeOH	40 °C	6 hours	1:10	13,04
37.	Orange peels	EtOH	r.t.	2 hours	1:5	3,67
38.	Orange peels	EtOH	r.t.	2 hours	1:10	5,33
39.	Orange peels	EtOH	r.t.	4 hours	1:5	3,63
40.	Orange peels	EtOH	r.t.	4 hours	1:10	6,67
41.	Orange peels	EtOH	r.t.	6 hours	1:5	5,93
42.	Orange peels	EtOH	r.t.	6 hours	1:10	5,88
43.	Orange peels	EtOH	40 °C	2 hours	1:5	9,75
44.	Orange peels	EtOH	40 °C	2 hours	1:10	11,44
45.	Orange peels	EtOH	40 °C	4 hours	1:5	8,17
46.	Orange peels	EtOH	40 °C	4 hours	1:10	11,59
47.	Orange peels	EtOH	40 °C	6 hours	1:5	7,82
48.	Orange peels	EtOH	40 °C	6 hours	1:10	10,50
49.	Orange peels	EtOH:H_2O (80:20)	r.t.	2 hours	1:5	7,50
50.	Orange peels	EtOH:H_2O (80:20)	r.t.	2 hours	1:10	16,72
51.	Orange peels	EtOH:H_2O (80:20)	r.t.	4 hours	1:5	17,30
52.	Orange peels	EtOH:H_2O (80:20)	r.t.	4 hours	1:10	16,14
53.	Orange peels	EtOH:H_2O (80:20)	r.t.	6 hours	1:5	12,48
54.	Orange peels	EtOH:H_2O (80:20)	r.t.	6 hours	1:10	16,03
55.	Orange peels	EtOH:H_2O (80:20)	40 °C	2 hours	1:5	13,13
56.	Orange peels	EtOH:H_2O (80:20)	40 °C	2 hours	1:10	15,84
57.	Orange peels	EtOH:H_2O (80:20)	40 °C	4 hours	1:5	14,05

58.	Orange peels	EtOH:H_2O (80:20)	40 °C	4 hours	1:10	17,40
59.	Orange peels	EtOH:H_2O (80:20)	40 °C	6 hours	1:5	11,40
60.	Orange peels	EtOH:H_2O (80:20)	40 °C	6 hours	1:10	16,51

3.1.2. HPLC Analysis of Orange Peels Extract

Hesperidin reach extract was subjected into Thermo Finnigan Surveyor HPLC with UV-detector (280 nm), Cosmosil C18 250:4.6:5 µm. The results showed that ethanol:water mixture (80:20) is the best solvent for hesperidine isolation (Figure 3.1-3.4).

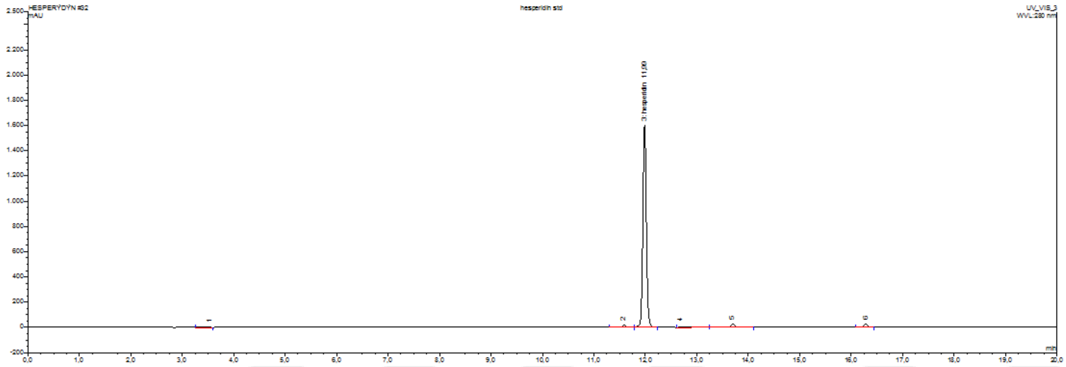


Figure 3.1. HPLC Chromatogram of Hesperidine

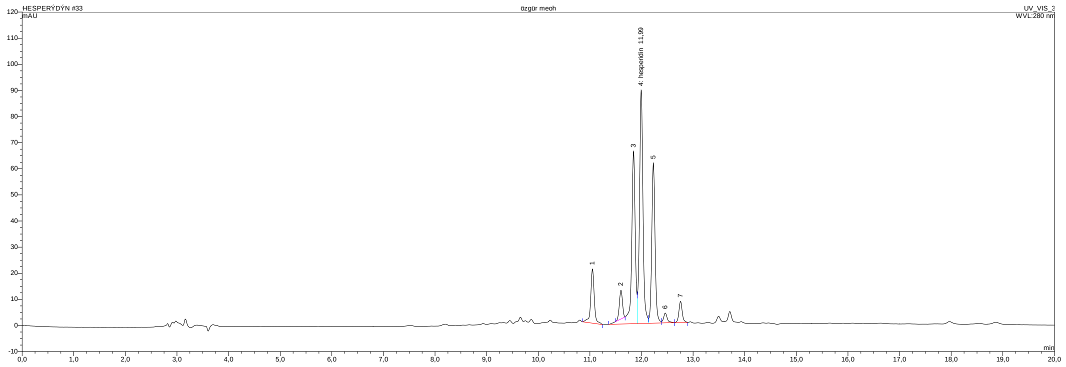


Figure 3.2. HPLC Chromatogram of Methanol Extract

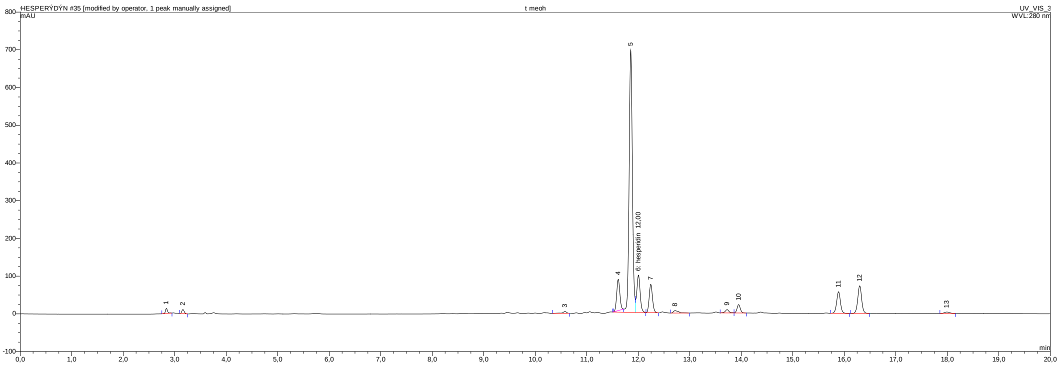


Figure 3.3. HPLC Chromatogram of Ethanol Extract

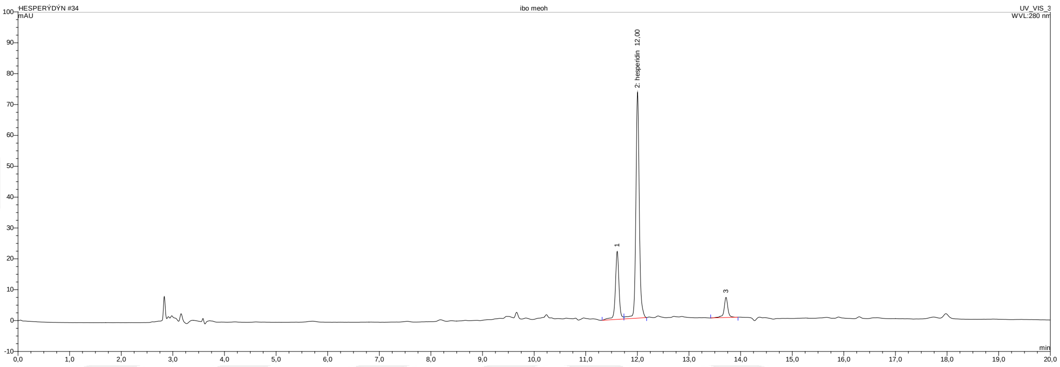


Figure 3.4. HPLC Chromatogram of Ethanol:Water (80:20) Extract

According to the extraction results and HPLC analysis, the optimum extraction conditions were found with ethanol: water (80:20) solvent system, 1:10 peel: solvent ratio and 4 hours extraction at 40 °C.

