MICROWAVE EXTRACTION OF PHENOLIC COMPOUNDS FROM CAPER AND OLEASTER

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JULY 2012

Approval of the thesis:

MICROWAVE EXTRACTION OF PHENOLIC COMPOUNDS FROM CAPER AND OLEASTER

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ABSTRACT

MICROWAVE EXTRACTION OF PHENOLIC COMPOUNDS FROM CAPER AND OLEASTER

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July 2012, 166 pages

The main objective of this study was to extract phenolic compounds from caper and oleaster by using microwave. The effects of microwave power (400 and 700 W), extraction time (5-20 min), solid to solvent ratio (1:10, 1:20 and 1:30) and solvent type (water, ethanol and different ratios of ethanol-water mixture) on total phenolic content, antioxidant activity and concentration of the phenolic compounds in the extracts were investigated. In conventional extraction, extraction time and solvent type were used as independent variables. Microwave extraction was compared with conventional extraction.

In caper and oleaster extracts that were obtained by using microwave, total phenolic compounds ranged between 5.0-52.4 and 2.6-43.0 mg GAE/g dry material while the antioxidant activity were between 0.5-2.0 and 0.2-3.7 mg DPPH/g dry material, respectively. Higher total phenolic content was observed with increasing solvent

amount. The highest total phenolic content was obtained when 50% ethanol-water mixture was used as solvent. Microwave power did not affect total phenolic content significantly.

The highest total phenolic contents were obtained when 400 W microwave power, 50% ethanol-water mixture and solid to solvent ratio of 1:30 were used in the extraction from both caper and oleaster. The best extraction time for caper was 5 min while it was 15 min for oleaster. The main constituents were rutin and kaempferol for both caper and oleaster extracts.

For caper and oleaster there was no difference between extraction methods in terms of concentration of phenolic compounds. However, extraction time decreased significantly by using microwave, as compared to conventional extraction.

Keywords: Microwave extraction, caper, oleaster, phenolic compounds, antioxidant activity

ÖZ

KAPARİ VE İĞDEDEKİ FENOLİK BİLEŞİKLERİN MİKRODALGA İLE ÖZÜTLENMESİ

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Temmuz 2012, 166 sayfa

Bu çalışmanın amacı, kapari ve iğde bitkilerindeki fenolik maddelerin mikrodalga ile özütlenmesidir. Özütlerde mikrodalga gücünün (400 ve 700 W), özütleme süresinin (5-20 dakika), katı madde-çözgen oranının (1:10, 1:20 ve 1:30) ve çözgenlerin (su, etanol ve değişik oranlardaki etanol-su karışımı) toplam fenolik madde miktarına, antioksidan aktivitesine ve fenolik madde konsantrasyonuna etkisi araştırılmıştır. Konvansiyonel özütlemede özütleme süresi ve çözgen çeşidi bağımsız değişkenler olarak seçilmiştir. Mikrodalga özütleme metodu ile elde edilen sonuçlar konvansiyonel özütleme metoduyla elde edilen sonuçlarla karşılaştırılmıştır.

Mikrodalga kullanılarak elde edilen kapari ve iğde özütlerinde, toplam fenolik madde miktarları sırasıyla 5.0-52.4 ve 2.6-43.0 mg GAE/g kuru madde, antioksidan miktarları sırasıyla 0.5-2.0 ve 0.2-3.7 mg DPPH/g kuru madde arasında bulunmuştur. Çözgen miktarı arttıkça toplam fenolik madde miktarı artmıştır. En yüksek fenolik madde miktarı çözgen olarak %50 etanol-su karışımı kullanıldığında elde edilmiştir.

Mikrodalga gücünün fenolik madde miktarına olan etkisi istatistiksel olarak önemsiz bulunmuştur.

En yüksek toplam fenolik madde miktarı 400 W mikrodalga gücü, %50 etanol-su karışımı ve 1:30 katı madde- çözgen oranı kullanıldığında, özütleme süresi kapari için 5 dakika, iğde için 15 dakikada elde edilmiştir. Kapari ve iğde bitkilerindeki ana bileşenler rutin ve kaempferol olarak bulunmuştur.

Özütleme metotları arasında kapari ve iğdedeki fenolik bileşen konsantrasyonları açısından fark yoktur, ancak mikrodalga kullanılması özütleme süresini konvansiyonel metoda göre önemli derecede azaltmaktadır.

Anahtar sözcükler: Mikrodalga ile özütleme, kapari, iğde, fenolik bileşenler, antioksidan aktivitesi

To my family

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge my supervisor, Prof. Dr. S. Gülüm Şumnu for her guidance, inspiration, enthusiasm, patience, kindness and moral support. I would also thank to my co-advisor, Prof. Dr. Serpil Şahin for her valuable discussions and suggestions.

Special thanks to Alev İnce for her technical guidance, encouragement, and endless patience. She made my graduate years easier and enjoyable. The memories in the laboratory will be unforgettable for me.

I would also thank my sister, Elif for her patience. It is time to start to spend much more time together.

I express my gratefulness to Prof. Dr. M. Musa Özcan for his guide in obtaining of caper.

I express sincere appreciation to Mr and Mrs Arsak for their helps in my thesis-writing period, their moral support and encouragement when it was most required.

It would have been difficult to write this thesis without the precious people around me, my home mate, my colleagues, my school mates and other friends. Thank you very much to you all.

Lastly, and most importantly, I wish to thank my parents, A. Gülgün Durmaz and Orhan Durmaz. They have always supported and encouraged me in everything in my life.

I would also like to thank The Scientific and Technological Council of Turkey for the financial support during my thesis.

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INTRODUCTION

Phytochemicals are bioactive substances found naturally in plants. A lot of phytochemicals, which are responsible for color and organoleptic properties, had been identified by scientist and food chemists. Since extracts of plants are believed to prevent several diseases and be beneficial for human health, they need to be clearly identified and developed for the food market. Studies on this issue are important because these studies are transmitted to the consumer through the media (Biesalski et al., 2009). These anticarcinogenic phytochemicals are carotenoids, chlorophyll, flavonoids, polyphenolic compounds, sulfides, protease inhibitors and indoles.

1.1. Phenolic Compounds

Phenolic compounds, which are secondary metabolites of plants, are one of the main subgroups of phytochemicals. Since they are commonly present in plants frequently and include more than 8000 different compounds (Liazid, 2007), human diet mainly comprises these compounds. Polyphenols are found in many foods, such as fruits, vegetables, beverages, cereals, legumes, nuts and herbal products (Shahidi, 2004).

There is a great demand for consumption of phenolic compounds in food industry due to their health benefits (Parr and Bolwell, 2000). They have multifunctional roles because of their great diversity in distribution, concentration and variety of structural types (Zucker et al., 1967).

The classification of phenolic compounds can be seen from Figure 1.1.

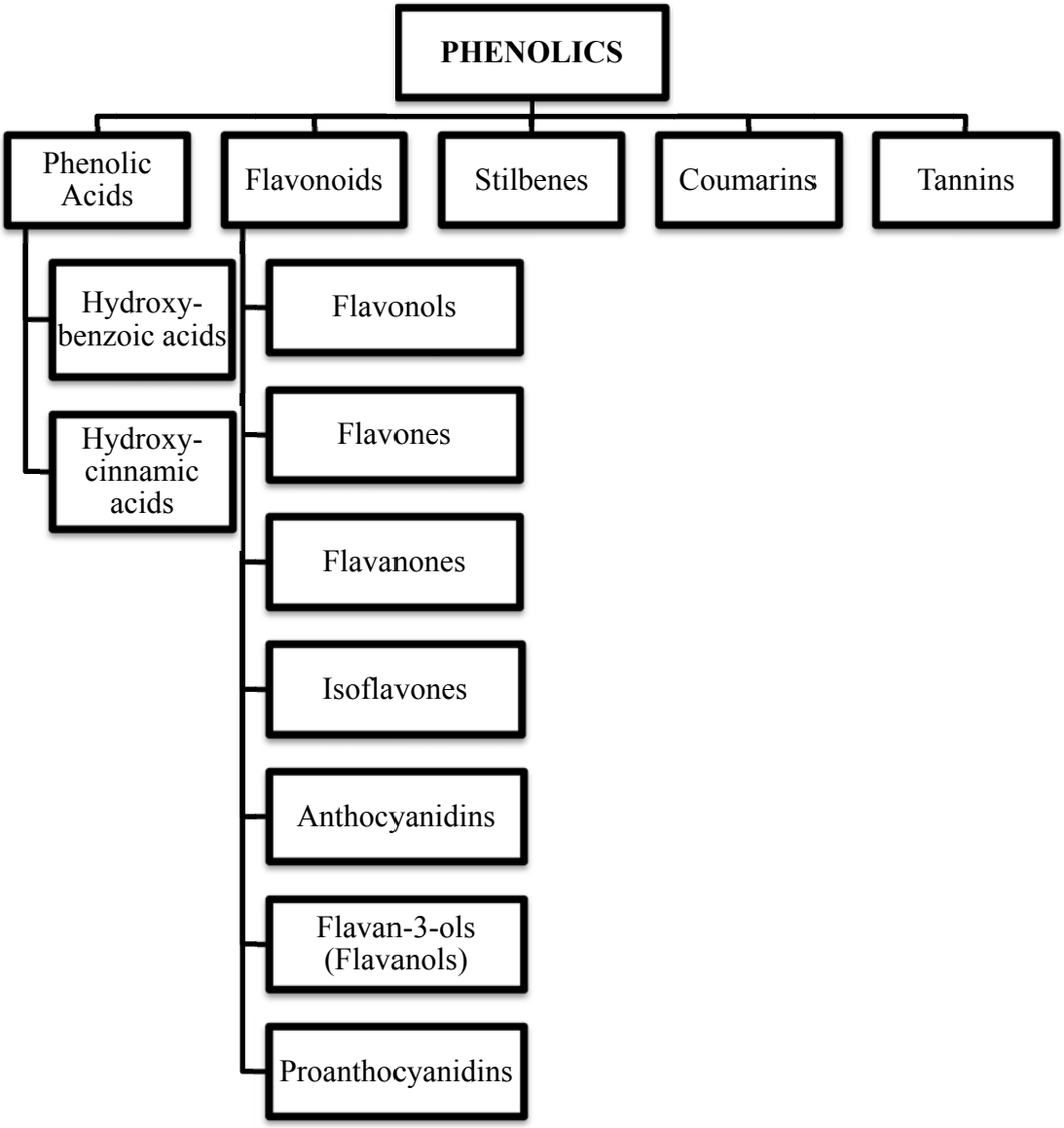

Figure 1.1. Classification of Phenolic Compounds (Erdman et al., 2007)

Among these phenolic compounds, phenolic acids and flavonoids are the main classes of polyphenols.

1.1.1. Phenolic Acids

Phenolic acids are widely distributed in plants. Approximately, one-third of the phenolic compounds in plants are composed of phenolic acids (Hayat et al., 2009), which can be mainly categorized as hydroxycinnamic acids and hydroxybenzoic acids.

Caffeic acid (3,4-dihydroxycinnamic), ferulic acid (4-hydroxy-3-methoxycinnamic), sinapic acid (4-hydroxy-3,5-dimethoxycinnamic) and p-coumaric (4-hydroxy-cinnamic) acid are hydroxycinnamic acids, while gallic(3,4,5-trihydroxybenzoic) acid, vanillic(4-hydroxy-3-methoxybenzoic) acid, syringic (3,5-dimethoxybenzoic) acid and protocatechuic (3,4-dihydroxybenzoic) acid are hydroxybenzoic acids.

Chlorogenic acid and p-coumaric acidare water soluble and highly polar compounds that are found in grape juice (Singleton et al., 1966). Chlorogenic acid is the main phenolic compound in potato tubers, consisting 90 % of total phenolic content (Rosa, 2010).

Ferulic acid and caffeic acid are other groups of phenolic acids. Some foods, such as wheat, corn, rice, tomatoes, spinach, cabbage and asparagus contain ferulic acid. Olive oil, coffee, white grapes and white wine are the examples of some foods that have caffeic acid (Rice-Evans et al., 1996).

Caffeic acid is very common in plants, often occurring as a caffeoylquinate or chlorogenic acid. Caffeic acid and p-coumaric acid or their derivatives have been reported in grapes and wine by several researchers (Singleton and Esau, 1969). Ribéreau-Gayon (1963) reported about 1-15 mg/liter of caffeic acid and 0.3-30 mg/liter of p-coumaric acid in crushed grapes or wine.

Sinapic acidis a widely investigated antioxidative compound and it is found in vinegar, rapeseed, mustard and canola seed (Gavez et al., 1994; Thiyam et al., 2006; Cai and Arntfield, 2001).

Gallic acid is a hydroxybenzoic acid that is commonly found in food materials. It is one of the main phenolics in black tea. It is mostly used to indicate the total phenolic content, namely gallic acid equivalents.

Syringic acid is found in olive oil, açai palm (Papadopoulos and Boskou, 1991; Pacheco-Palencia et al., 2008).

Protocatechuic acid is a dihyroxybenzoic acid that is found in açai oil, mushrooms (Pacheco-Palencia et al., 2008; Delsignore et al., 1997).

Vanillic acidhas a pleasant and creamy odor; therefore it is used as flavoring and scent agent. It is an oxidized form of vanillin. Açai oil and a herb called “dong quai” includes vanillic acid (Pacheco-Palencia et al., 2008; Duke, 1992).

1.1.2. Flavonoids

Many of the food material contain flavonoids in different quantities and chemical structure. Dragsted et al. (1997) investigated the average intake of flavonoids from food in Denmark and found about 100 mg per day of flavonoid consumption.

The subclasses of flavonoids are shown in Figure 1.1.

1.1.2.1. Flavonols

Flavonols are major group of flavonoids in terms of percentage of existence in foods. Outer parts of the fruits and vegetables are richer in flavonol content, because sunlight activates their synthesis (Manach et al, 2004).

Flavonols have been studied for a long time. When Neubauer recovered quercetin by extracting grapes, it was 1873. Flavonols are present in lots of plant tissues. According to US Department of Agriculture (2007a), caper, which contains 490 mg flavonols/100 g fresh weight of caper, is the main source of flavonols. Quercetin,

kaempferol, myricetin, isorhamnetin are some of the flavonols in which kaempferol and quercetin are the main ones.

While kaempferol is found mostly in endive, leek broccoli, radish, grapefruit and black tea; onion, lettuce, cranberry, apple skin, olive, tea and red wine are known to have quercetin (Rice-Evans et al., 1996). Miean and Mohamed (2001) investigated on flavonoid content of 62 edible tropical plants and found highest quercetin (1497.5 mg/ kg of dry weight) and kaempferol (832 mg/ kg of dry weight) contents in onion leaves. They found the quercetin and kaempferol concentrations of carrot as 55 mg/ kg of dry weight and 140 mg/ kg of dry weight, respectively.

Quercetin is generally found in various vegetables, fruits and herbs. Also, it is known to be in wine and tea leaves (Pietta, 1998; Ross and Kasum, 2002; Mahmoud et al., 2000; Yang et al., 2000). Among many flavonoids that are found in plants, it is the most abundant and most biologically active one. The estimated average human intake of quercetin is 20-500 mg per day and this high amount of consumption makes this flavonoid popular in antioxidant studies (Bedir et al. 2002; Myhrstad et al., 2002; Pedrielli et al., 2001; Tanaka, 1994).

1.1.2.2. Flavones

Apigenin, luteolin, tangeretin, chrysin and nobiletin are the flavones that are mostly found in green plant tissues. Celery stalks and parsley leaves are good sources of flavones (Manach et al., 2004).

1.1.2.3. Flavanones

Naringenin, hesperetin, naringin, eriodictyol and isosakuranetin are some of theflavanones. Kefford and Chandler (1970) stated that these flavanonescause a bitter taste in fruits and fruit juices. Citrus fruits and their products contain higher amounts of flavanones. In fact, in citrus fruits 50 to 80 % of total flavonoids consist of naringin, neoeriocitrin and hesperidin (Kanes et al., 1992). For example, the major

flavanones in grapefruits are naringenin, naringin and narirutin (Rouseff et al., 1987). Bocco et al. (1998) studied on peels and found that naringin, neoeriocitrin and neohesperidin are mostly found flavanones in lemon peel.

1.1.2.4. Isoflavones

Isoflavones are another class of flavonoids. Genistein, daidzein, glycitein, glycitein, genistin and formononetin are some of the isoflavones. These substances are present in plants mostly in the glucoside form. Manach et al. (2004) stated that they occur almost exclusively in leguminous plants. According to US Department of Agriculture (2007b), soya bean and its soya products, like soya milk, tofu and tempeh are main dietary source of these substances and they are responsible for the herb-like flavor, astringency and bitterness (Huang et al., 1979).

1.1.2.5. Anthocyanidins

Anthocyanidins and glucosidically bound form of them, namely anthocyanins are subgroup of flavonoids. Epigenidin, cyaniding, delphinidin and pelargonidin are the most frequently found substances among 31 known anthocyanidins (Anderson and Jordheim, 2006). They are responsible for bright red, blue and violet colors of foods (Mazza and Miniati, 1994). There are a lot of studies on red, blue and purple pigments (Harborne, 1967). Red grapes, red apples, pomegranates, currants, all kind of berries, plums, red radishes, eggplants,and red onions are examples that contain this kind of flavonoids.

1.1.2.6. Flavan-3-ols (Catechins)

Flavan-3-ols are also referred as flavanols and catechins in literature. Catechin, epicatechin, gallocatechin, epigallocatechin and theaflavin are the mostly foundflavan-3-ols in foods. Many commonly consumed fruits and vegetables contain low concentrations of flavan-3-ols, such as apples, grapes, berries, peaches, apricots, bananas, nuts and beer (US Department of Agriculture, 2007a; Harnly et al., 2006).

However, tea, cocoa bean and chocolate are the richest sources of these substances (Manach et al., 2004). In fact, major compound of tea leaves are flavan-3-ols (Wickremasinghe, 1978; Stagg and Millin, 1975).

1.1.2.7. Proanthocyanidins

This type of flavonoids formed as a result of polymerization of flavan-3-ols to condensed tannins. They may lead to discoloration of foods, although they are colorless, even in enzymatic browning reactions (Shahidi, 2004). Luh et al. (1960) stated that proanthocyanidins gives pinkish color to pears. Procyanidinsare the main proanthocyanidins in foods.The main dietary sources of proanthocyanidins are cinnamon and sorghum (US Department of Agriculture, 2004).

1.1.3. Effects of Phenolic Compounds on Human Health

Polyphenols have a lot of functions, such as antimutagenic, anti-inflammatory, antiproliferative, signal transduction, antioxidant and synergistic relationships with other antioxidants and therefore they are considered as chemopreventive agents (Yuting et al., 1990; Craig, 1996; Cody et al., 1988). Rafat et al. (1987) and Serafini et al. (1996) found that these compounds are attractive as antioxidant because of their functions including radical scavenging and metal chelating. The polyphenolic compounds found in plants may show anticarcinogenic or cardioprotective action since they act as antioxidants (Rice-Evans et al., 1996). Moreover, polyphenols in foods act as protective material against oxidative stress and chronic actinic damage in skin (Purba et al., 2001).

Middleton and Kandaswami (1992) studied on effects of flavonoids on immune and inflammatory cell functions and found that flavonoids enhanced the activity of the immune system. They also demonstrated a protective effect against DNA damage (Noroozi et al., 1998; Anderson et al., 2000). In another study on cancer it was found that flavonoids allowed the death of prostate cancerogenic cells by altering cell cycle regulators (Bhatia and Agarwal, 2001).

Isoflavones show antioxidative and antiproliferative activities and they have inhibitory effects on tumor invasion and angiogenesis (Adlercreutz and Mazur, 1997; Uckun et al., 1995). Besides, they are important due to their antiestrogenic properties (Zheng et al., 1999) since increased level of estrogens in blood is a sign for breast cancer (Bernstein et al., 1990; Toniolo et al., 1995).

Quercetin increases the killing rate of tumor cells during heat therapy. At the same time it reduces the toxicity and carcinogenic effects of substances in the body (Bloch, 1997). Another beneficial effect of quercetin is on angiogenesis, which is a very important process in cancer treatment due to forming new blood vessels by using existed ones (Berbari et al., 1999; Tosetti et al., 2002).

In the androgen independent prostate tumor cell line, quercetin and kaempferol enable complete suppression of growth (Knowles et al., 2000; Hiipakka et al., 2002).

1.1.4. Effect of Storage and Processing Methods on Phenolic Compounds

Several studies were performed about the effects of processing on total phenolic content and specific antioxidant compounds. Investigators found different results on this issue. Tsao et al. (2006) stated that concentration of phenolic compounds in a food material decreases as a result of processing. Especially heat processes, such as drying, pasteurization, frying, boiling and microwave cooking cause a decrease in total antioxidant activity (Gil-Izquierdo et al., 2002; Guyot et al., 2003; Aziz et al., 1998). Lee et al. (2008) examined the flavonoid losses of onion during several processing. They calculated the decrease in flavonoid content as 33 %, 14-20 %, 14 % and 4 % when they used frying, boiling, steaming and microwaving processes, respectively. Sun-drying also adversely affects phenolics, such that it decreased total phenolic content of pears by 64 % (Ferreira et al., 2002). Moreover, extension of thermal processing caused a degradation of lycopene in the study of Jacob et al. (2010). In the study of Dutra et al. (2008), it was commented that compound stability was affected from heating process due to chemical and enzymatic decomposition and

the thermal decomposition was the main mechanism causing the reduction of polyphenol contents.

Contrary to investigators who argued that processing has a destructive effect on phenolics, some of the studies showed that this argument was not always true. For example, anthocyanin and anthocyanogen content of wine produced by pressing of red grapes after crushing were found as 16 and 100 mg/liter respectively, whereas they were found as 26 and 320 mg/liter by maceration overnight at ambient temperature (Anon.,1966a).In another study, heat treated tomatoes exhibited higher antioxidant activity as compared to fresh tomatoes (Wang et al., 1996). Alothman et al. (2009) found that polyphenols increase after UV irradiation processing of fresh-cut fruits and explained it by facilitation higher extractability with UV treatment.

Moreover, during cold storage of apple for up to 9 months, no change was observed in terms of phenolic content (Burda et al., 1990; Golding et al., 2001). Connor et al. (2002) also expressed the similar results for blueberries. Gil et al. (1999) also found that total flavonoid content remained stable during storage in air and after modified-atmosphere packaging.

As a result, in a positive or negative way, the levels of phenolic content may change with some factors, such as ripening, maturation, fruit development, storage and processing (Maga, 1978; Hanna et al., 1991; Ayaz et al., 1997). In those situations, phenolics are also responsible for dark color and unpleasant taste of foods.

1.2. Antioxidants

As a result of aerobic metabolism damages, free radicals are generated and they cause oxidative damage which leads to mutations (Feig et al., 1994). Free radicals can damage DNA by changing nucleic acids and cause cancer. In the cell membrane, loss of fluidity and lysis of cell after lipid peroxidation of unsaturated lipids, are the effects of free radicals on the metabolism. They are either produced as a result of mitochondrial respiration or exogenous factors. They are highly reactive because of their unpaired electrons in their outermost orbits. This reactivity causes some chemical reactions which damage cells. Unless an antioxidant or another free radical binds a free radical, defense mechanism of the body cannot stop these reactions. Therefore, human body defends itself against these damages by using antioxidants from foods. The researches on the effects of free radicals on cancer and benefits of antioxidants derived from diet create a great interest on antioxidant containing foods (McLarty, 1997).

Antioxidants are basically described as natural compounds which inactivate the free radicals. The inactivation mechanism is expressed as:

$$RO^{\bullet} + AH \rightarrow ROH + A^{\bullet}$$

Antioxidants have lots of beneficial effects. Antioxidant compounds such as vitamin C and E, flavonoids and carotenoids can hinder the tumor formation in the body (Gerster, 1995; Bilton et al., 2001). Generally, more than one antioxidant compound, in other words various antioxidants together show better effects, probably because of the synergistic effect of the compounds (Pastori et al., 1998; Amir et al., 1999). Antioxidant intake both helps normal tissues functions and protects these tissues from the adverse effects of chemotherapy (Pietta, 1998). Thus, they are mentioned as health protecting compounds.

The importance of antioxidants has been understood more and more with experimental researches and also epidemiological and clinical results. The phenolic compounds, like flavonoids and vitamin E are well-known antioxidants. Foods are natural sources of these antioxidants. There are other sources of antioxidants, other than foods, namely synthetic antioxidants. 2,6-di-tert-butyl-4-methylphenol (BHT) is

one of the example of this kind of antioxidants. However, natural antioxidants are more preferable than synthetic ones due to their powerful, safe and efficient properties.

Different methods can be used in order to determine the antioxidant activity of food materials. DPPH method, which was used in this study, is a rapid, simple and inexpensive method to measure antioxidant capacity of food. It involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH).It is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. This method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity will help us understand the functional properties of food.

In this method, DPPH is reduced by an antioxidant and as a result, absorbance in 517 nm decreases and color changes. The remaining [$DPPH^{\cdot}$] is proportional with antioxidant concentration (Prior et al., 2005).

The mechanism of DPPH method is shown in Figure 1.2.

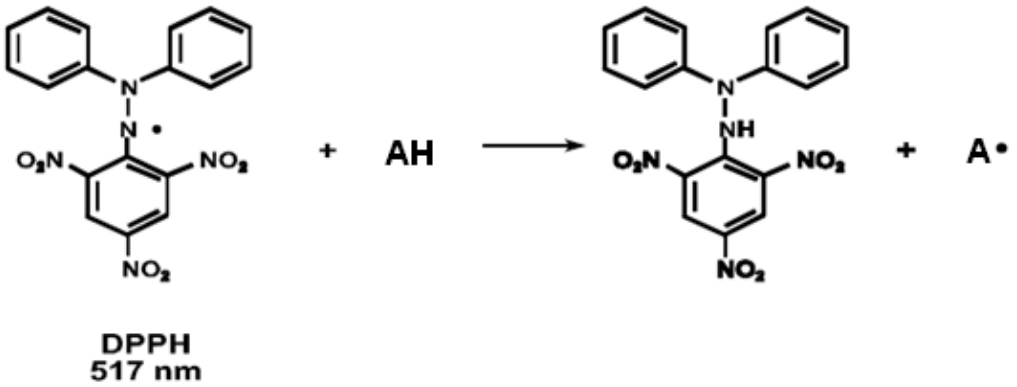

Figure 1.2. The structure of DPPH and its reduction by an antioxidant

1.3. Extraction of Phenolic Compounds

Extraction is a process for separation of substances from a matrix. It is the most important step in obtaining of phenolic compounds. There are numerous ways for extraction of phenolic compounds, such as Soxhlet, microwave, maceration, ultrasound and supercritical extractions. These extraction methods affect the extract both by quantity and quality.

1.3.1. Soxhlet Extraction

Soxhlet extraction is one of the most applicable conventional methods in extraction studies. In this method, sample and solvent is placed into a round bottom flask. A hot plate heats the solvent. After solvent evaporates, the solvent vapor condenses through condenser and floods back into the flask. In other words, solvent is heated to reflux. The refluxing solvent repeatedly washes the solid that results in leaching of components into the solvent. Then, the desired compounds dissolve in the warm solvent.

This cycle, which consists of heating, evaporation, condensation of solvent, must be allowed to repeat many times, mostly over hours till the end of the dissolution of target compound.

In this method, heating principle is basic. It occurs with conductive heating from the heater. Heat is transferred molecule by molecule from the outside. Flask is heated first and then temperature of the solution increases. Convective currents are take place within the solvent. In fact, the temperature of the heating surface of the flask is higher than that of the solvent.

Conventional extraction has the advantage of being cheap in terms of equipment. However it is proved by a lot of studies that conventional method takes long time. For example, Proestos and Komaitis (2008) stated conventional extraction as “time consuming process” in their study after comparison of conventional and microwave

extraction. Long time extraction increases the variable cost in terms of electricity cost. Therefore, in the long term it is not a very economical method. Furthermore degradation of compounds and low extraction efficiency during essential oil extraction were expressed by Ferhat et al. (2007).

1.3.2. Microwave Extraction

In recent years, new extraction techniques have become very popular in the fields of phenolic, volatile and nonvolatile compounds, due to their advantages upon conventional ones. They are preferred due to decreasing extraction times, solvent consumption and energy savings and increasing efficiency, convenience and practicality.

Microwave heating takes place in dielectric materials such as foods, due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz (Decareau, 1985).

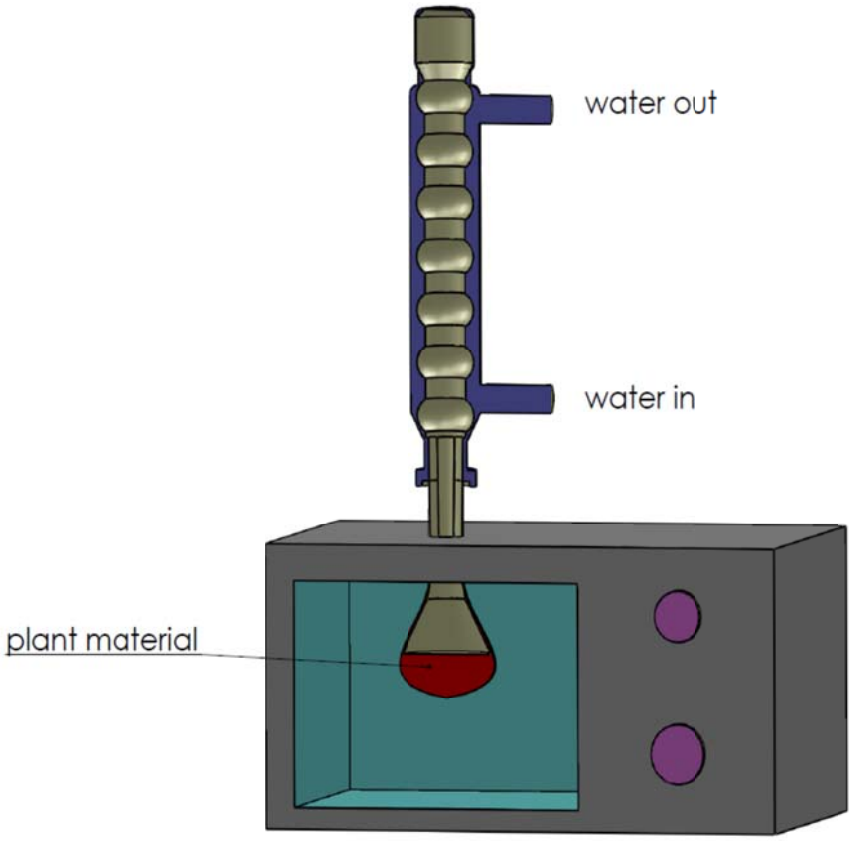

Figure 1.3. Drawing of Microwave Extraction

Microwave extraction is one of the most commonly studied novel processing methods that is used to heat solvent and solid samples by microwave energy, thus compounds are leached and diffuses into the solvent. This method can be an alternative to conventional extraction. The difference of this technique is its heating mechanism which occurs in two ways, namely ionic migration and dipole rotation. Both of these heating effects of microwave enhance the product recovery. **Ionic migration** is the movement of ions in the electric field of microwaves (Sahin and Sumnu, 2006). This heating principle is dominant especially if there are ions in the solvent, for example if the solvent is salt containing water. Polar molecules, such as polyphenols and polar solvents create a **dipole movement**, in other words they

arrange themselves within the field. Solvent temperature increases by dipole rotation of solvent in the microwave field. As a result of temperature increase in solvent, solubility of the target compounds that will be extracted increases. Gfrerer and Lankmayr (2005), and Venkatesh and Raghavan (2004) concluded that the rapid temperature rise and fast extraction process are the result of dipole movements in microwave extraction. Moreover, absorption of microwave energy by plant material results in high pressure gradient inside the plant (Bayramoglu et al., 2008). This internal heating and pressure accelerate the diffusion of phenolic compounds from sample into the solvent. Lay-Keow and Michel (2003) explained the release of molecules from sample to solvent with the rupture of the plant cells by microwaves.

One of the most important advantages of using microwave is its uniform and rapid heating mechanism. In contrast to conventional heating, heat is generated within the material and whole material is heated almost about the same rate.

In addition, microwave is an energy saving process. According to the Energy Center of Wisconsin, the benefits of microwaves include, "Energy efficiency of 50% (vs. 10% to 30% for fuel fired processes)." According to California Energy Commission, “microwave ovens use around 50 % to 65 % less energy than conventional ovens”.

Another advantage of microwave oven for using in industry is its floor space which is 20–30 % less than conventional units. Moreover, there is no extra time requirement for warm-up and cool-down; it has a principle of instant on and off.

Microwave extraction efficiency depends on some factors. Microwave power and extraction time are two of these parameters (Egizabal et al., 1998; Kaufmann et al., 2001). Moreover, properties of materials determine the efficiency of microwave heating. Therefore, selection of the solvent is highly important in microwave extraction. Proestos and Komaitis (2008) added solubility, dielectric constant and dissipation factor of solvent to this independent parameters list. Solvent must have the ability to solve the phenolic compounds. Since, phenolic compounds contain OH groups in their structures; they can be more soluble in polar solvents. This claim was also confirmed by Wang and Weller (2006). Dielectric constant (ε′) of the solvent,

which is a parameter that shows the absorption ability of microwaves, must be high so that it can absorb more microwave energy. This term is completed with another term, called dielectric loss factor (ε´´), which corresponds to efficiency of absorbed energy converted into heat. Dissipation factor or tangent loss (δ), which is another parameter of a dielectric material, is a numerical value that combines dielectric constant and dielectric loss factor. Kok and Boon (1992) describes the dissipation factor as "the ability of a sample to absorb microwave energy and dissipate that energy in the form of heat". That is, a high dissipation factor means higher microwave energy absorption and higher heating rate. It can be defined as;

$$\tan \delta = \varepsilon'' / \varepsilon'$$

All of these properties of the solvent are effective on microwave absorption and heating. In accordance with this formula, although water has a high dielectric constant, since its dissipation factor is low; its heating rate may be lower as compared to other solvents.

Some polar materials, such as water, methanol, ethanol, acetonitrile and acetone are used as solvent in microwave extraction. The dielectric properties of some solvents that are used in extraction methods are presented in Table 1.1.

Table 1.1 Dielectric properties of solvents

Materials	**Dielectric Constant (ε´)**	**Dielectric Loss Factor (ε´´)**	**Dissipation Factor (tan δ)x10^4**
Water	76.7	12	1570
Ethanol	24.3	6.1	2500
Methanol	23.9	15.3	6400
Acetone	21.1	11.5	5555

Choosing the convenient solvent for polyphenol extraction affects the total phenolic content extracted (Xu and Chang, 2007). Ethanol is a good solvent for extraction of phenolic compounds, since it is safe for human consumption (Shi et al., 2005). In addition, it has higher dissipation factor than water.

Among these solvents methanol and acetone are not suitable for extraction of food materials, methanol is denoted as a toxic alcohol by National Institute for Occupational Safety and Health and acetone is described as toxic by Agency for Toxic Substances & Disease Registry.

The other factor that can have an impact on phenolic compound extraction is solvent to solid ratio. Increasing this ratio enhanced phenolic yields (Cacace and Mazza, 2003a; Pinelo et al., 2005a). However, in order to find an optimum value, there should be a balance in solvent amount. If the solid-solvent ratio is less, the concern must be saturation of solution. If the solid-solvent ratio is high, the concern becomes high cost of extraction (Pinelo et al., 2006).

Particle size of the sample is the other parameter that is effective on extraction of phenolics. Yield of phenolic compounds increases with decreased particle size (Pinelo et al., 2005b; Nepote et al., 2005). Mechanical treatment, excessive stirring, etc., speeds the phenol extraction (Ough and Amerine, 1960). Disrupted skin cells cause diffusion of skin phenolics towards inside of the grape and larger amount of phenolics may appear in the pulp or juice. Phenolic content increases with disruption of cells of solid tissues, such as berry (Singleton and Esau, 1969).