3.1.3. Structural Identification of Hesperidin

The structure of hesperidin was elucidated by ^{1}H-NMR, ^{13}C-NMR and FT-IR (Table 3.2, Spectrum 3.1-3.2-3.3). On the basis of ^{1}H-NMR and ^{13}C-NMR spectral data and the other physical properties the isolated was identified and established as hesperidin (IUPAC name is (2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-3,4-dihydro-2H-chromen-7-yl-6-*O*-(6-deoxy-α-L-mannopyranosyl)-*β*-D-glucopyranoside) ($C_{28}H_{34}O_{15}$) (Figure 3.5).

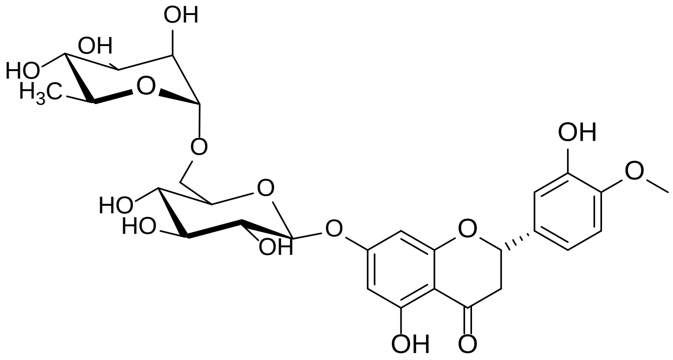
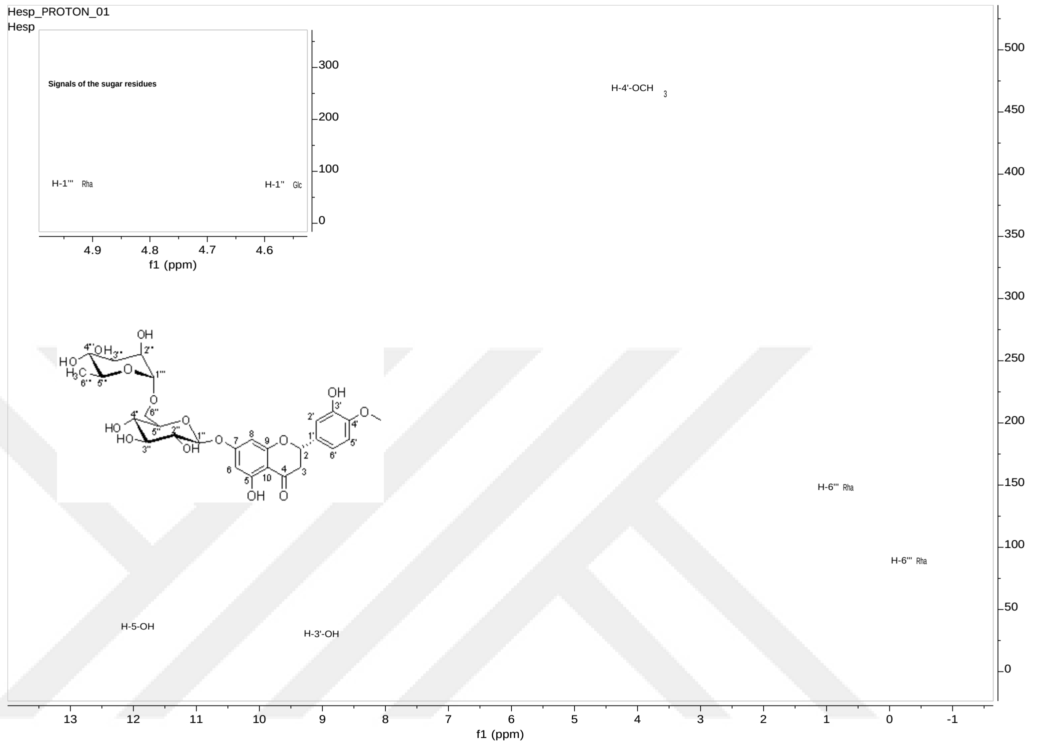


Figure 3.5. The Structure of Hesperidin

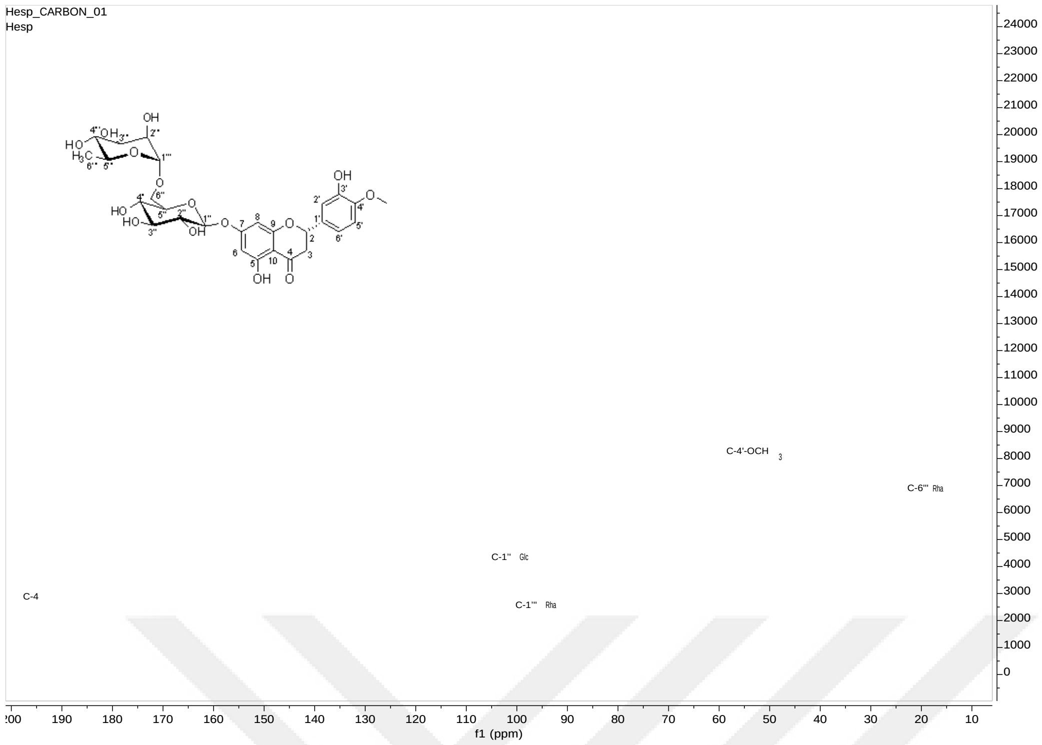
Table 3.2. ^{1}H- and ^{13}C-NMR Assignments of Hesperidin (400/100 MHz, in DMSO)