There are respectable amount of studies on microwave extraction in literature. Bioactive compounds, especially phenolic compounds and essential oils have been efficiently extracted from various food materials by using microwave energy. The common point of all these studies is that these studies demonstrated the advantages of microwave as compared to other extraction methods. For instance, Nkhili et al. (2009) studied on extraction of polyphenols from green tea. They compared microwave extraction and conventional extraction and found microwave extraction more advantageous in terms of extraction time, energy consumption and

environmental aspects. Moreover, they got higher total phenolic content with microwave and attribute this to less thermal degradation due to lower extraction time. Wang and Weller (2006) also praised microwave extraction for its rapid delivery of energy and homogeneous heating mechanism.

In literature, there are publications about microwave extraction of phenolic compounds from grape and pomegranate seeds (Hong et al., 2001; Abbasi et al., 2008). Du et al. (2009) performed studies on extraction of polyphenols from medicinal plants by using microwave and ionic solvents. Spigno and Faveri (2009) showed that microwave extraction is a potential alternative to conventional extraction in extraction of tea phenols because of less time requirement and savings both in energy and solvent consumption. In the same year, Hayat et al. (2009) studied phenolic acid extraction from citrus mandarin peels by using microwave and found similar results. In their study, microwave extraction gave higher total phenolic acid content and antioxidant activity as compared to rotary extraction. The optimum extraction time was found as short as 49 seconds.

Beejmohun et al. (2007) studied microwave extraction of phenolic compounds from flaxseed. In their study higher phenolic contents were obtained after 3 min of microwave extraction process, although traditional extraction lasted for 6 h in total. Liazid et al. (2007) investigated stability of phenolic compounds during microwave extraction. They studied microwave extraction at different temperatures, such as 50, 75, 100, 125, 150 and 175 ^{0}C.The maximum values of most of the phenolic compounds were found at 100 ^{0}C and they concluded that phenolics can be extracted up to 100 ^{0}C without degradation. Furthermore; they stated that shorter extraction times reduced adverse effects of enzymatic activity and degradation of phenolics.

Martino et al. (2006) showed that microwave extraction decreased time and solvent consumption while providing higher recoveries during coumarin and o-coumaric acid extraction of sweet clovers. Antioxidants of longan peels were extracted both by using microwave with 1:10 solid to solvent ratio for 30 min and by using Soxhlet apparatus with 1:16 solid to solvent ratio for 2 h; and then total phenolic contents

were found as 96.78 mg/g and 90.35 mg/g dry weight by microwave and Soxhlet extraction, respectively (Pan et al., 2008).

Considering the advantages of using microwave, its popularity in extraction has recently been increased as compared to other methods.

1.4. Caper

Caper, Latin name *Capparis Ovata*, which is grown in Turkey widely is not consumed much enough. The capers of the highest quality in the world are grown in Turkey. This Mediterranean plant grows in most of the areas, except cold regions like Erzurum and altitude of more than 1000 meters. Capers in brine are exported to Europe and America and its fruits are consumed only in the regions it grows (Ozcan, 1999).When the fact that international trade income of caper is 24,5 million dollars by the year 2003 is considered, the importance of this plant come into focus much more (Sat and Cil, 2006).

The caper which is as valuable as meat due to its high protein content (24 %), is also rich in terms of minerals and vitamins (Cosge et al., 2005). Therefore, it has a great importance in a balance diet. Furthermore, caper displays antioxidant characteristics due to various chemical compounds it has. This plant prevents harmful effects of cancerogenic materials and at the same time it has effective materials that repress cancerous cells. Studies that were performed by International Cancer Research Institute stated that caper was one of the plants that are used during preparation of extracts exhibiting antitumor activity (Anonymous, 1997). Some chemical compounds in caper have diuretic and chlorothiazide effects. The seeds of caper have active substances that regulate liver, spleen and kidney functions; cure asthma and hemorrhoid diseases; and also exhibit aphrodisiac property. The fruits of caper include painkiller and aphrodisiac effect, while skins of it contain anti-inflammatory substances (Tansı et al., 1997). In another study, it is detected that caper can be used in diabetes (Yaniv, 1987). Akgul (1996) proved that caper can be used as cosmetical additive that is effective on skin and hair diseases.

In one of the studies, Ozcan (1999) investigated the physical and chemical properties of raw and brined capers. In another study, sensory analyses of capers during storage in water with different concentration of salt were studied (Ozcan, 2001). El-Ghorab et al. (2007) used buds and leaves of caper that grow in Turkey in order to determine chemical composition and antioxidant activity and they found 86 compounds in the extracts of caper buds and 100 compounds in the extracts of caper leaves. Inocencio et al. (2000) examined flavonoids in caper and found quercetin and kaempferol as significant compounds. Unver et al. (2009) performed a study on phenolic compounds and antioxidant activity of different plants, and obtained the total phenolic compound of *Capparis Ovata* as 185.54 mg GAE/ g fresh material. It is concluded as a result of the antioxidant and antiradical activity studies on caper by Nadaroglu et al. (2008) that caper can be used as natural antioxidant source.

All of the parts of caper are valuable. Branches, buds and fruits of caper are used in food industry; while leaves, aged branches and roots are used in pharmaceutical, paint and cosmetic industry. Nevertheless, the caper plant cannot be appraised in Turkey adequately. It is important that capers, which are rich in phenolic compounds, should be processed with novel techniques, to enlarge the consumption areas of this plant.

1.5. Oleaster

Oleaster, Latin name *Elaeagnus angustifolia L.*, is a tree that is cultivated in warm climate regions. As appearance it looks like a date, it is dry and inside is white with a little bit sweetness. It is also known as Russian olive. The total production of oleaster in Turkey is about 6000 tons (Durmuş and Yigit, 2003). It is known that oleaster, which can grow in various climatic and environmental conditions, has important environmental effects on erosion control and wind stoppage (Christiansen, 1963; Little, 1961). Besides, it is valuable in terms of health. Oleaster is advised to be consumed by the people who have kidney disorders. Baytop (1984) mentioned that it can be used as diuretic and fever-reducing drugs in traditional medicine. Moreover, it

is used for preventing intestine disorders and mouth rust (Danoff-Burg, 2002). In a study of Ahmadiani et al. (2000), *Elaeagnus angustifolia* fruit extracts showed a significant effect as anti-inflammatory and analgesic. Gurbuz et al. (2003) performed a study that shows oleaster has a gastroprotective activity, in other words anti-ulcerogenic effect.

In Turkey, oleaster is consumed as an appetizer during winter. It is a healthy plant that can be used as natural antioxidant. It can be demanded by conscious societies, since modern societies tend to consume natural sources.

The studies on oleaster are limited in literature. Bekker and Glushenkova (2001) investigated compounds in oleaster, by collecting all the studies on *Elaeagnaceae* family between the years 1950 and 2000. They focused on essential oil, flavonol and fat content in flower part of the plant; and carotenoids, flavonol, tannin and polyphenolic carboxylic acid compounds in leaves. Kusova et al. (1988) examined glycosidic compounds and caffeic acid content of oleaster. Ayaz and Bertoft (2001) studied sugar and phenolic acid composition of oleaster fruits; and found fructose and glucose as dominant sugars in the plant. They established seven kind of phenolic acids in which 4-hydroxybenzoic and caffeic acid were the most abundant ones. In another study, flavonol and polyphenolic carboxylic acid compositions in the young branches of oleaster were analyzed by using high performance liquid chromatography and mass spectrometry (Bucur et al., 2009). In this study, p-coumaric acid was found as the most abundant compound.

1.6. The Objectives of the Study

Phenolic substances are known to have a lot of beneficial effects for health. They have high antioxidant activity. Consumers prefer natural antioxidants instead of synthetic ones. Therefore, some of the foods gain importance as the source of natural antioxidants. Extraction of these valuable compounds from foods is done with several methods. Soxhlet extraction is one of the most applicable conventional method in extraction of phenolic compounds but in this method sample is supposed

to high temperatures for a long time which causes economical loss and destruction of phenolic compounds. Microwave is an economical and efficient method for extraction.

The main objective of this study was to extract phenolic compounds from caper and oleaster which are rich in nutritional value by using microwave. In this study, the best microwave extraction conditions were determined by analyzing total phenolic content, antioxidant activity and concentration of the phenolic compounds in the caper and oleaster extracts. Microwave extraction results were compared with the results obtained from the conventional extraction method. It was aimed to show that microwave is an alternative technique for extraction of phenolic compounds.

Although caper and oleaster grow in the most of the regions of Turkey and their nutritional value is high, the consumptions of these plants are very low. There is no study on obtaining phenolic compounds and determination of antioxidant properties of caper and oleaster extracted by using microwave in the literature. The results of this study will provide additional information to the limited literature about these plants and microwave extraction.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

In this study, the caper (*Capparis Ovata*) plants were picked from the fields in Konya, Turkey and only the buds of the caper were used. The oleaster (*Elaeagnus angustifolia*) plants were picked from trees in Izmir, Turkey.

Folin Ciocalteu's phenol reagent (2 N, MERCK), sodium carbonate (SIGMA-ALDRICH), $DPPH^{\cdot}$ (1,1-Diphenyl-2-picrylhydrazyl, SIGMA), ethanol (SIGMA) and methanol (gradient grade, for HPLC, ≥99.9%, SIGMA-ALDRICH) were used as reagents to perform the analysis.

Standards of gallic acid, caffeic acid, quercetin, rutin, vanillin, kaempferol, guaiacol, ferulic acid, vanillic acid, p-coumaric acid, trans-3-hydroxycinnamic acid, hydrocinnamic acid, benzoic acid, 4-hydroxybenzoic acid, sinapic acid, chlorogenic acid and 3-4-dihydroxybenzoic acid were purchased from Sigma-Aldrich.

2.2. Methods

2.2.1. Preparation of the Samples

The buds of the caper were stored at –80°C in the deep freezer. Then, they were dried by using freeze drier (Alpha 1-2 LD Plus, Osterode am Harz, Germany) at -53°C and 0.02 kPa for 42 h. Dried samples were grinded by using kitchen-type grinder (Arzum, AR-151 Mulino, Istanbul, Turkey). The dried and ground samples were also kept at –80°C.

The oleasters were used without doing any pretreatment. They were not dried and stored approximately at 20 °C.

The moisture contents of the freeze-dried capers and oleasters were analyzed at moisture analyzer (Ohaus MB45, Nanikon, Switzerland).

2.2.2. Extraction of Phenolic Compounds

2.2.2.1. Microwave Extraction

Extraction process was performed in a laboratory scale microwave oven (Ethos D, Milestone, Italy) that contains a heating unit, a condenser above it and an adaptor between the flask and the condenser. Microwave oven set up can be seen in Figure 1.4.

Samples of 2.5 grams and the solvent were put into an extraction flask that has a capacity of 1 L at different solid to solvent ratios and then the flask was placed into the oven. Power and time were adjusted by using the control panel of microwave oven.

Microwave power, solvent type, solid to solvent ratio and extraction time were the independent variables during the microwave extraction process. 700 W and 400 W were chosen as microwave power levels. Water, ethanol and water:ethanol mixture at different concentrations (25:75, 50:50 and 75:25 (v/v)) were chosen as solvent types because of their high dielectrical properties that enables powerful absorption and heating. Three different solid to solvent ratio, which were 1:10, 1:20, 1:30 were experienced. Extraction time intervals were determined by performing preliminary experiments. They were chosen as 5, 10 and 15 min for capers and 10, 15, 20 min for oleasters.

After the extraction was completed, extracts were filtered under vacuum and then the filtrate was kept in dark colored bottles of 50 ml capacity at refrigerator.

All extractions were done in two replicates.

2.2.2.2. Conventional Extraction

Conventional extraction was done by using a conventional heater (Şimşek Laborteknik, PI-404, 4x1000, Ankara, Turkey). The solvent and samples were added into a flask and the flask was put on the hot plate of the conventional heater.

The oleasters and the buds of caper plant were conventionally extracted by using 1:30 solid to solvent ratio that gives the best result in microwave experiments. The solvent types were the same as in microwave extraction and different times were performed to find the best extraction time.

After the extraction was completed, extracts were filtered under vacuum and then the filtrate was kept in dark colored bottles of 50 ml capacity at refrigerator.

All extractions were done in two replicates.

2.2.3. Analysis of Extracts

2.2.3.1. Determination of Total Phenolic Content

In this study, in order to determine the total phenolic content, the Folin-Ciocalteu method (Singleton and Rossi, 1965) was used. This method is based on the principle that phenolic substances reduces Folin-Ciocalteu reagent in the presence of sodium carbonate. This reduction causes a color change between 745 and 765 nm.

According to this method, 0.5 ml diluted samples were put into a tube and 2.5 ml of 0.2 N Folin- Ciocalteu were added. After vortex mixing, they were allowed to rest for 5 min, in a dark place at room temperature for incubation. After that, 2 ml of 75 g/L sodium carbonate was added. After vortexing again, the mixture was kept for 1 h in the dark place at room temperature. The absorbance measurement was performed at 760 nm using spectrometer (PG Instruments Ltd, T70 UV/VIS Spectrometer, Leichester, England).

The results were given as mg gallic acid equivalent/ g dry material. All the standard/calibration curves were prepared by using gallic acid solution at different concentrations.

All of the spectrometric measurements were done in three replicates and the average value was used in the calculations of total phenolic content.

Calibration curves are given in Appendix A.

2.2.3.2. Determination of Antioxidant Activity

The antioxidant activity of the caper buds and oleaster extracts were determined by using DPPH method (Brand-Williams et al., 1995). This method is based on the principle that the DPPH radicals are reduced by antioxidants. This reduction causes color change.

According to this method, 0.025 g DPPH˙, which is a dark purple radical, was dissolved in 1 L methanol. Then 3.9 ml of this solution was added to a cuvette that has 0.1 ml of extract. The absorbances were measured after keeping for 2 h in a dark place at room temperature. The waiting time was determined as 2 h to reach the constant reaction level. The remaining DPPH˙ in the samples were expressed by equation (2.1).

$$[DPPH^{\cdot}]_{remaining} = ([DPPH^{\cdot}]_{blank} - [DPPH^{\cdot}]_{sample}) \quad (2.1)$$

Where $[DPPH^{\cdot}]_{blank}$ is the concentration of DPPH blank measured after 2 h the blank was prepared and $[DPPH^{\cdot}]_{sample}$ is the concentration of DPPH measured after 2 h the sample and DPPH solution was mixed.
The remaining [DPPH˙] is proportional with antioxidant concentration (Prior et al., 2005).

The absorbance measurements were performed at 517 nm using spectrometer (PG Instruments Ltd, T70 UV/VIS Spectrometer, Leichester, England).

Calibration curve was prepared with 5, 10, 15, 20, 25 ppm concentrations of DPPH˙ by using methanol. Calibration curve is given in Appendix A.6.

Since the DPPH˙ solution is very sensitive to light, the volumetric flask where the solution was prepared and the cuvettes which had the sample-DPPH˙ solution mixture, were covered with aluminum foil.

2.2.3.3. Determination of Phenolic Compound Concentrations

High pressure liquid chromatographic (HPLC) technique was used for determination of the phenolic compound concentrations. The HPLC equipment (Shimadzu UFLC, Columbia, USA) was used with the reversed phase column (Agilent Zorbax SB-C18, 250x4.6 mm, 5 μm particle size, Santa Clara, USA). The model of the pump was LC-20AD, autosampler was SIL-20A HT, degasser was GDU-20A5, diode array detector was SPD-M20A and the column oven was CTO-20A. The two mobile phases used were, 5 % acetic acid (CH3COOH) in distilled water (A) and 90 % methanol solution that was prepared with distilled water (B). During the preparation of the standards, 90 % methanol solution was used also. Calibration curves were obtained for each phenolic acid individually and the coefficient of determination values (R^2) were obtained to be greater than 0.98. All of the standards, samples and mobile phases were analyzed after passing through the 0.45 μm filter. For the determination of the wavelengths, the ones which gave the peak values for each of the standard (through the 190 and 800 nm) were chosen.

2.2.3.3.1. HPLC conditions for extracts of caper buds

The phenolic acids of quercetin, rutin, 2-methoxy-4vinylphenol, kaempferol, gallic acid, thymol and guaiacol and vanillin were analyzed at 255, 256, 260, 264, 270, 275 and 281 nm, respectively. Injection volume was 10 μL/min, elution rate was 1 ml/min and temperature was 40 ^{0}C. Table 2.1 shows the gradient program with respect to time.

Table 2.1 Gradient program for extracts of caper buds

time(min)	A%	B%
0.01	100	0
1.0	98	2
4.0	94	6
5.0	88	12
6.0	80	20
7.0	75	25
8.0	70	30
9.0	65	35
15.0	64	36
20.0	63	37
25.0	62	38
30.0	61	39
32.0	61	39
35.0	60	40
40.0	55	45
45.0	50	50
50.0	42	58
53.0	35	65
55.0	28	72
58.0	20	80
60.0	10	90
63.0	40	60
65.0	80	20
68.0	100	0

2.2.3.3.2. HPLC conditions for extracts of oleasters

The phenolic acids of 4-hydroxybenzoic acid, rutin, 3,4-dihyroxybenzoic acid and vanillic acid, kaempferol, gallic acid, benzoic acid, trans-3-hyroxycinnamic acid, p-coumaric acid, ferulic/caffeic/sinapic acid and chlorogenic acid were analyzed at 252, 254, 260, 264, 270, 272, 278, 308, 322 and 326 nm, respectively. Injection volume was 10 μL/min, elution rate was 0.5 ml/min and temperature was 40 ^{0}C. Table 2.2 shows the gradient program with respect to time.

Table 2.2. Gradient program for extracts of oleasters

time(min)	%A	%B
0.01	100	0
1.0	98	2
4.0	94	6
5.0	88	12
6.0	80	20
7.0	75	25
8.0	70	30
9.0	65	35
12.0	60	40
15.0	50	50
20.0	30	70
25.0	23	77
27.0	20	80
29.0	20	80
34.0	18	82
37.0	16	84
39.0	16	84
43.0	15	85
46.0	14	86
50.0	12	88
53.0	12	88
56.0	10	90
57.0	10	90
58.0	12	88
60.0	13	87
64.0	15	85
67.0	17	83
70.0	20	80
72.0	70	30
74.0	100	0

2.2.4. Statistical Analysis

Four way analysis of variance (ANOVA) was performed to determine whether there is a statistically significant difference between different parameters (microwave power, solvent type, solid to solvent ratio and extraction time) and between two extraction methods. If significant difference was found ($p \leq 0.05$), Duncan's multiple comparison method was used for comparison of means.

Statistical Analysis Software (SAS 9.1) program was used throughout the analysis.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Extraction of Phenolic Compounds from Caper

3.1.1. Effect of Microwave Extraction on Total Phenolic Content

When microwave extraction at different conditions were used, total phenolic contents of caper extracts were found to be between 5.0 and 52.4 mg GAE/g dry material. Data are shown in Appendix B.1. Figure 3.1 shows the change of total phenolic content of caper extracts obtained for different extraction times and solid to solvent ratios, when the solvent type was water and power was kept constant at 400 W. As can be seen from the figure, total phenolic content remained almost constant with respect to extraction time. Similar tendency was observed when different solvent types were used (Figure 3.2 and 3.3). The reason for this may be the immediate diffusion of phenolic compounds that are found in caper into the solvents used in this study. In addition, very fast heating mechanism of the microwave may be another reason for this (Sahin and Sumnu, 2006). The decrease in the total phenolic content was observed after long extraction times such as 60 min (Figure 3.4). This may occur because of the deterioration of phenolic substances under prolonged temperature exposure. According to the statistical analysis, time was not found to be a significant ($p \leq 0.05$) parameter (Table D.1). Since there is no significant difference between extraction times of 5, 10 and 15 min, optimum extraction time was chosen as 5 min which was the shortest time. Similar result was observed in microwave extraction of phenolic compounds of melissa and nettle (Ince, 2011). In this study, it was shown that phenolic substances could be extracted in very short times such as 5 min by using microwave extraction and there was no change in total phenolic content with respect to time.

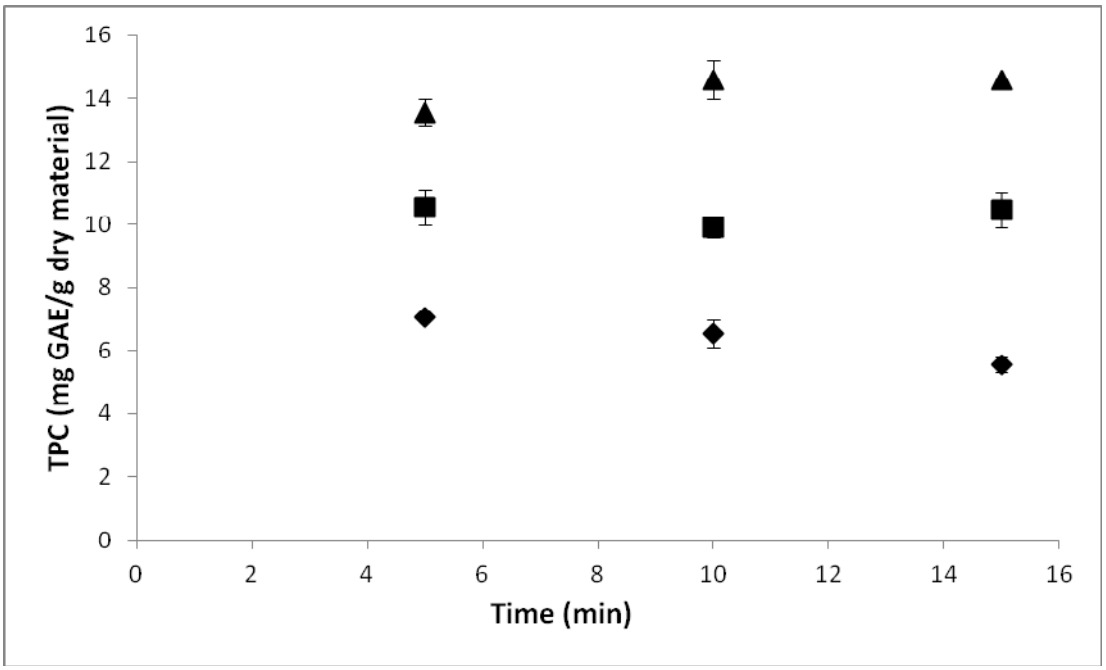

Figure 3.1. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with water at 400 W

♦1:10[c*] ■ 1:20[b] ▲1:30[a]

*Solid to solvent ratios having different letters (A, B, C) are significantly different.

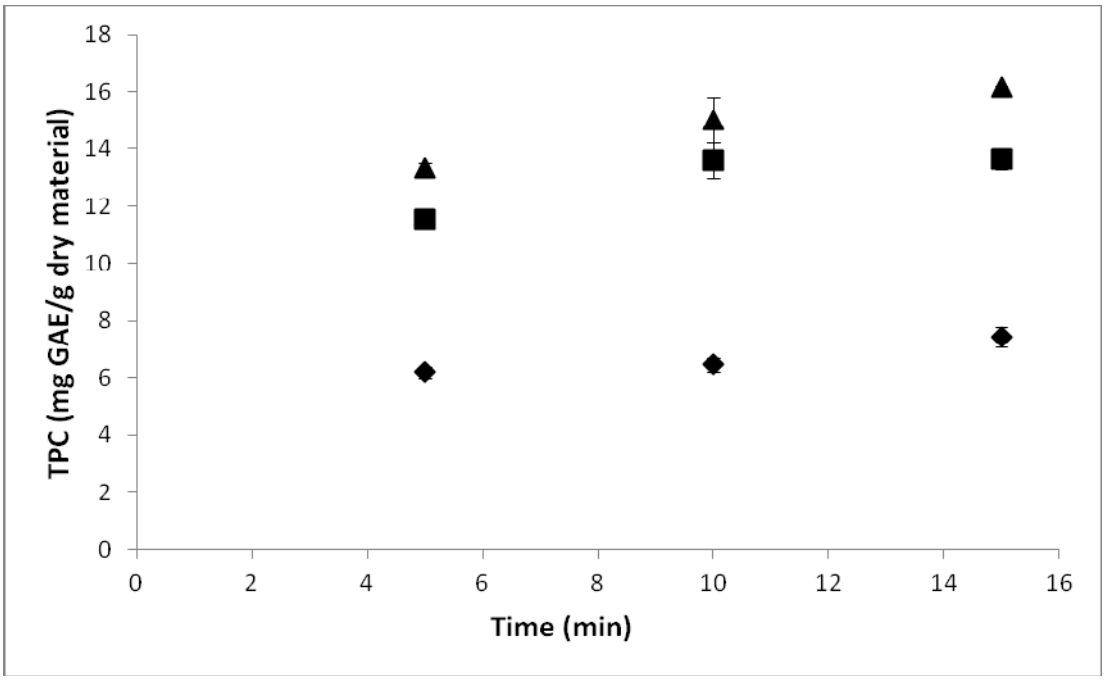

Figure 3.2. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with ethanol at 400 W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

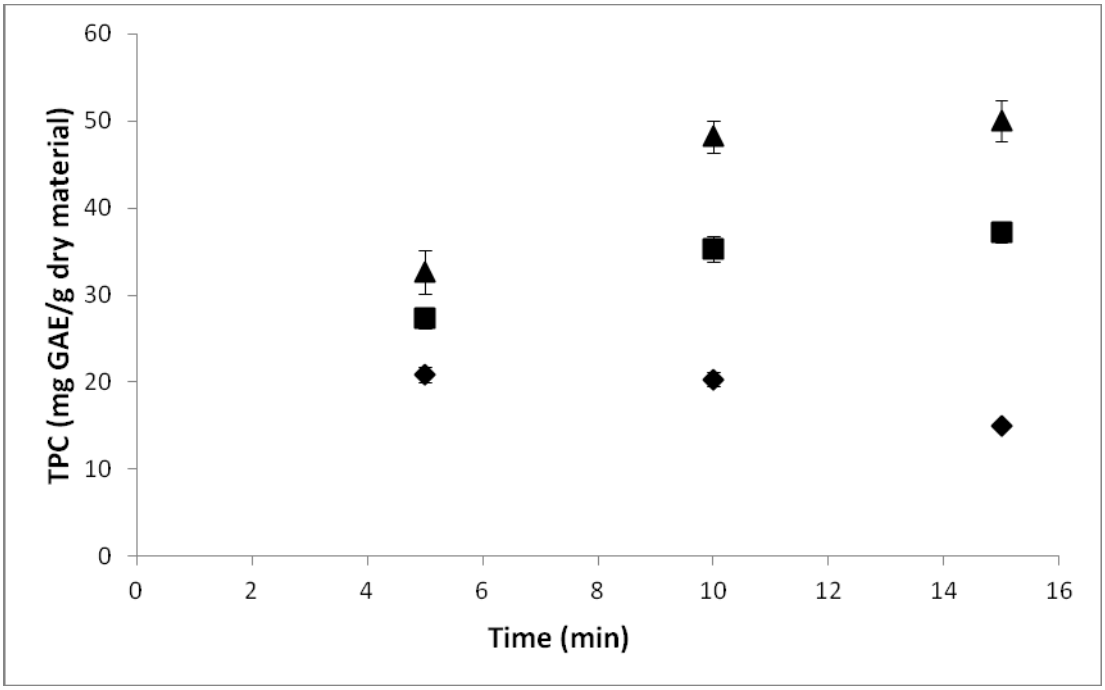

Figure 3.3. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with 50 % ethanol-water mixture at 400 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

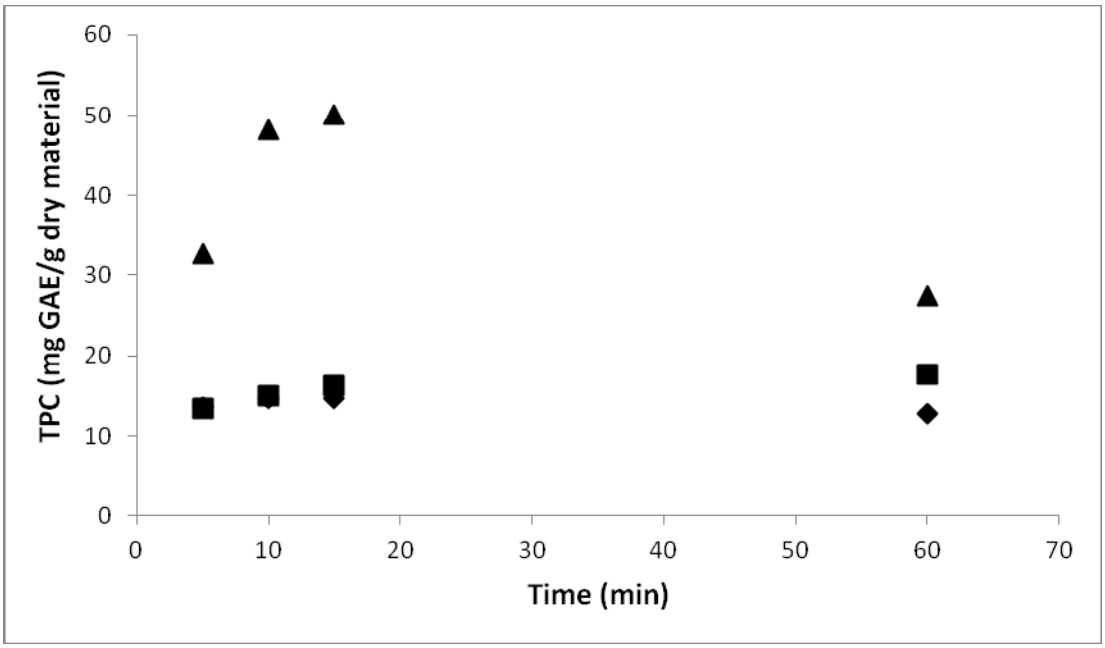

Figure 3.4. Effects of extraction time on total phenolic contents of caper extracts obtained by microwave extraction at 400 W with 1:30 solid to solvent ratio
♦water[b] ■ ethanol[b] ▲50 % ethanol-water mixture[a]

According to ANOVA results (Table D.1), there was a significant difference between solid to solvent ratios of 1:10, 1:20 and 1:30. Total phenolic content increased with increased solvent amount (Figure 3.1-3.3). Similar results were obtained by other researchers (Richter et al., 1996; Alekovski et al., 1998; Xiao et al., 2008; Sayyar et al., 2009; Bi et al., 2010; Yan et al., 2010). The highest total phenolic concentration was obtained when 30 ml solvent/g solid was used. This can be explained by higher concentration gradient with higher amount of solvent (Adil et al., 2008; Cacace and Mazza, 2003b).

The experiments of 1:40 and 1:50 of solid to solvent ratios were performed for the ethanol-water mixture only in order to determine the change of total phenolic concentration when higher amount of solvent was used. The effect of different solid to solvent ratios on concentration of total phenolic substances extracted with water-ethanol mixture by using microwave at 400 W for 5 min can be seen in Figure 3.5. According to the statistical analysis (Table D.2), there was no significant difference between 1:30, 1:40 and 1:50 solid to solvent ratios; hence experiments were proceeded with 1:10, 1:20 and 1:30 ratios.

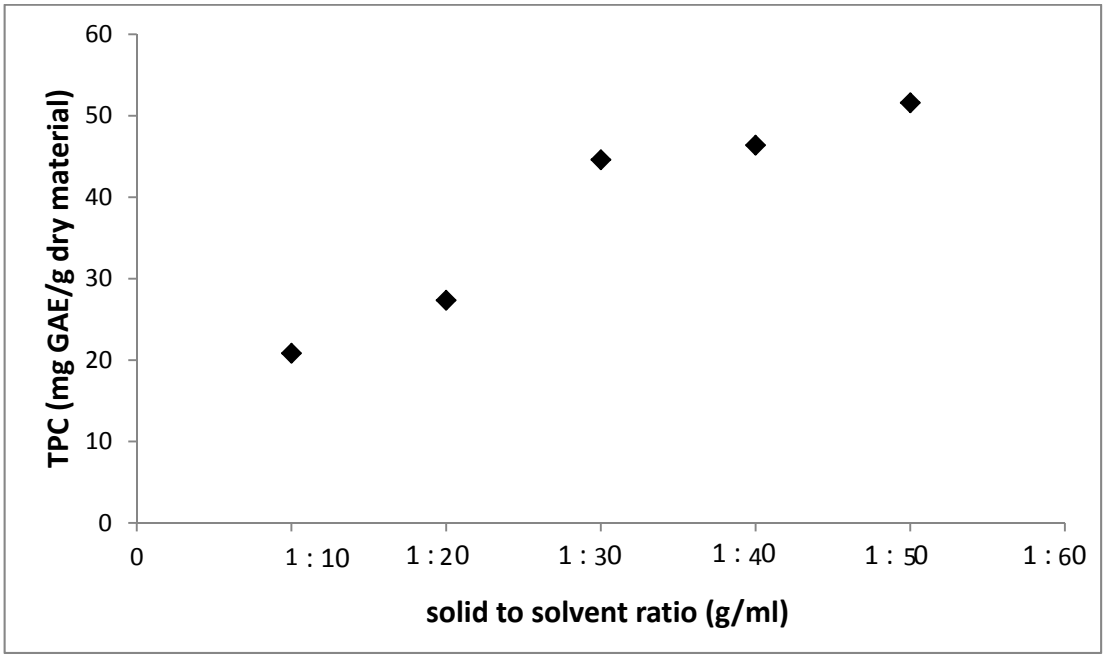

Figure 3.5. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with 50 % ethanol-water mixture at 400 W and for 5 min

Different ratios of ethanol and water mixtures (25:75, 50:50, 75:25 (v/v)) were also tried in order to determine the optimum percentage of ethanol:water mixture. Statistically, concentrations of total phenolic substances were found to be lower when ethanol-water mixtures at ratios of 25:75 and 75:25 were used as compared to 50:50 ratio (Figure 3.6). Hence the experiments were performed with 50:50 ethanol-water mixture ratio. Rostagno et al. (2007) also found 50% as the best ethanol-water mixture for the extraction of isoflavones by using microwave.

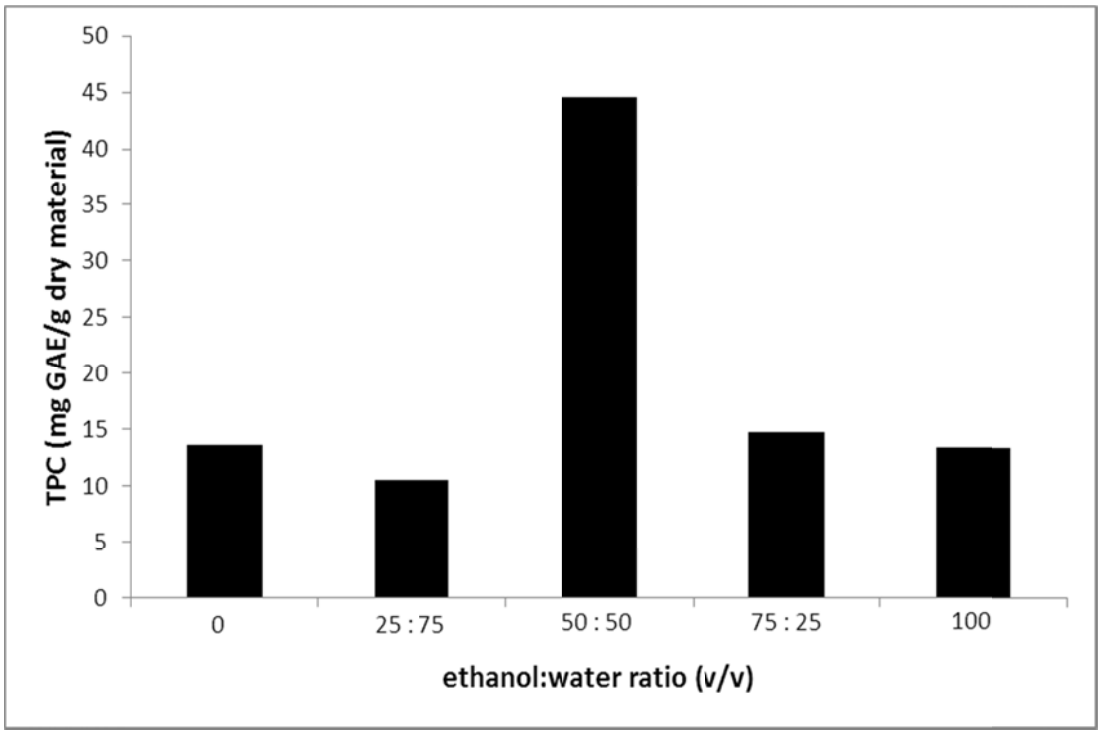

Figure 3.6. Effects of different ethanol to water ratios on total phenolic contents of caper extracts obtained by microwave extraction with 1:30 solid to solvent ratio at 400 W and for 5 min

According to ANOVA results (Table D.1), the effects of different solvent types on total phenolic content were significantly different. The highest phenolic concentration was obtained when ethanol-water mixture was used (Figure 3.7). This is related to dielectric properties of solvent. Dielectric properties have an important role for interaction of the food with microwave energy. It is known that ethanol-water mixture has higher dielectric properties than pure water and pure ethanol (Mudgett, 1995). The synergistic effect that is revealed by ethanol-water mixture can

be explained by hydrogen bonds between water and ethanol that increases the dielectric properties. As a result, this solvent type can be heated faster in microwave oven than the other solvents and extraction yield can increase.