Position	δ_C	**δ_H (*J* in Hz)**
2	78,8	5,40; dd (11,0; 5,0)
3	103,8	3,08; 2,82 dd (17,0:11,0:5,0)
4	197,42	-
5	163,5	-
6	101,0	6,11; s
7	165,57	-
8	96,8	6,13; s
9	162,0	-
10	103,8	-
1'	131,4	-
2'	114,5	6,93; s
3'	146,9	-
4'	148,9	-
5'	112,6	6,91; d (8,6)
6'	118,4	6,89; d (8,6)
4'-OCH_3	56,1	3,73
1''	101,0	4,54; d (7,5)
2''	73,4	4,19 dd (7,5; 9,0)
3''	76,7	3,38-3,50 dd (9,0; 9,0)
4''	71,2	3,38-3,50 dd (9,0; 9,0)
5''	75,9	3,38-3,50 m
6''	68,7	3,72; 3,89
1'''	99,7	4,97; d (1,5)
2'''	70,0	3,38-3,50 dd (9,0; 9,0)
3'''	70,7	3,38-3,50 dd (9,0; 9,0)
4'''	72,5	3,38-3,50 m

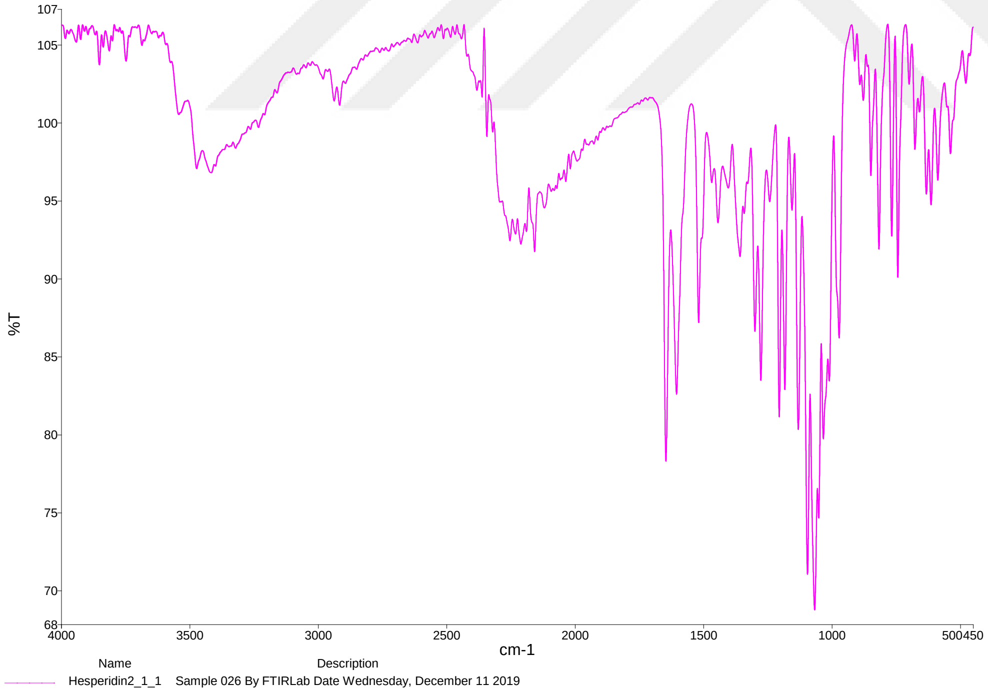
5'''	68,8	3,72; 3,89
6'''	18,3	1,09



Spectrum 3.1. ^{1}H-NMR spectrum of **Hesperidin** (400 MHz, DMSO)



Spectrum 3.2. ^{13}C-NMR spectrum of **Hesperidin** (100 MHz, DMSO)



Spectrum 3.3.FT-IR spectrum of **Hesperidin**

3.2. Synthesis and Structural identification of Hesperetin

3.2.1. The Hydrolysis Conditions of Hesperidin

Hesperetin was obtained from hydrolysis of hesperidin in different conditions such as acid type, solvent systems and time. Based on the experimental results, optimum hydrolysis conditions were selected. According to the hydrolysis results, the optimum hydrolysis conditions were found with methanol, hydrochloric acid (9%) and 6 hours (Table 3.3).

Table 3.3. Hydrolysis Yields of hesperidin with different conditions

	Solvent	Acid	Time	Yield %
1.	**MeOH**	**HCl**	**2 hours**	**6,38**
2.	**MeOH**	**H_2SO_4**	**2 hours**	**6,38**
3.	MeOH	HCl	4 hours	4,32
4.	MeOH	H_2SO_4	4 hours	6,38
5.	MeOH	HCl	6 hours	4,32
6.	MeOH	H_2SO_4	6 hours	6,38
7.	MeOH	HCl	8 hours	4,32
8.	MeOH	H_2SO_4	8 hours	6,38
9.	MeOH	HCl	10 hours	4,32
10.	MeOH	H_2SO_4	10 hours	6,38
11.	MeOH:Toluen	HCl	2 hours	11,23
12.	MeOH:Toluen	H_2SO_4	2 hours	11,23
13.	MeOH:Toluen	HCl	4 hours	11,23
14.	MeOH:Toluen	H_2SO_4	4 hours	11,23
15.	MeOH:Toluen	HCl	6 hours	11,23
16.	MeOH:Toluen	H_2SO_4	6 hours	11,23
17.	MeOH:Toluen	HCl	8 hours	11,23
18.	MeOH:Toluen	H_2SO_4	8 hours	11,23
19.	MeOH:Toluen	HCl	10 hours	11,23
20.	MeOH:Toluen	H_2SO_4	10 hours	11,23

3.2.2. Structural Identification of Hesperetin

The structure of **Hesperetin** was elucidated by ^{1}H-NMR, ^{13}C-NMR and FT-IR (Table 3.4, Spectrum 3.4-3.6). On the basis of ^{1}H-NMR and ^{13}C-NMR spectral data and the other physical properties the isolated was identified and established as hesperetin (IUPAC name is (2S)-5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one) ($C_{16}H_{14}O_6$) (Figure 3.6).

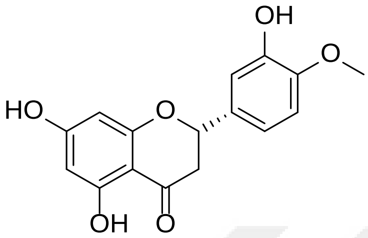
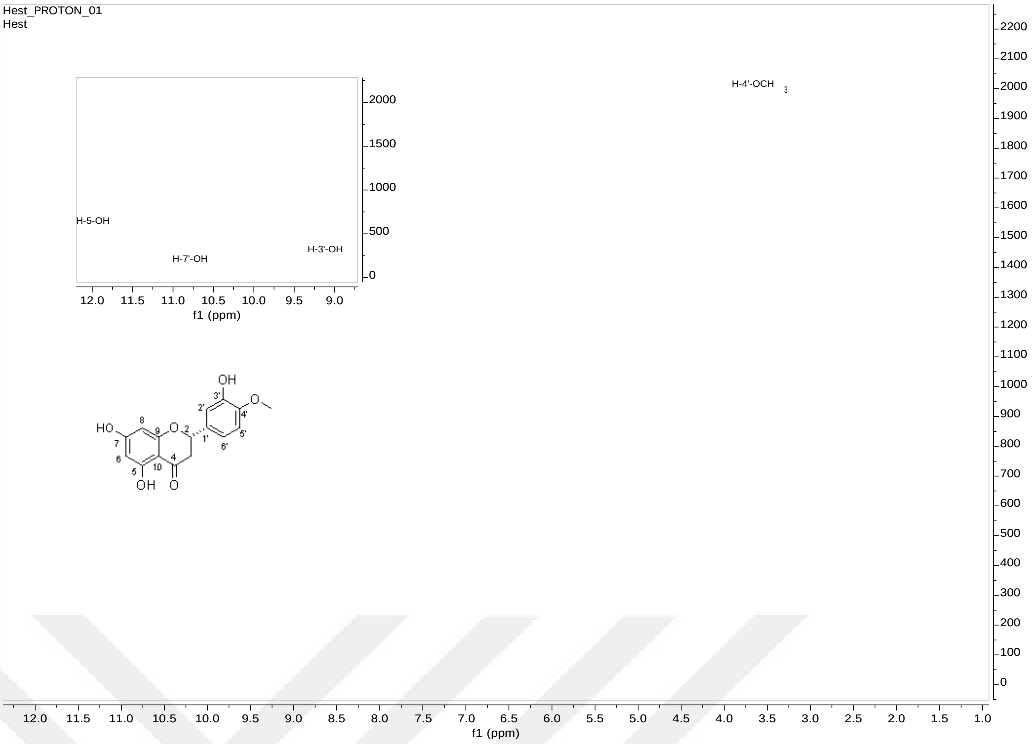


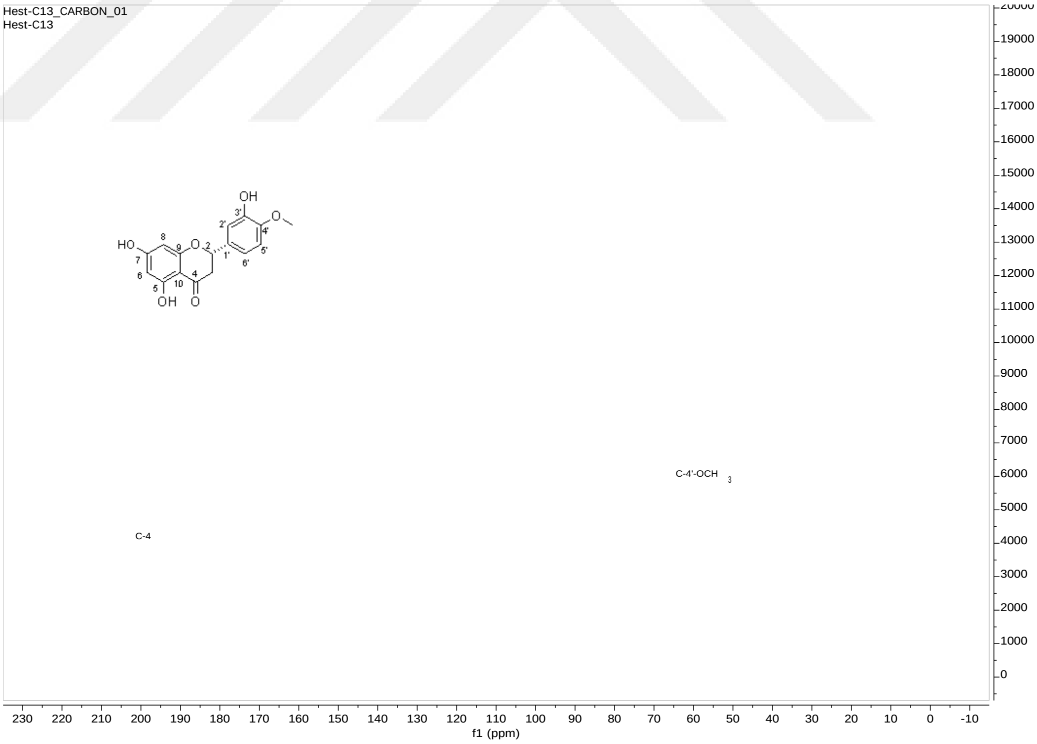
Figure 3.6. The Structure of Hesperetin

Table 3.4. ^{1}H- and ^{13}C-NMR Assignments of Hesperetin (400/100 MHz, in DMSO)

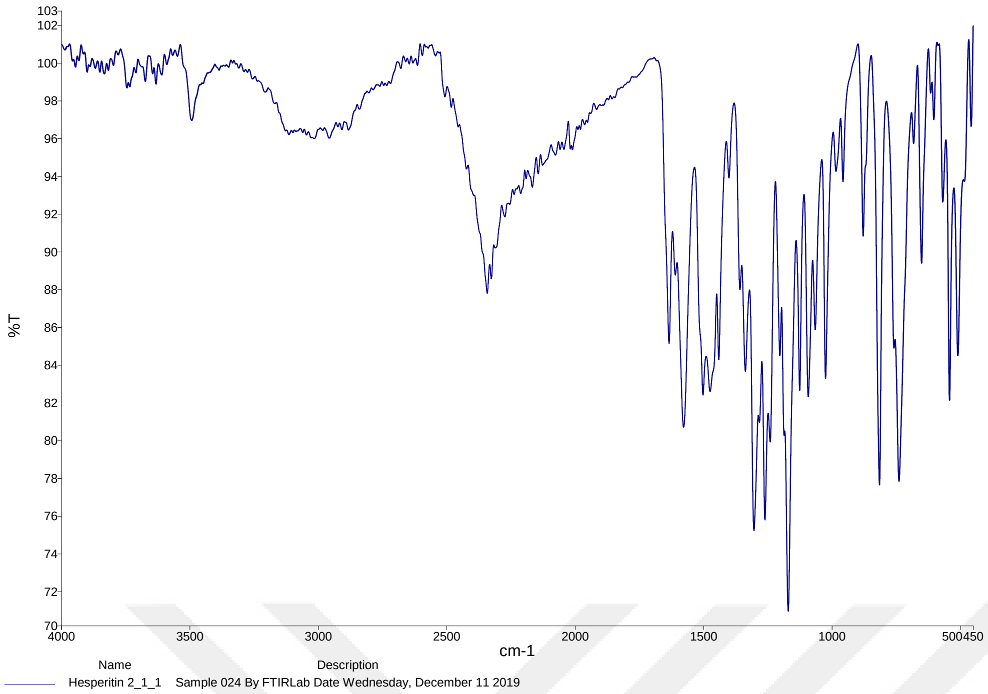
Position	**δ_C**	**δ_H (*J* in Hz)**
2	78.8	5.40; dd (11.0; 5.0)
3	103.8	3.08; 2.82 dd (17.0:11.0:5.0)
4	197.42	-
5	163.5	-
6	101.0	6.11; s
7	165.57	-
8	96.8	6.13; s
9	162.0	-
10	103.8	-
1'	131.4	-
2'	114.5	6.93; s
3'	146.9	-
4'	148.9	-
5'	112.6	6.91; d (8.6)
6'	118.4	6.89; d (8.6)
4'-OCH_3	56.1	3.73



Spectrum 3.4. ^{1}H-NMR spectrum of **Hesperetin** (400 MHz, DMSO)



Spectrum 3.5. ^{13}C-NMR spectrum of **Hesperetin** (100 MHz, DMSO)



Spectrum 3.6. FT-IR spectrum of **Hesperetin**

3.3. Structural Identification of Diosmin

The structure of diosmin was elucidated by ^{1}H-NMR, ^{13}C-NMR and FT-IR (Table 3.5, Spectrum 3.7-3.9). On the basis of ^{1}H-NMR and ^{13}C-NMR spectral data and the other physical properties the isolated was identified and established as diosmin (IUPAC name is 5-Hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-oxo-4H-chromen-7-yl6-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside ($C_{28}H_{32}O_{15}$) (Figure 3.7).