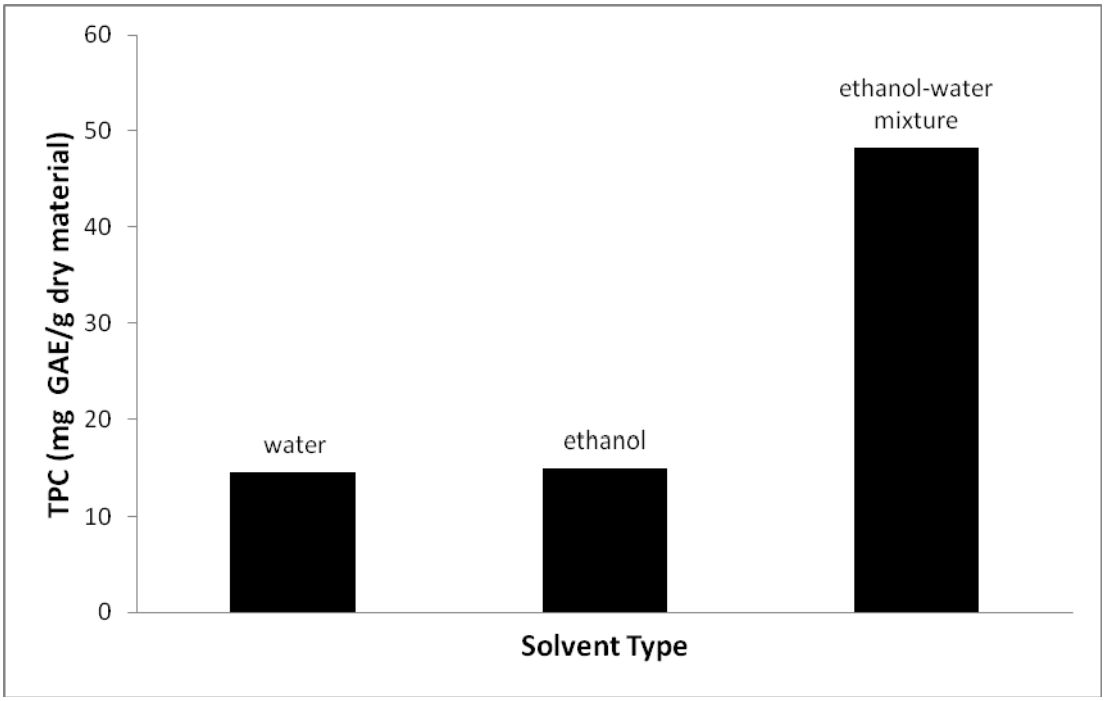

Figure 3.7. Effects of different solvent types on total phenolic contents of caper extracts obtained by microwave extraction with 1:30 solid to solvent ratio at 400 W and for 10 min

The results of total amount of phenolics when 700 W of microwave power was used were similar with the ones obtained with 400 W (Figure 3.8-3.10). The influence of microwave power on total phenolic content was found to be insignificant (Table D.1). Hong et al. (2001), who studied microwave-assisted extraction of phenolic substances from grape seeds, also found that power had no significant effect on the yield and on the polyphenol content of the extracts. In another recent study about the potato peel extracts, it was also found that microwave power had no significant impact on the concentration of phenolics (Singh et al., 2011).

In conclusion, considering the total phenolic content, when solvent to solid ratio increased total phenol concentration also increased and the highest value was obtained at 1:30 solid to solvent ratio. The highest total phenolic content was

obtained when ethanol:water mixture was used in 1:1 ratio as solvent. The best microwave power and extraction time were chosen as 400 W and 5 min, respectively, since higher microwave power and longer time were not significantly effective on increasing total phenolic content. The total phenolic content was 32.6 mg GAE/g dry material for the extraction of capers with 50% ethanol-water mixture, at 1:30 solid to solvent ratio, at 400 W microwave power for 5 min.

Unver et al. (2009), investigated phenolic content of some different caper species, and found the total phenolic content of *Capparis Ovata* as 185.54 mg GAE/g fresh material, which was almost equal to 30 mg GAE/g dry material. This result is similar with the result that was found in this study.

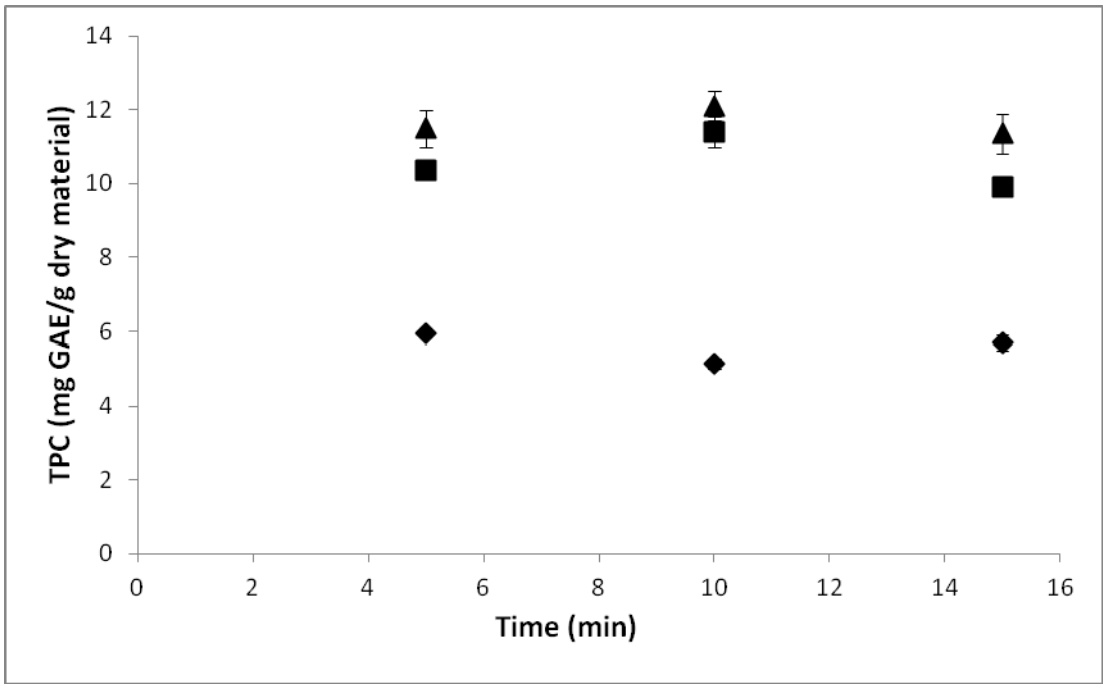

Figure 3.8. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with water at 700 W
♦1:10[c] ■ 1:20[b] ▲1:30[a]

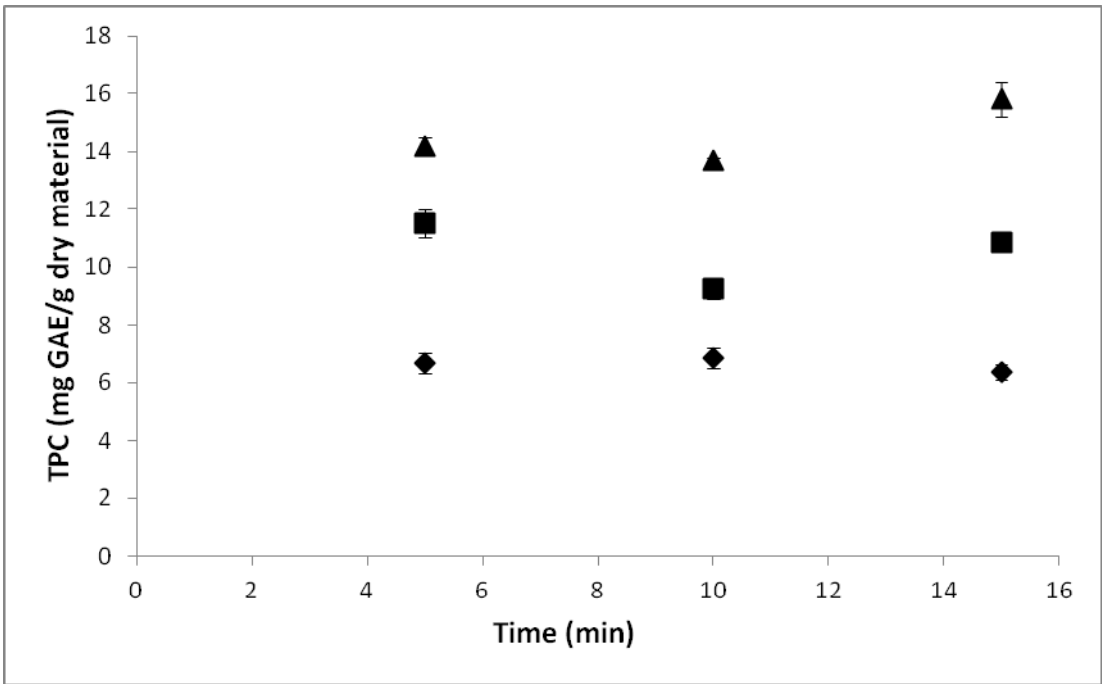

Figure 3.9. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with ethanol at 700 W
♦1:10[c] ■ 1:20[b] ▲1:30[a]

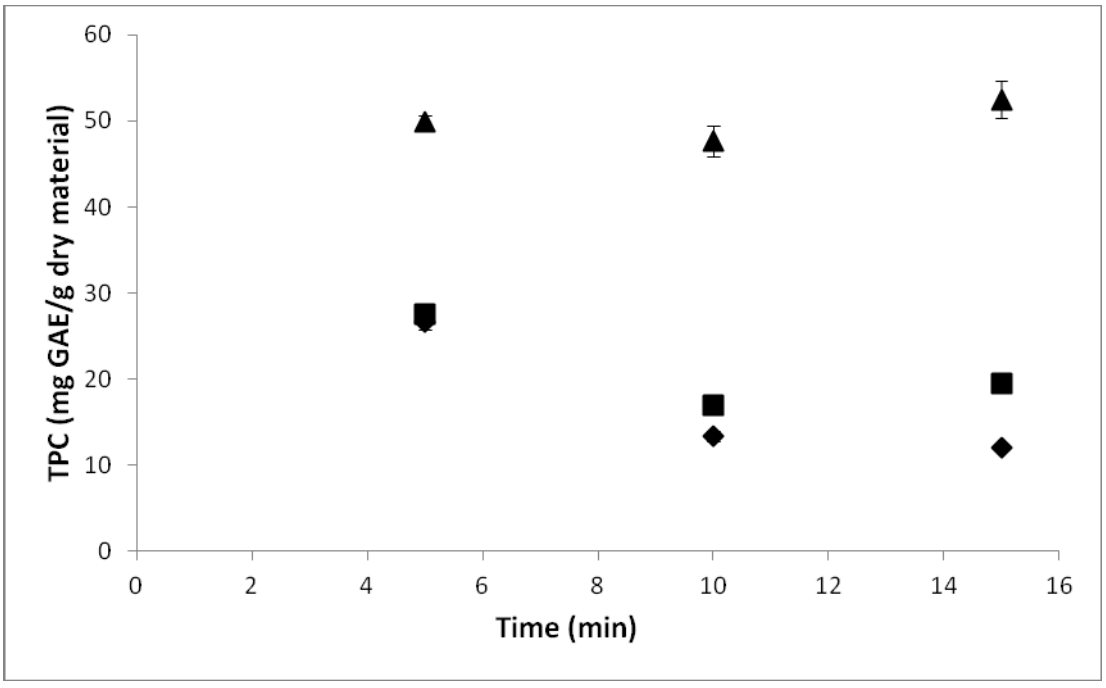

Figure 3.10. Effects of different solid to solvent ratios on total phenolic contents of caper extracts obtained by microwave extraction with 50 % ethanol-water mixture at 700 W ♦1:10[c] ■ 1:20[b] ▲1:30[a]

3.1.2. Comparison of Microwave and Conventional Extraction in terms of Total Phenolic Content

In the conventional extraction experiments, 1:30 solid to solvent ratio was used for the comparison of microwave method with the conventional method, since the total phenolic concentration was the highest at the solid to solvent ratio of 1:30 as compared to 1:10 and 1:20. Total phenolic contents of capers which were extracted by using conventional method were found to be between 14.1 and 41.1 mg GAE/g dry material. The graphs that show the conventional extraction of total phenolic content as a function of time for different solvents can be seen in Figure 3.11, 3.12 and 3.13. Data are shown in Appendix B.2.

The best extraction time was 1 h for conventionally extracted capers in water (Figure 3.11). When water was used, the amount of total phenolic content obtained at this condition was similar to the ones obtained by using microwave at 400 W for 5 min for solid to solvent ratio of 1:30. In other words, using microwave extraction for 5 min can substitute conventional extraction for 1 h. This time difference can be explained by increased mass transfer between solid and solvent due to high pressure gradient inside the cell which builds up as a result of microwave extraction.

It can be seen from Figure 3.12 and 3.13 that the best extraction times were 4 h in conventional extraction with ethanol and ethanol-water mixture. The maximum total phenolic content could be reached at 4 h of extraction time and a decrease was observed after this time (Table D.3). The reason for this decrease may be the destruction of phenolic compounds when the extraction time was extended. Total phenolic content was 41.1 mg GAE/g dry material when ethanol-water mixture and conventional set-up was used, while it was 48.2 mg GAE/g dry material when microwave at 400 W for 10 min was used.

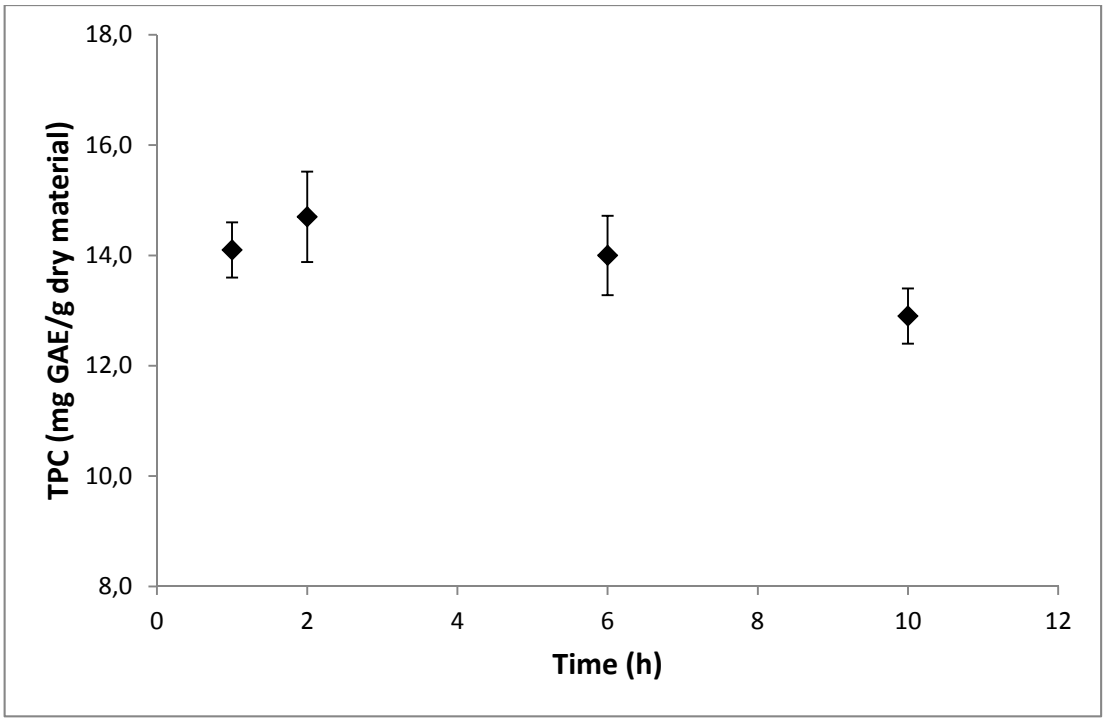

Figure 3.11. Change in total phenolic content of caper extracts obtained by conventional extraction in water with respect to time

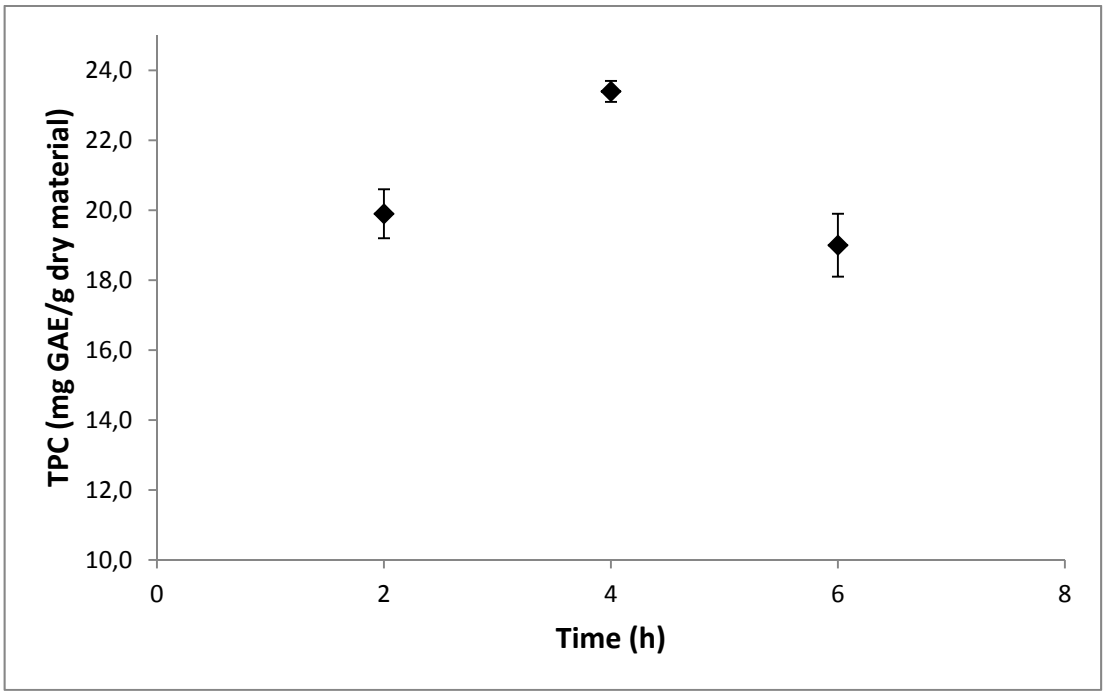

Figure 3.12. Change in total phenolic content of caper extracts obtained by conventional extraction in ethanol with respect to time