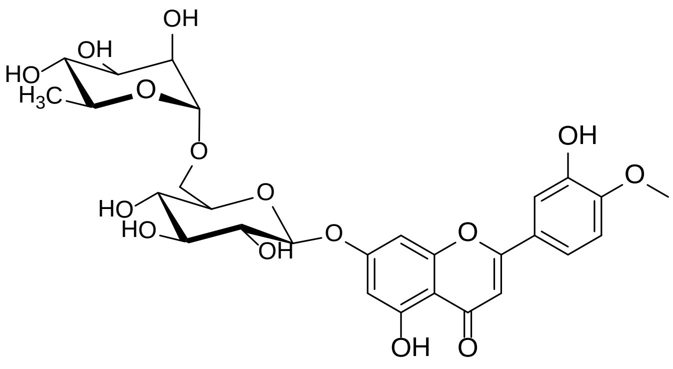
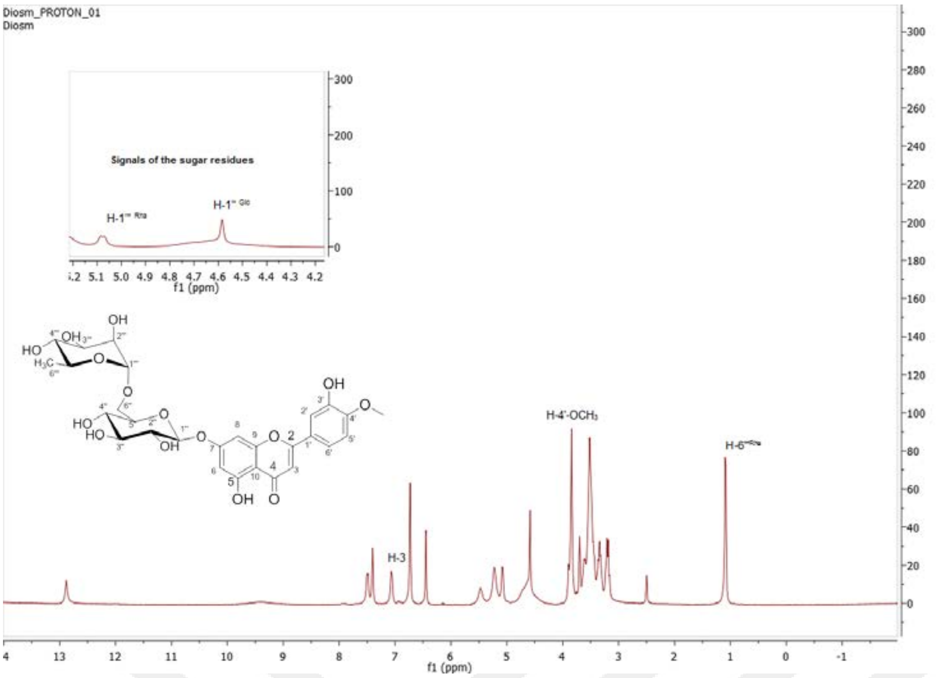


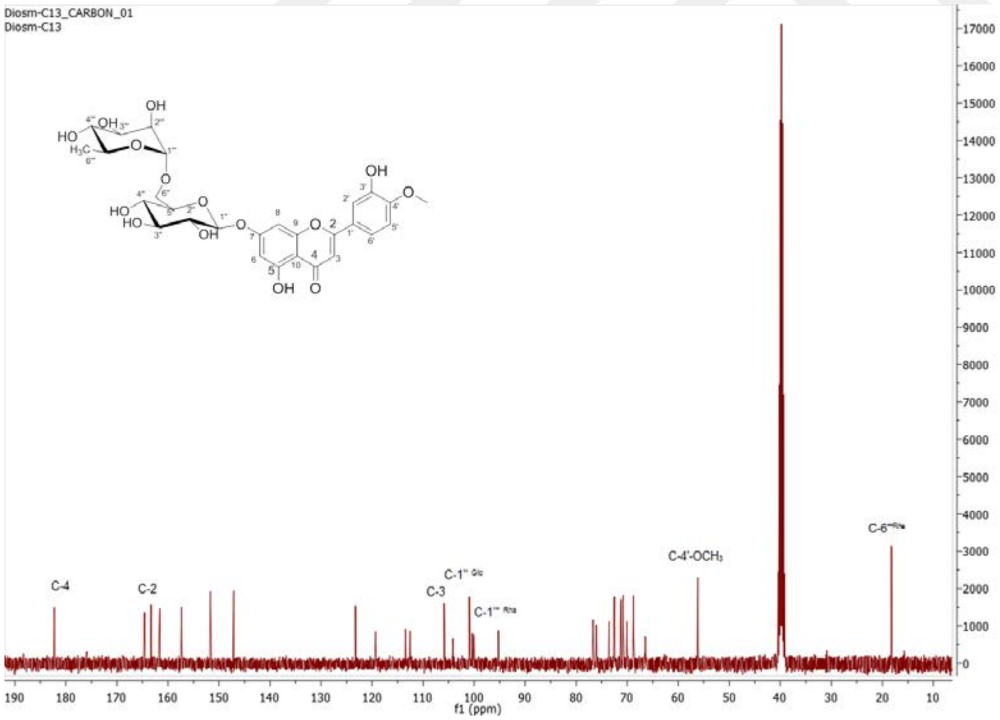
Figure 3.7. The Structure of Diosmin

Table 3.5. ^{1}H- and ^{13}C-NMR Assignments of Diosmin (400/100 MHz, in DMSO)