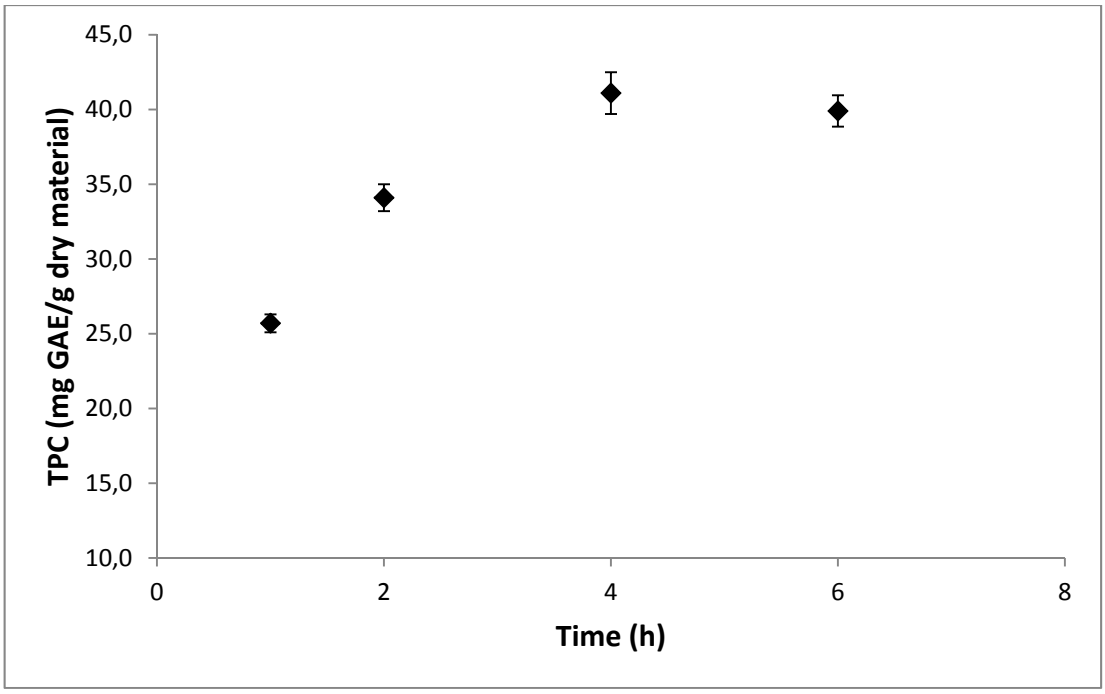

Figure 3.13. Change in total phenolic content of caper extracts obtained by conventional extraction in 50 % ethanol-water mixture with respect to time

3.1.3. Effect of Microwave Extraction on Total Antioxidant Activity

Antioxidant activities of the caper extracts were found to be between 0.5 and 2.0 mg DPPH/g dry material by using microwave extraction, at different conditions. Figure 3.14-3.16 shows the antioxidant activities of caper extracts obtained at 400 W microwave power; when water, ethanol and ethanol-water mixture (1:1 v/v) were used as solvents in different solid:solvent ratios (1:10, 1:20, 1:30 g/ml) with respect to time. Similar trend in total phenolic content graphs can be seen in antioxidant activity graphs. Increase in solvent to solid ratio resulted in higher antioxidant activity. As a result of statistical analysis, antioxidant activity of the extract obtained by 1:30 solid to solvent ratio was higher than that obtained with 1:20 solid:solvent ratio and antioxidant activity of the extract obtained by 1:20 solid:solvent ratio was found to be higher than that obtained with 1:10 solid:solvent ratio. The effect of extraction time on antioxidant activity was found to be insignificant, similar to the results of total phenolic content (Table D.4).

The maximum antioxidant activities were obtained when ethanol and water mixture was used as solvent. The reason of obtaining higher antioxidant activity with ethanol-water mixture was the synergetic effect of solvents on affecting antioxidant activity.

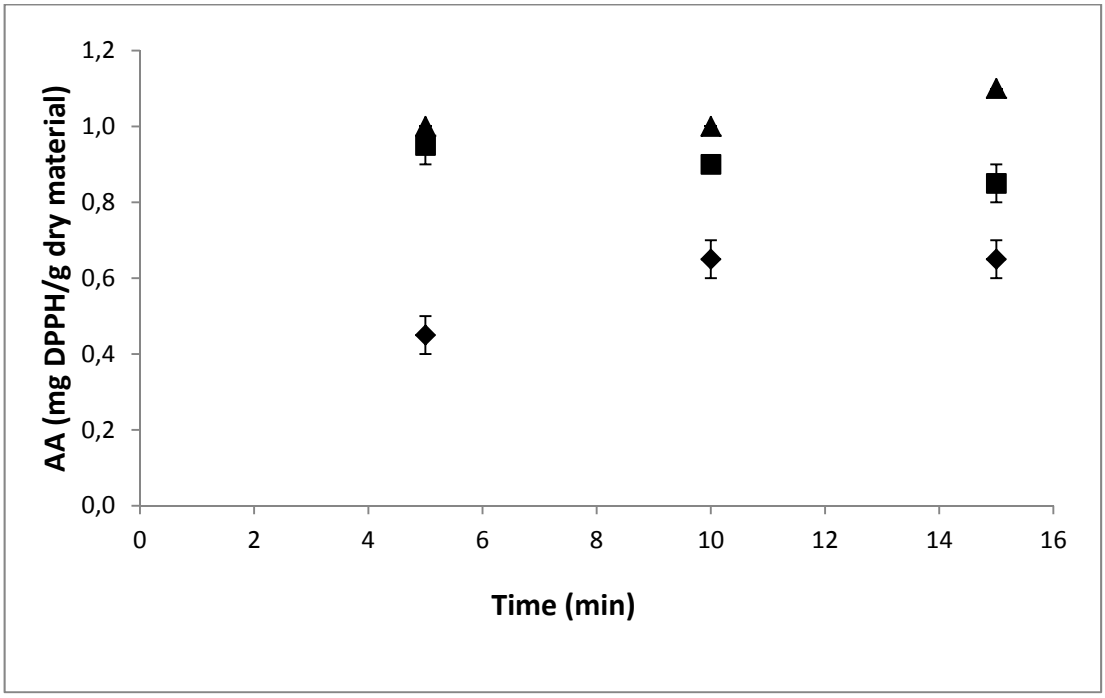

Figure 3.14. Effects of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with water at 400 W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

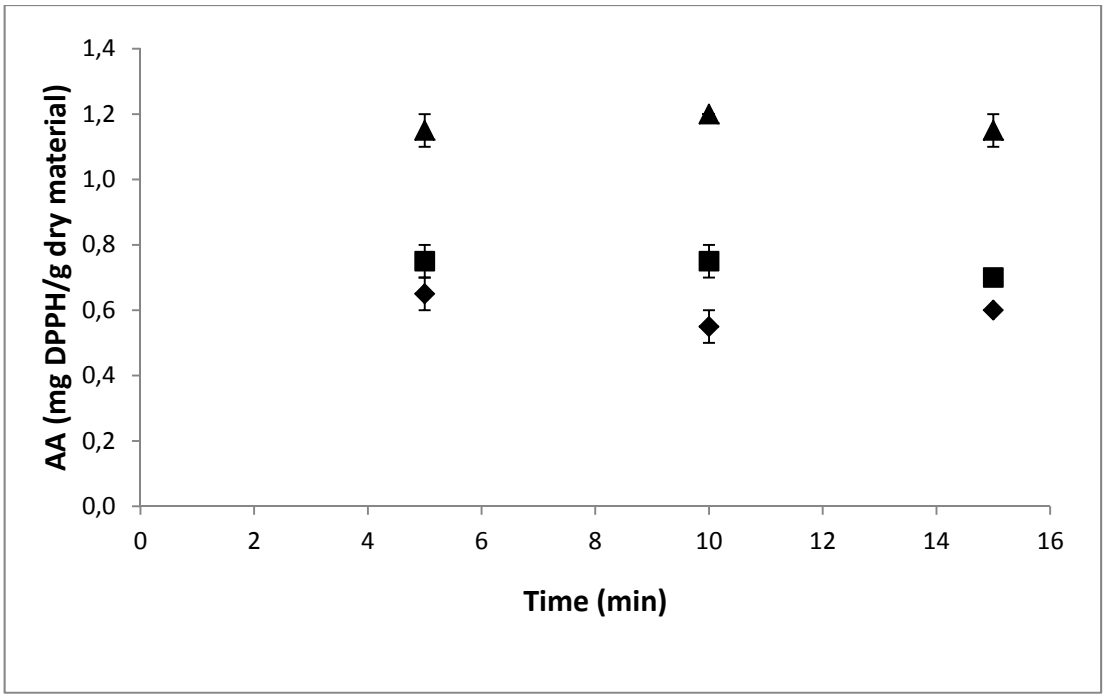

Figure 3.15. Effects of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with ethanol at 400 W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

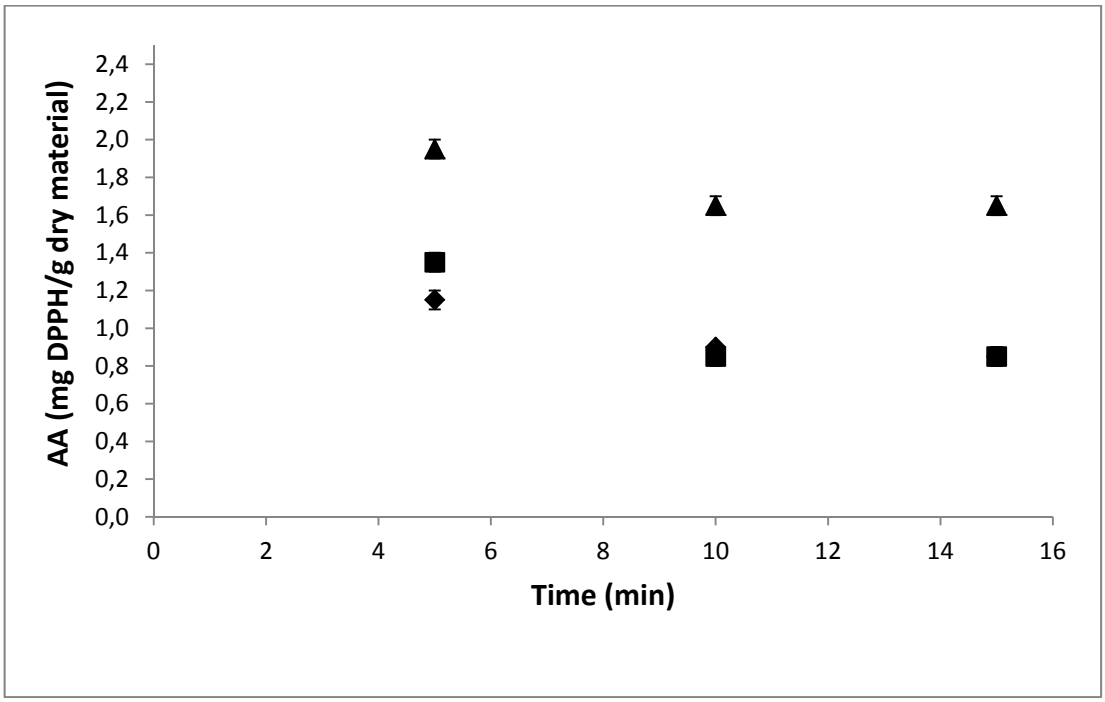

Figure 3.16. Effects of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with 50 % ethanol-water mixture at 400W ♦1:10[c] ■ 1:20[b] ▲1:30[a]

The graphs that show the antioxidant activities of phenolic compounds of capers extracted with 700 W microwave power can be seen in Figure 3.17, 3.18 and 3.19. Increase in solvent to solid ratio increased the antioxidant activity also when 700 W microwave power was used. The most effective solvent was found as ethanol-water mixture. Similar to total phenolic content, there was no significant difference between 400 W and 700 W microwave powers (Table D.4).

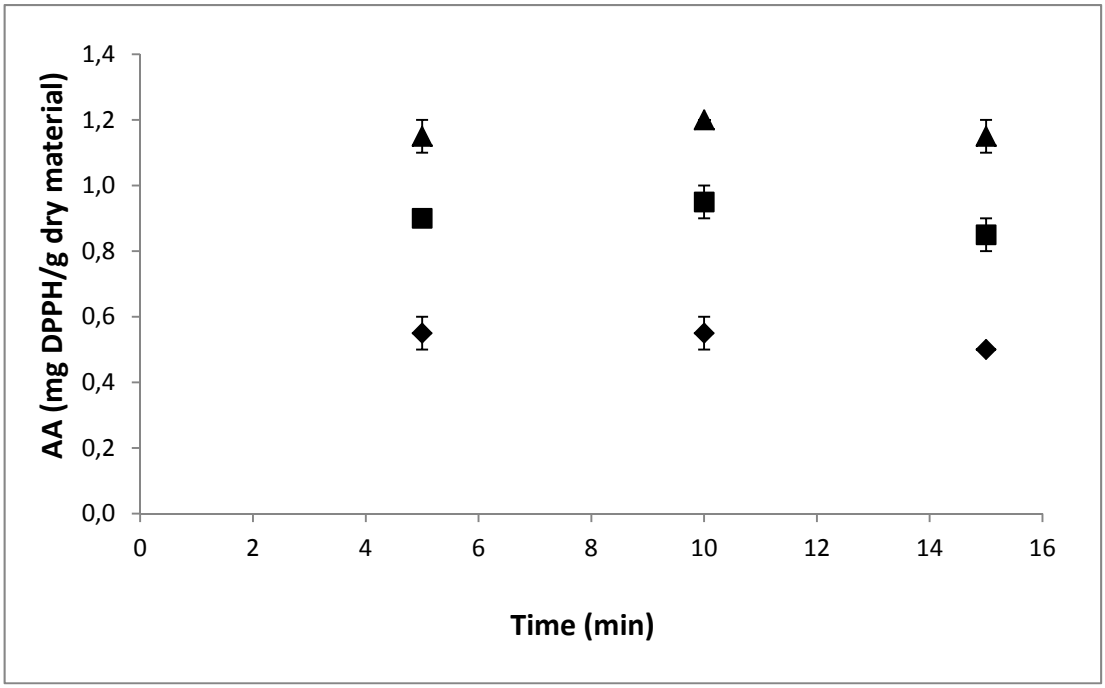

Figure 3.17. Effects of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with water at 700 W
♦1:10[c] ■ 1:20[b] ▲1:30[a]

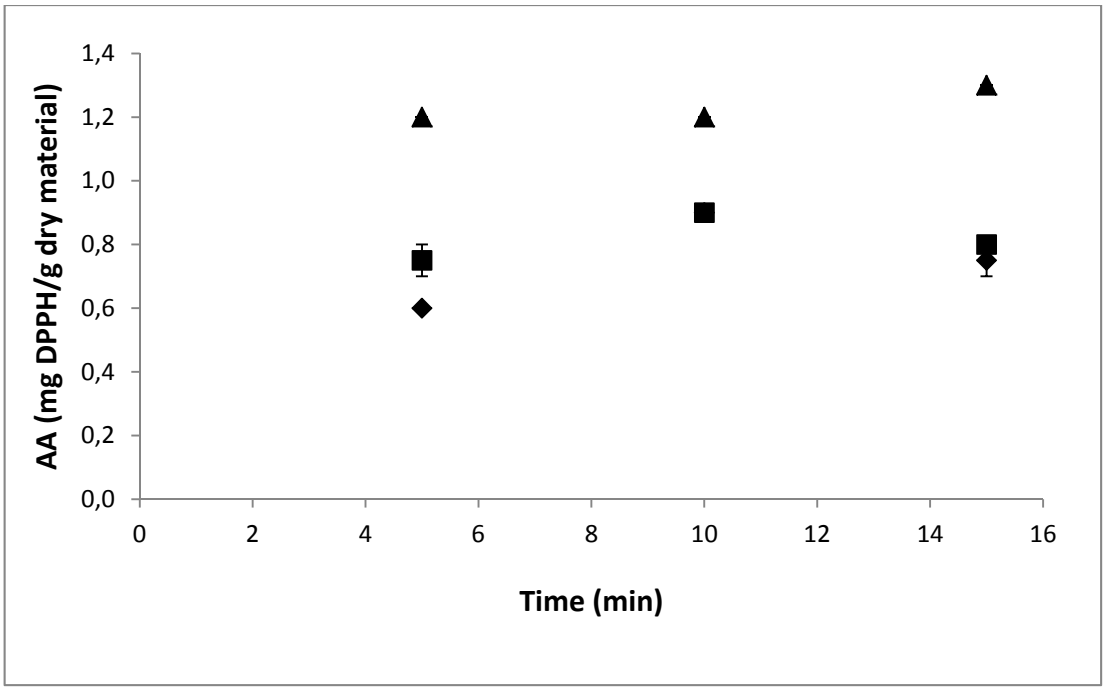

Figure 3.18. Effects of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with ethanol at 700 W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