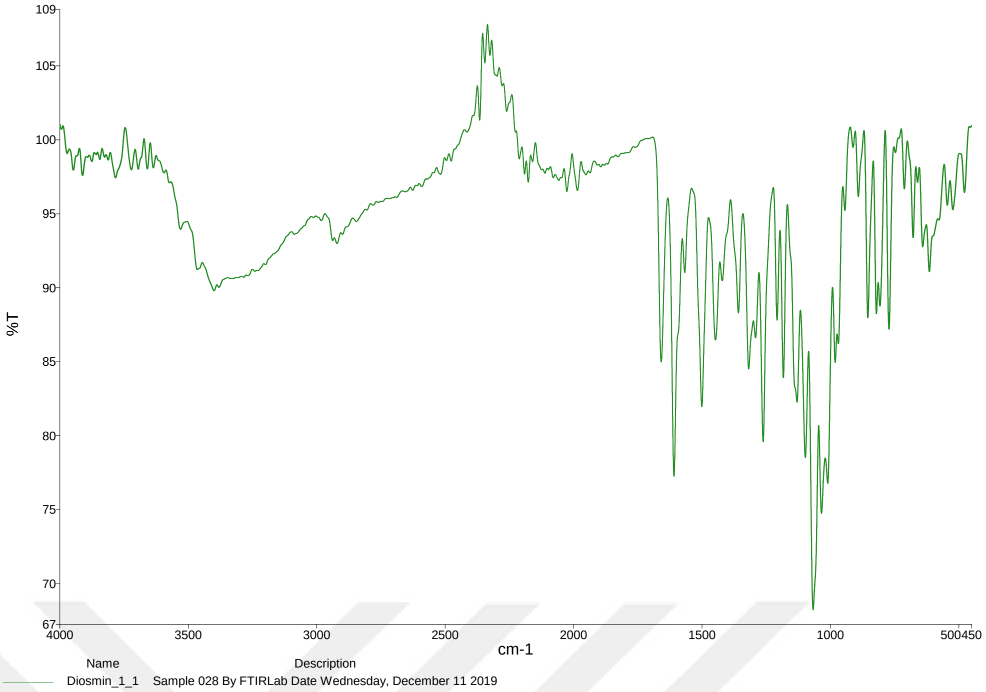
Position	δ_C	δ_H **(*J* in Hz)**
2	164.6	-
3	105.8	7.01; s
4	182.3	-
5	161.6	-
6	100.0	6.43; s
7	163.3	-
8	95.2	6.72; s
9	157.3	-
10	105.6	-
1'	123.2	-
2'	113.5	7.40; s
3'	147.1	-
4'	151.7	-
5'	112.5	7.06; d (8.6)
6'	119.3	7.46; d (8.6)
4'-OCH_3	56.6	3.88
1"	100.9	5.07; d (7.5)
2"	73.5	4.19 dd (7.5; 9.0)
3"	76.7	3.38-3,50 dd (9.0; 9.0)
4"	71.2	3.38-3.50 dd (9.0; 9.0)
5"	76.0	3.38-3.50 m
6"	68.7	3.72; 3.89
1'''	100.4	4.57; d (1.5)
2'''	70.0	3.38-3.50 dd (9.0; 9.0)
3'''	70.0	3.38-3.50 dd (9.0; 9.0)
4'''	72.5	3.38-3.50 m
5'''	68.8	3.72; 3.89
6'''	18.2	1.08



Spectrum 3.7. ^{1}H-NMR spectrum of **Diosmin** (400 MHz, DMSO)



Spectrum 3.8. ^{13}C-NMR spectrum of **Diosmin** (100 MHz, DMSO)



Spectrum 3.9. FT-IR spectrum of **Diosmin**

3.4. Structural Identification of Diosmetin

The structure of diosmetin was elucidated by ^{1}H-NMR, ^{13}C-NMR and FT-IR (Table 3.6, Spectrum 3.10-3.12). On the basis of ^{1}H-NMR and ^{13}C-NMR spectral data and the other physical properties the isolated was identified and established as diosmetin (IUPAC name is 5,7-Dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4H-chromen-4-one. ($C_{16}H_{12}O_6$) (Figure 3.8).

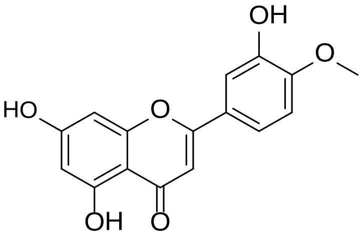
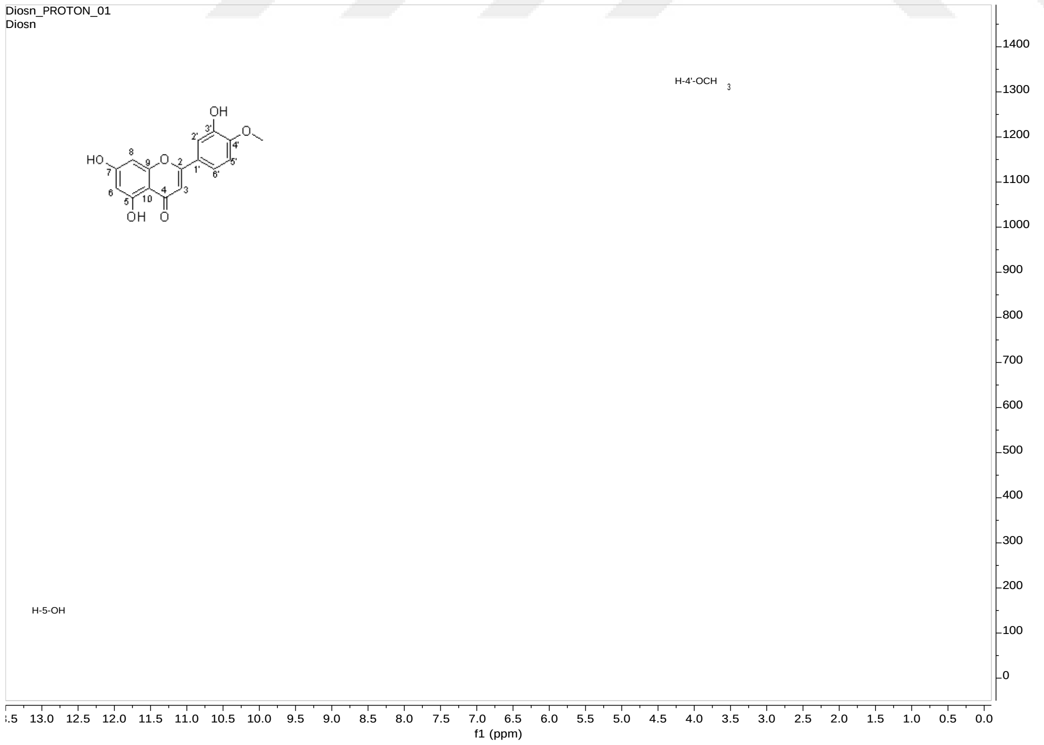