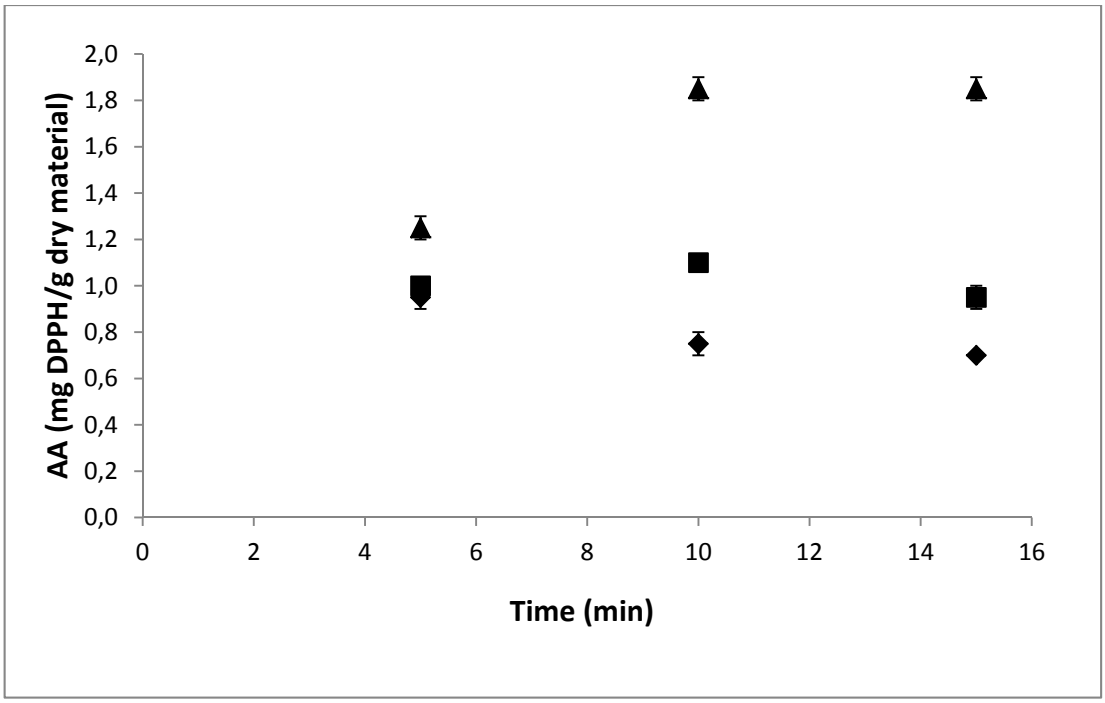

Figure 3.19. Effect of different solid to solvent ratios on antioxidant activity of caper extracts obtained by microwave extraction with 50 % ethanol-water mixture at 700W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

3.1.4. Comparison of Microwave and Conventional Extraction in terms of Antioxidant Activity

Figure 3.20 shows the change in antioxidant activity with respect to time when conventional extraction method was used. When the solvent was water, the maximum antioxidant activity was 1.2 mg DPPH/g dry material. This amount was reached in 2 h by conventional extraction method, while the same amount was reached in 5 min by microwave extraction method.

The maximum antioxidant activity was found as 2.8 mg DPPH/g dry material, when 30:1 ml/g ratio of ethanol-water mixture to solid was used in conventional extraction method. This result was not statistically different than the 2.0 mg DPPH/g dry material which was obtained by 400 W microwave extraction for 5 min with 1:30 solid to solvent ratio (Table D.5).

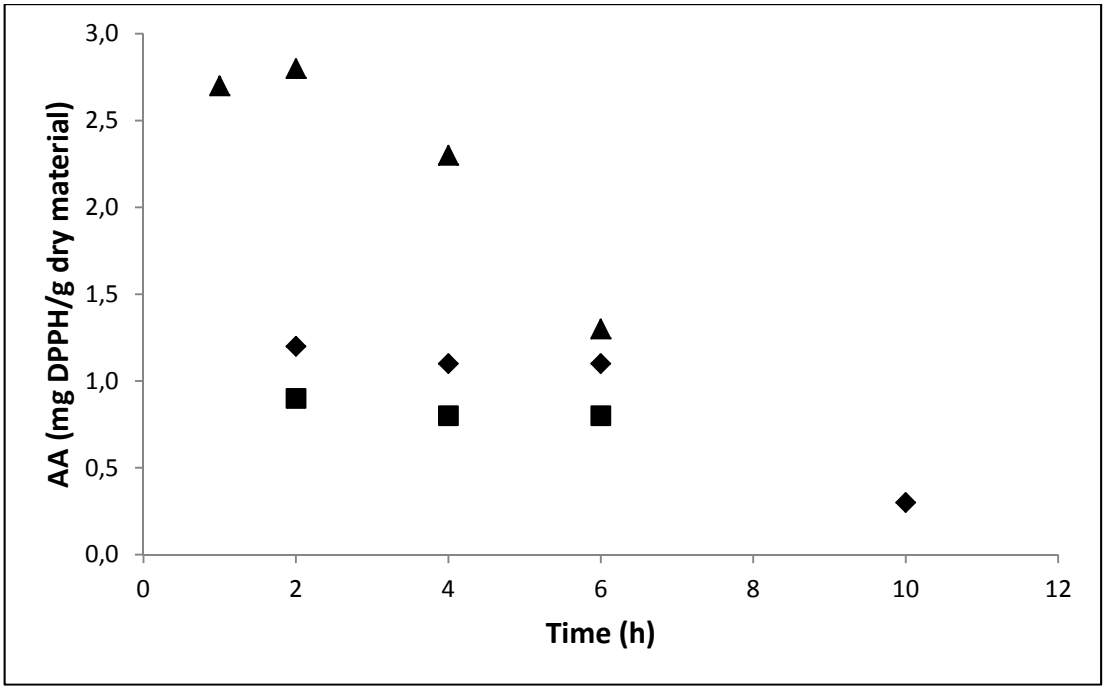

Figure 3.20. Change in antioxidant activity of caper extracts in different solvents obtained by conventional extraction with respect to time

♦ water[b] ■ ethanol[b] ▲ 50 % ethanol-water mixture[a]

3.1.5. Comparison of Microwave and Conventional Extraction in terms of Concentration of Phenolic Acids

In high pressure liquid chromatography, the extracts which gave the maximum total phenolic compounds in microwave method (1:30 solid to solvent ratio, 5 min extraction time and 400 W microwave power) and in conventional method (1:30 solid to solvent ratio, 2 h of extraction with water and 4 h of extraction with ethanol and ethanol-water mixture) were analyzed.

Table 3.1 shows the concentration of 7 kinds of phenolic compounds that is found in the extracts, namely quercetin, rutin, kaempferol, gallic acid, guaiacol, thymol and vanillin. The amounts were expressed as mg/g dry material, except for guaiacol. The unit for guaiacol is µl/g dry material.

Table 3.1. Concentration of phenolic compounds of caper extracts in different solvents for the best parameters*

Extraction Method	Solvent Type	quercetin	rutin	kaempferol	gallic acid	guaiacol	thymol	vanillin
Microwave	Water	0.9	11.4	2.4	1.4	1.6	5.0	0.4
Microwave	Ethanol	-	8.5	3.1	0.6	0.9	2.9	0.3
Microwave	Ethanol-Water Mixture	0.9	15.4	3.4	0.7	2.2	2.4	0.4
Conventional	Water	-	12.9	2.4	1.6	1.8	3.2	0.4
Conventional	Ethanol	0.9	12.0	3.4	0.6	0.4	5.2	0.3
Conventional	Ethanol-Water Mixture	0.9	17.7	3.5	0.6	2.2	3.3	0.4

*For microwave extraction; 400 W, 5 min and 1: 30 solid to solvent ratio were used.

For conventional extraction; 1:30 solid to solvent ratio was used.

The maximum amount of phenolic compound was found as rutin. Rutin, also known as vitamin P, is an important natural phenolic compound, because it has antioxidant, anti-inflammatory and anticarcinogenic effects and it decreases the fragility of blood vessels (Ihme et al., 1996). Tlili et al. (2010) investigated the amount of rutin in caper cultivated in different regions, and found as 13.5 mg/g fresh material in *Capparis Spinosa*, which was approximately equivalent to 2 mg/g dry material.

Following rutin, kaempferol gave the maximum phenolic concentration. Tomas-Barberan and Clifford (2000), in their study about flavonoid content of *Capparis Spinosa*, found the rutin as main phenolic compound and the kaempferol as the second highest content, as it was found in this study.

In general, there was no difference between microwave and conventional extraction methods in terms of concentration of phenolic compounds. The solubilities of the phenolic compounds in solvents are different. Higher amounts of rutin and kaempferol were detected when ethanol and water mixture was used for extraction in both microwave extraction and conventional method. These compounds may dissolve more in ethanol-water mixture.

Although the highest total phenolic contents were analyzed with ethanol-water mixture, gallic acid was detected more in water extracts. This may be related with hydrophilic structure and water solubility of gallic acid.

Chromatograms prepared at different wavelengths can be seen in Appendix C.1.

3.2. Extraction of Phenolic Compounds from Oleaster

In total phenolic content determination, the disadvantage of Folin Ciocalteau method is the interference of phenolics with sugar present in the sample. A correction factor should be subtracted from apparent phenolic content in order to eliminate this interference. However, it is stated that the correction of sugar interference is only necessary if the sugar content is greater than 2% (w/v) in extract (Slinkard and Singleton, 1977). Oleaster contains 27.1% fructose and 22.3% glucose (Ayaz and Bertoft, 2001) which correspond to 1.8% sugar content (w/v) in the extracts. For this reason, sugar content of oleaster was ignored and no correction value was used during total phenolic content calculations.

Maturation level is an effective parameter on total phenolic content of oleasters. In nature, oleasters have firstly a yellowish color and become reddish during ripening. In this study, oleasters with random colors were picked up. After performing some experiments, it was decided to continue the experiments with the yellow oleasters, because yellow oleasters gave greater total phenolic content than reddish orange oleasters. This may be because of degradation of phenolic substances after exposure of sunlight and high temperatures for a long time on the tree. Another reason of the lower total phenolic content in reddish orange oleasters may be the increased sugar content of oleasters after becoming more mature. Sakamura and Suga (1987) investigated the changes in chemical components during ripening of oleaster fruits and found that the total phenolic content difference between yellow and reddish-orange color oleaster species is 50%.

In order to show the effect of maturity, the graphs of experiments done with reddish orange oleasters (mature ones), were also given in the following section after discussing the total phenolic results of yellow oleasters (unripe ones).

3.2.1. Effect of Microwave Extraction on Total Phenolic Content

Total phenolic contents of oleaster extracts were found to range between 2.6 and 43.0 mg GAE/g dry material when microwave extraction at different conditions was used. Data are shown in Appendix B.3.

Figure 3.21, 3.22 and 3.23 show the change in total phenolic content of oleaster extracts obtained for different extraction times, when power was kept constant at 400 W and the solvent type was water, ethanol and 50% ethanol-water mixture, respectively.

As can be seen from the figures, total phenolic content increased with respect to extraction time and then became constant or decreased. The decrease might be explained by the deterioration of some phenolic substances in oleaster during 30 min of extraction.

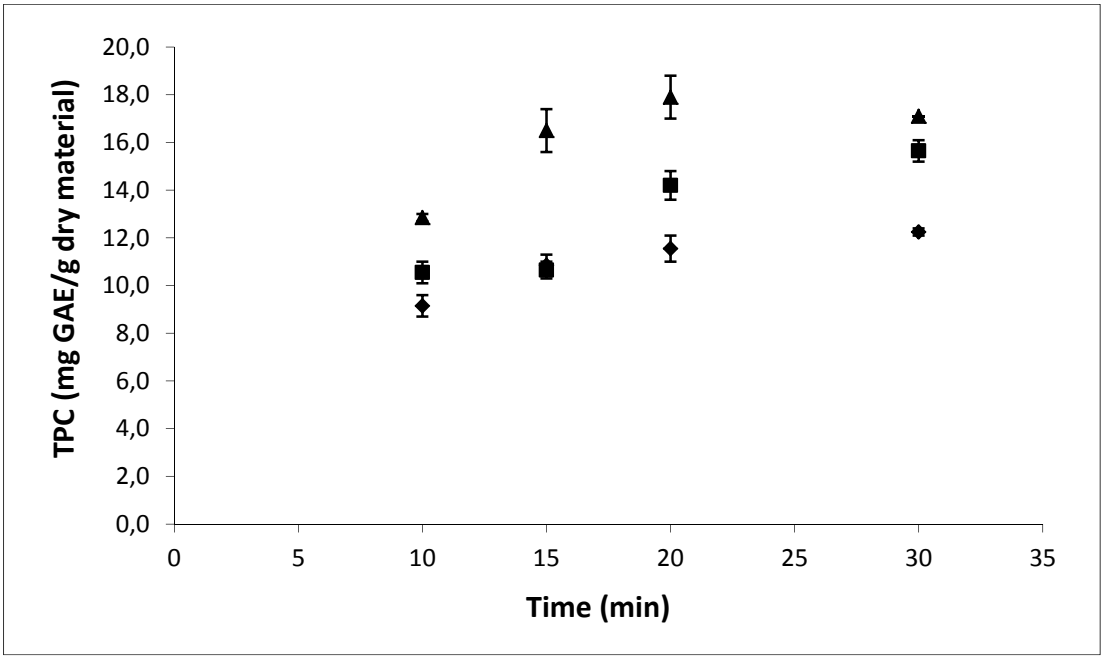

Figure 3.21. Effects of different solid to solvent ratios on total phenolic contents of oleaster extracts obtained by microwave extraction with water at 400 W

♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

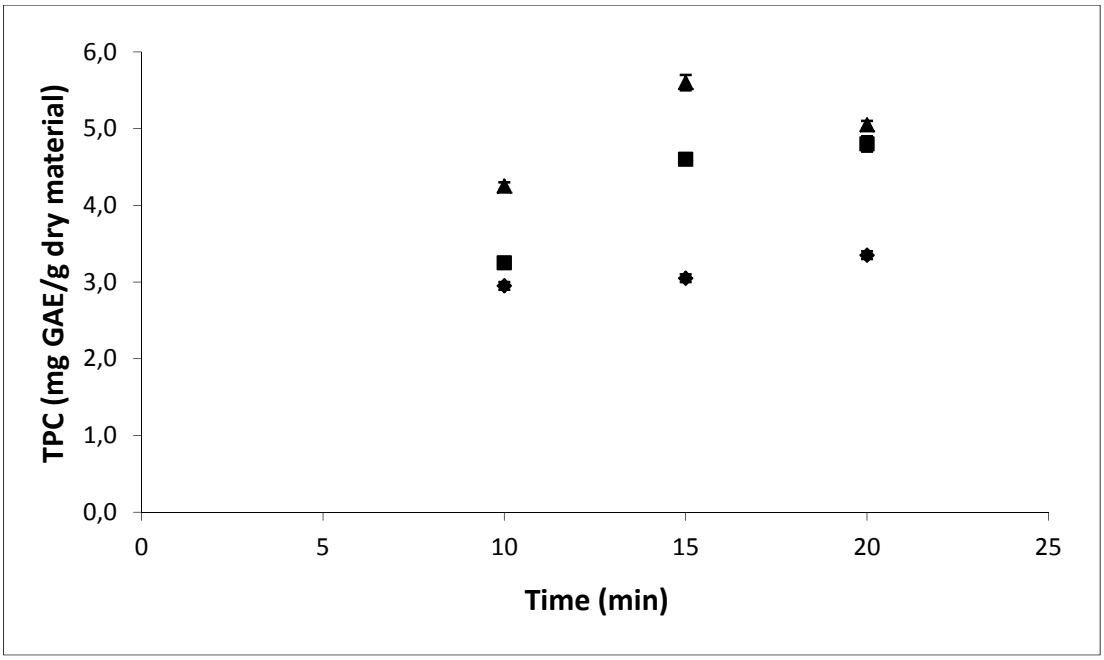

Figure 3.22. Effects of different solid to solvent ratios on total phenolic contents of oleaster extracts obtained by microwave extraction with ethanol at 400 W

♦1:10[c] ■ 1:20[b] ▲1:30[a]

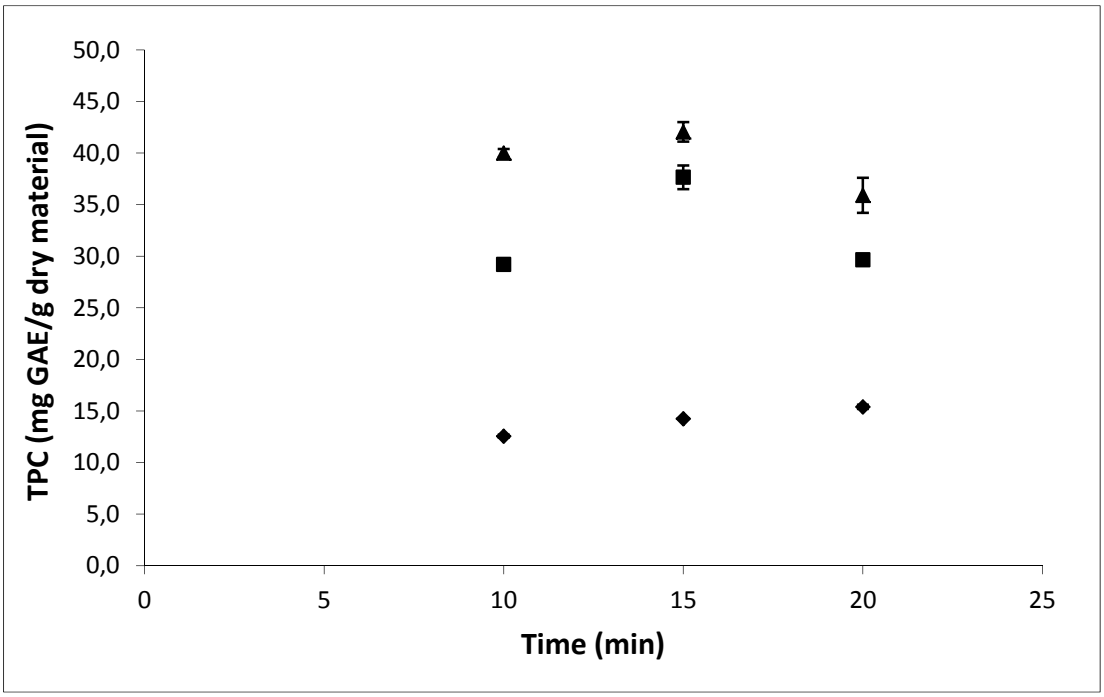

Figure 3.23. Effects of different solid to solvent ratios on total phenolic contents of oleaster extracts obtained by microwave extraction with 50 % ethanol-water mixture at 400 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

As can be seen from Figure 3.21-3.23, ethanol-water mixture gave higher total phenolic content. This might be explained by increased dielectric properties due to synergistic effect of ethanol and water mixture. This result was also similar with that of caper experiments.