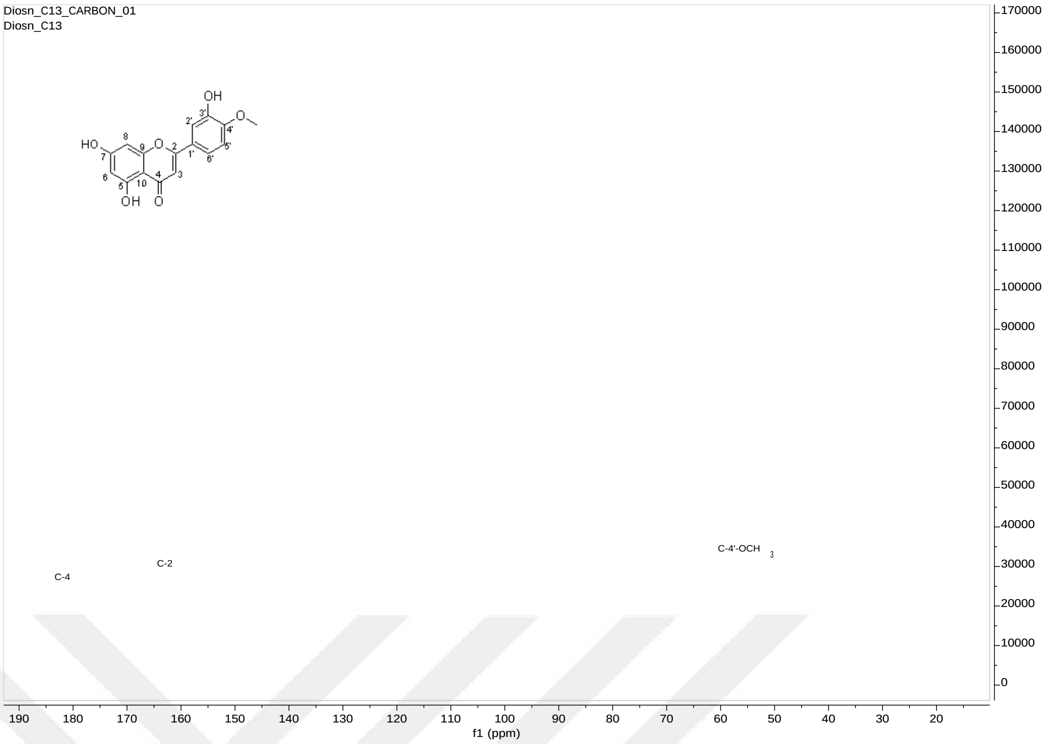
Figure 3.8. The Structure of Diosmetin

Table 3.6. ^{1}H- and ^{13}C-NMR Assignments of Diosmetin (400/100 MHz, in DMSO)

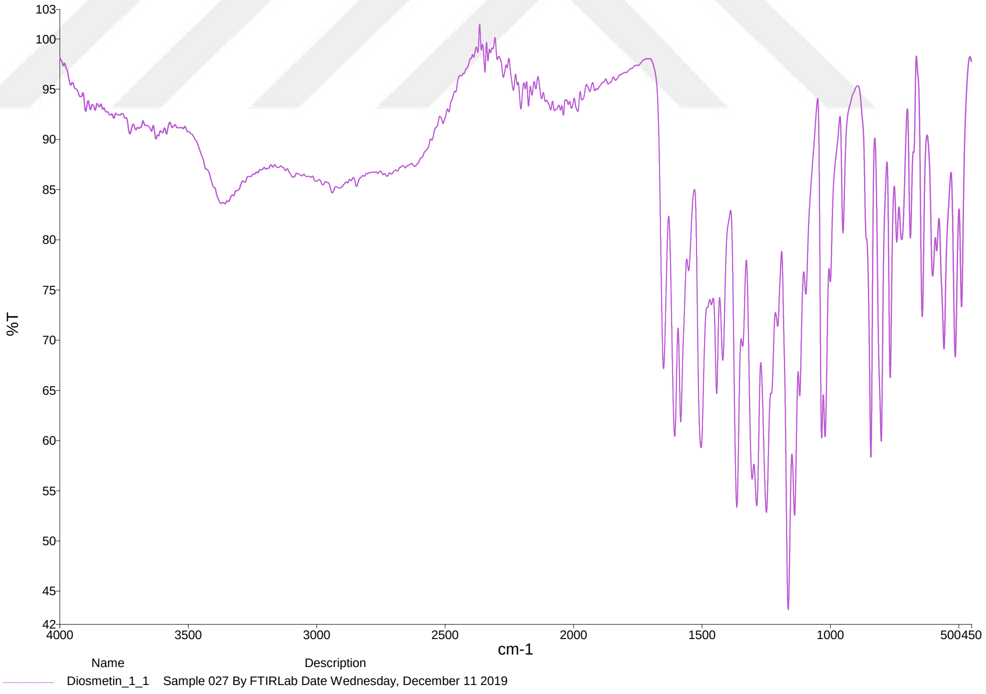
Position	**δ_C**	**δ_H (*J* in Hz)**
2	164.6	-
3	104.0	6.88; s
4	182.0	-
5	161.9	-
6	99.4	6.42; s
7	163.9	-
8	94.1	6.76; s
9	157.7	-
10	105.6	-
1'	123.5	-
2'	113.4	7.38; s
3'	147.0	-
4'	151.5	-
5'	112.0	7.03; d (8.6)
6'	118.7	7.48; d (8.6)
4'-OCH$_3$	56.1	3.83



Spectrum 3.10. ^{1}H-NMR spectrum of **Diosmetin** (400 MHz, DMSO)



Spectrum 3.11. ^{13}C-NMR spectrum of **Diosmetin** (100 MHz, DMSO)



Spectrum 3.12. FT-IR spectrum of **Diosmetin**

3.5. Structural identification of Compound 2

The structure of compound **2** was elucidated by ^{1}H-NMR, ^{13}C-NMR and FT-IR (Table 3.7, Spectrum 3.13-3.14-3.15). On the basis of ^{1}H-NMR and ^{13}C-NMR spectral data and the other physical properties the isolated was identified and established as 5-hydroxy-7-methoxy-2-(3,4-dimethoxyphenyl)-4H-chromen-4-one ($C_{18}H_{16}O_6$) (Figure 3.9).

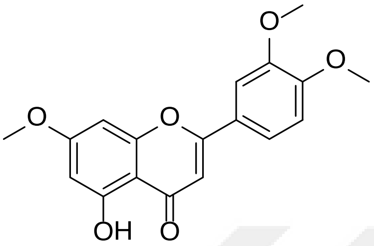
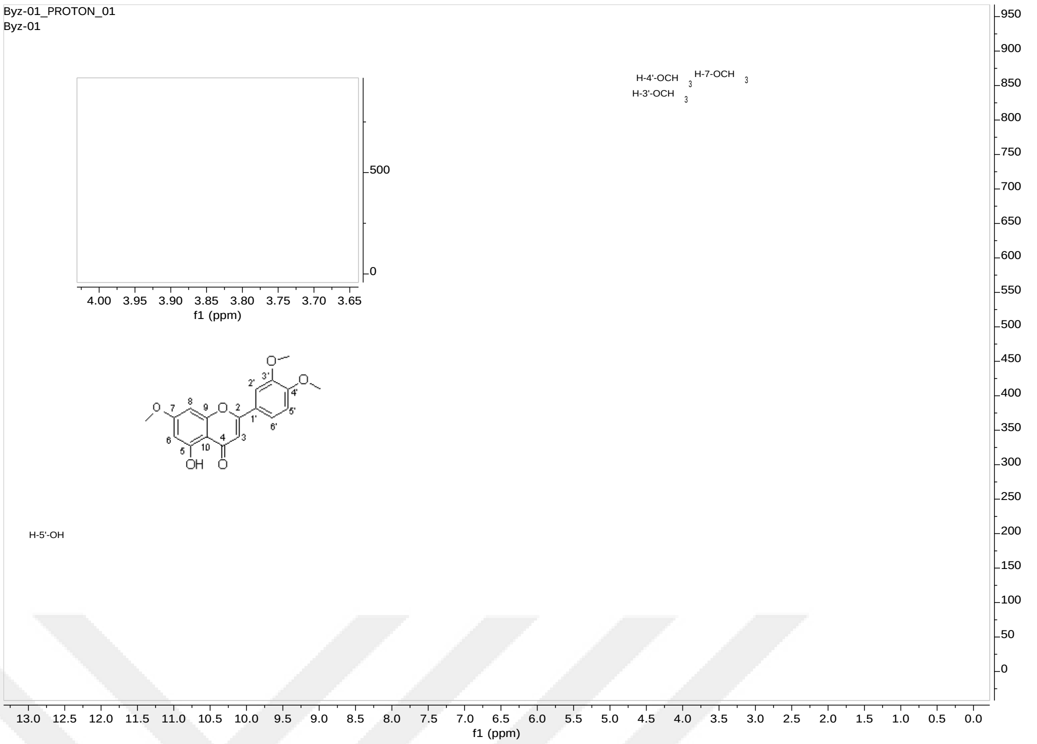


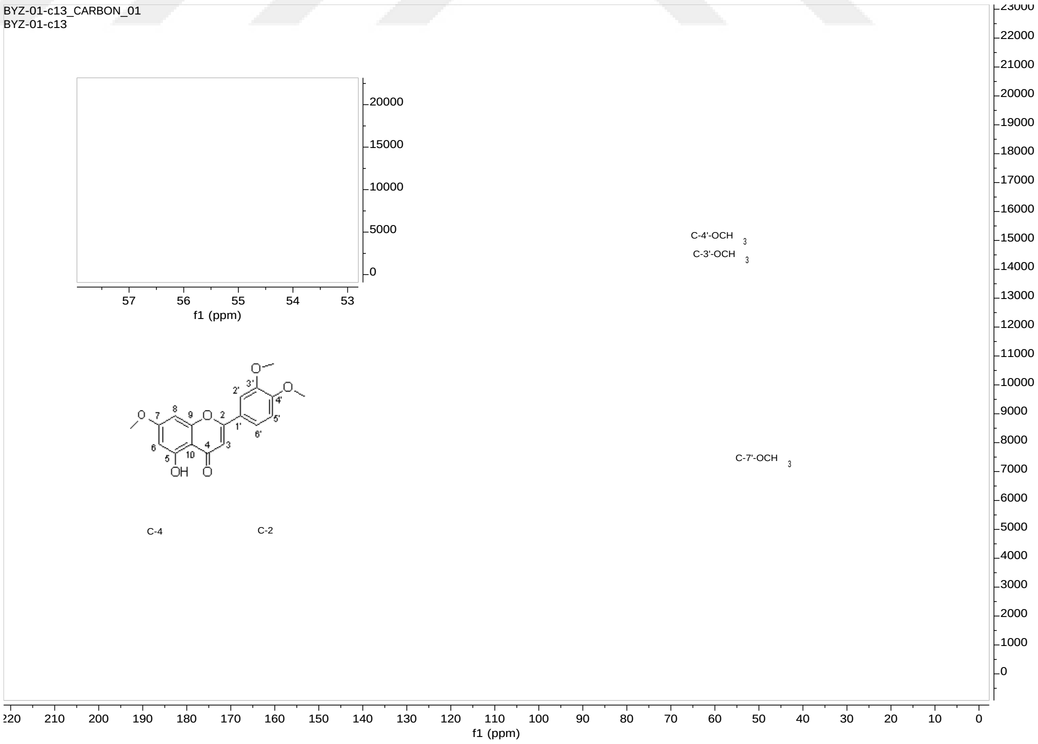
Figure 3.9. The Structure of Compound **2**

Table 3.7. ^{1}H- and ^{13}C-NMR Assignments of compound **2** (400/100 MHz, in CDCl3)

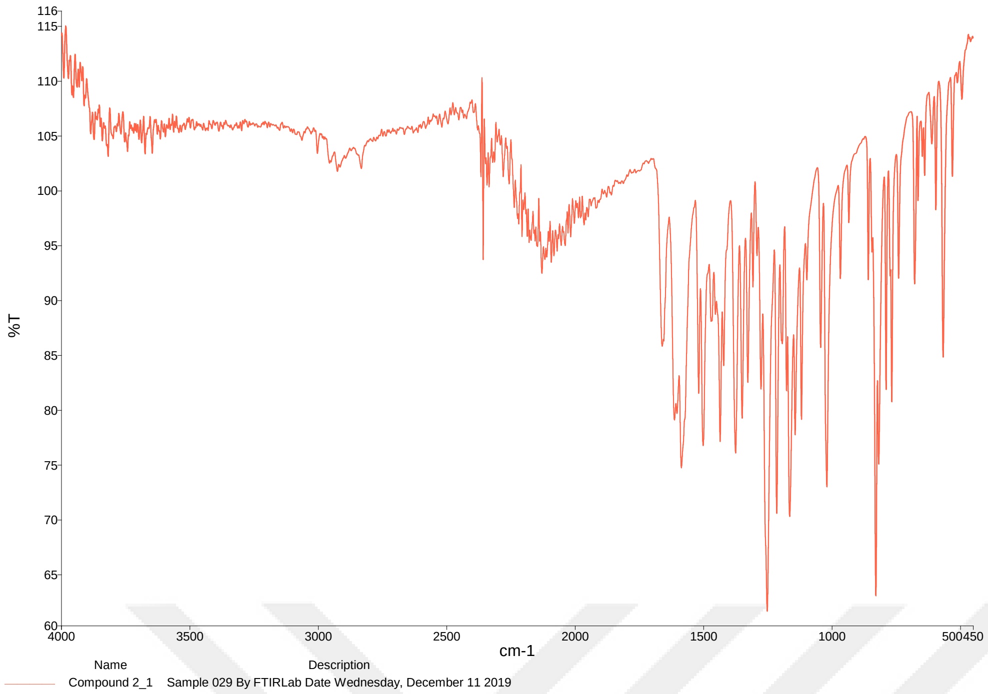
Position	δ_C	δ_H (*J* in Hz)
2	165.3	-
3	104.4	6.50; s
4	182.1	-
5	162.0	-
6	97.9	6.29; s
7	163.7	-
8	92.4	6.41; s
9	157.5	-
10	105.3	-
1'	123.5	-
2'	111.0	7.26; s
3'	149.1	-
4'	152.1	-
5'	108.6	7.03; d (8.6)
6'	119.9	7.48; d (8.6)
3'-OCH_3	55.9	3.91
4'-OCH_3	55.9	3.91
7'-OCH_3	55.6	3.82



Spectrum 3.13. ^{1}H-NMR spectrum of **Compound 2** (400 MHz, $CDCl_3$)



Spectrum 3.14. ^{13}C-NMR spectrum of **Compound 2** (100 MHz, $CDCl_3$)



Spectrum 3.15. FT-IR spectrum of **Compound 2**

4. CONCLUCIONS

In this thesis, isolation of hesperidin from orange peels grown in our country and obtained from waste condition was obtained as a result of extraction experiments under different conditions. After finding the optimum extraction conditions, hesperidin reach extract was purified by acidic precipitation in order to obtain hesperidin. Hesperetin, an aglycone of hesperidine, was obtained by acidic hydrolysis of hesperidine under different conditions such as acid type, temperature and time. Hesperidin were converted into their alpha beta unsaturated derivative, diosmin by elimination reaction. Diosmetin was synthesized by acidic hydrolysis of diosmin under different conditions Diosmetin was obtained by acidic hydrolysis of diosmin under different conditions, and compound **2** was synthesized by partial methylation reaction. All the synthesized and obtained compounds were purified and then their structural identifications were performed by spectroscopic techniques such as ^{1}H- , ^{13}C-NMR and FT-IR. Subsequently, the synthesis of the semisynthetic derivatives of these compounds with the wittig reaction was tested and the reaction did not occur.In the composition of drugs such as Daflon and Vendios which are sold in the pharmaceutical market in our country,hesperidine,hesperetin and semi-synthetic derivatives of diosmin and diosmetin are used as active ingredients.In the light of this information hesperidine and semi-synthetic derivatives of hesperetine have been prepared which have positive effects on the cardiovascular system which is our aim in our planned project.

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