In order to determine the best conditions giving the highest phenolic content, statistical analysis was performed with 50% ethanol-water mixture. Solid to solvent ratio of 1:30 gave statistically higher results than 1:20, and 1:20 ratio gave higher results than 1:10. These results were similar with the caper results. Higher solvent amount resulted in higher concentration gradient and higher driving force between solid and solvent (Adil et al., 2008; Cacace and Mazza, 2003b). Moreover, 15 min was found to be the best extraction time as a result of ANOVA results (Table D.6).

According to ANOVA results, microwave power was not found to be a significant ($p \leq 0.05$) parameter in extraction of phenolic compounds from oleaster. The best extraction times were chosen as 20 and 10 min when water and ethanol were used as solvents, respectively (Table D.7, Table D.8).

Figure 3.24, 3.25, 3.26 and 3.27 show the change in total phenolic content of "reddish orange" (mature) oleaster extracts obtained for different extraction times, and solvent types and microwave powers. Similar to unripe oleaster results the highest solvent to solid ratio resulted in the highest total phenolic content.

The only difference between the results of mature and unripe oleasters is that mature oleasters had lower total phenolic content as compared to unripe ones (Figure 3.21-3.23 and Figure 3.24-3.27).

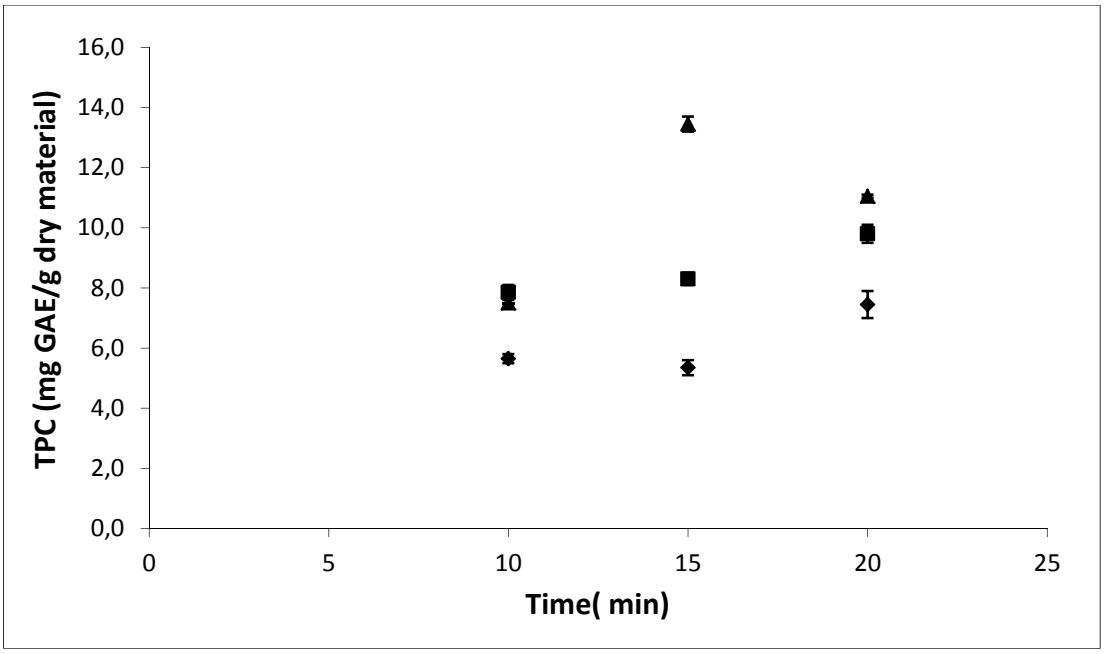

Figure 3.24. Effects of different solid to solvent ratios on total phenolic contents of mature oleaster extracts obtained by microwave extraction with water at 400 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

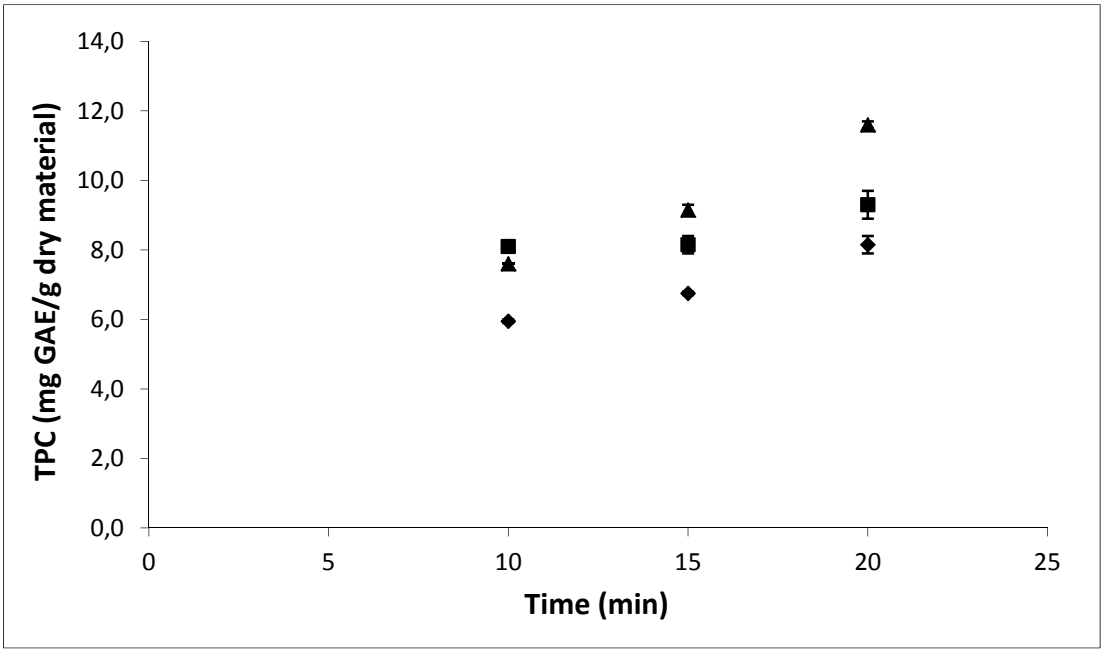

Figure 3.25. Effects of different solid to solvent ratios on total phenolic contents of mature oleaster extracts obtained by microwave extraction with water at 700 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

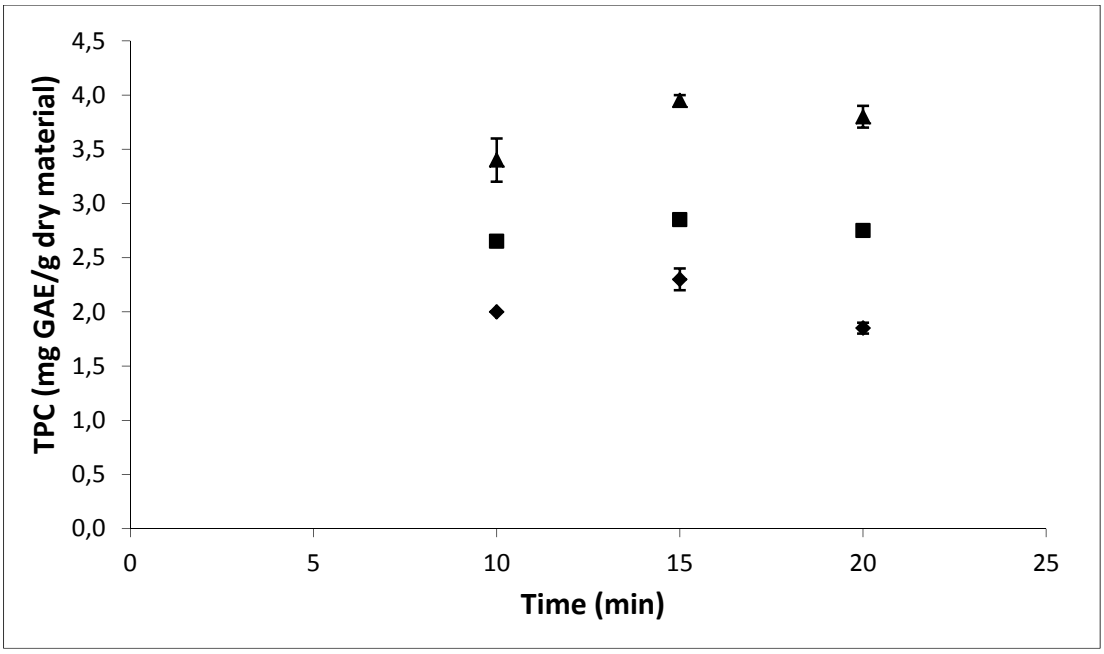

Figure 3.26. Effects of different solid to solvent ratios on total phenolic contents of mature oleaster extracts obtained by microwave extraction with ethanol at 400 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

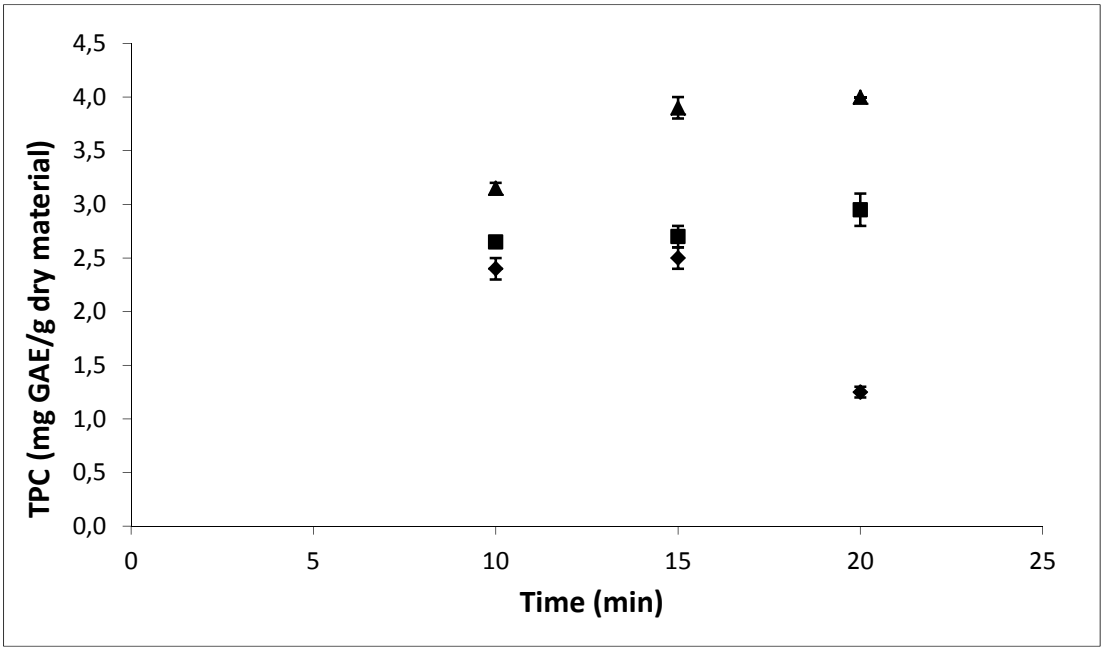

Figure 3.27. Effects of different solid to solvent ratios on total phenolic contents of mature oleaster extracts obtained by microwave extraction with ethanol at 700 W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

3.2.2. Comparison of Microwave and Conventional Extraction in terms of Total Phenolic Content

In the conventional extraction experiments, solid to solvent ratio of 1:30 was used for comparing microwave method with the conventional method since the total phenolic content was the highest at the solid to solvent ratio of 1:30 as compared to others. Total phenolic contents of oleasters which were extracted by using conventional method were found to be between 26.3 and 52.2 mg GAE/g dry material (Figure 3.28 and 3.29).

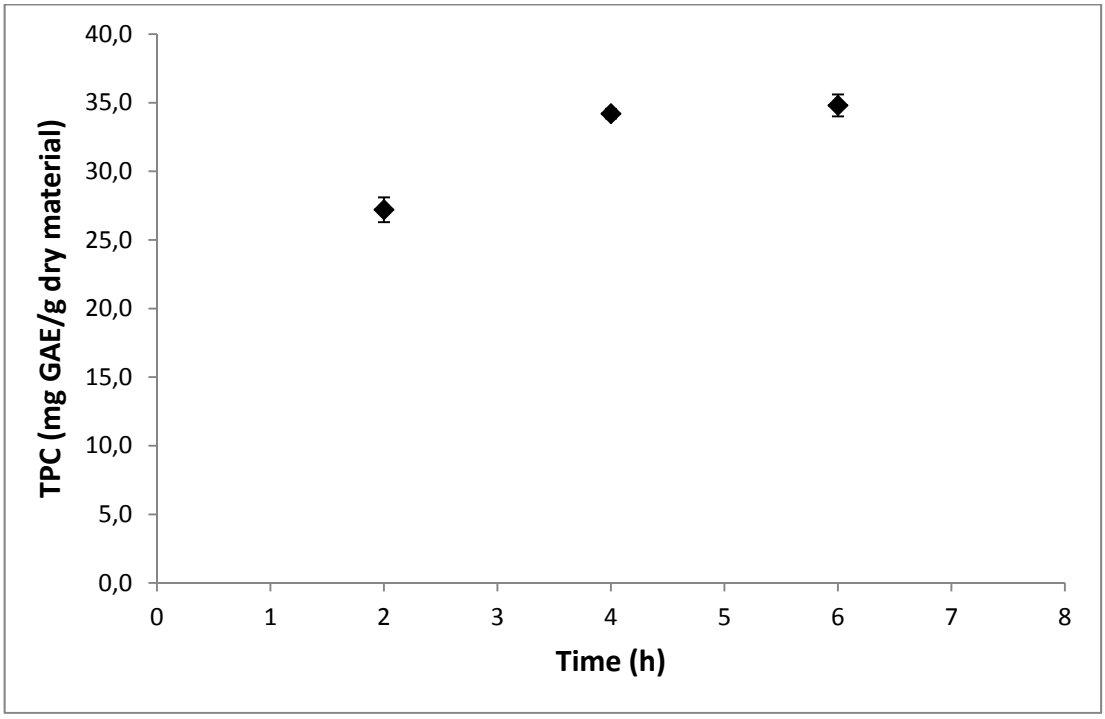

Figure 3.28. Change in total phenolic content of oleaster extracts obtained by conventional extraction in water with respect to time

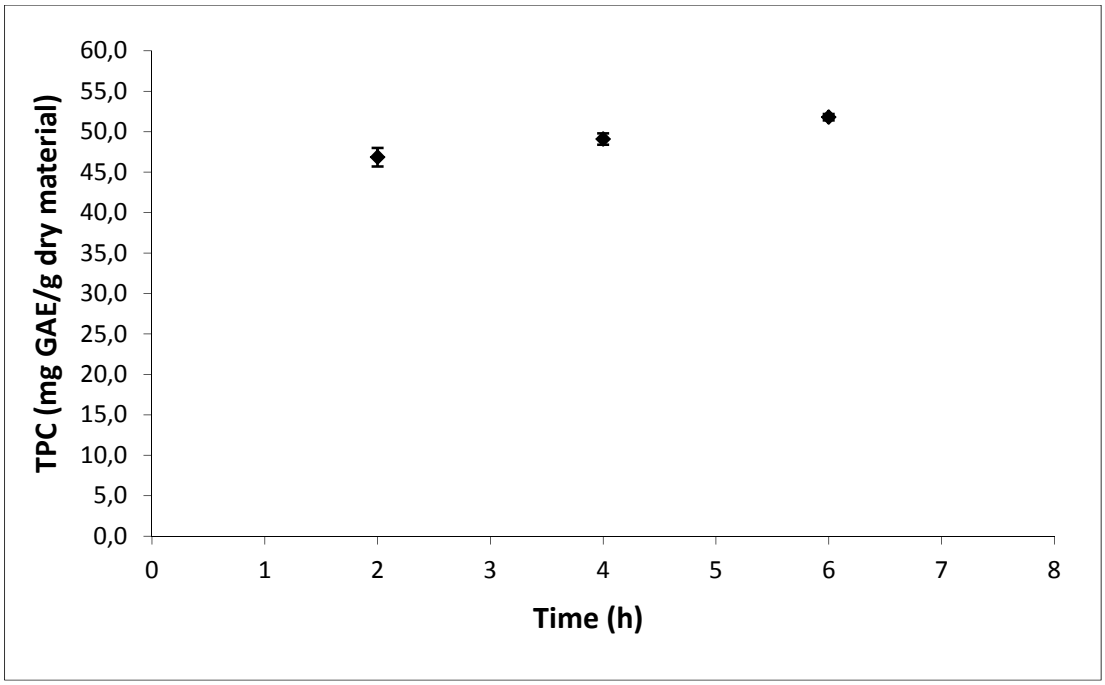

Figure 3.29. Change in total phenolic content of oleaster extracts obtained by conventional extraction in 50 % ethanol-water mixture with respect to time

According to the statistical analysis, the best extraction time was 4 h for conventionally extracted oleasters in water (Table D.9).

Similar to microwave extraction, there was a significant difference between solvent types also in conventional extraction. Total phenolic content increased when 50% ethanol and water mixture was used instead of using water as solvent. Since extraction time of 4 h was not statistically different from 6 h of extraction, the best extraction time was chosen as 4 h (Table D.10).

There was a significant difference between total phenolic content of extracts obtained by conventional method and that of the extracts obtained by microwave (Table D.11). Although conventionally extracted samples gave higher total phenolic content than the microwave extracted samples, time and energy consumption must also be taken into consideration while selecting the optimum method.

3.2.3. Effect of Microwave Extraction on Total Antioxidant Activity

Antioxidant activities of the oleaster extracts were found to be between 0.2 and 3.7 mg DPPH/g dry material by using microwave extraction at different conditions. The graphs that show the antioxidant activities of oleasters extracted with 400 W microwave power; and water, ethanol and 50 % ethanol-water mixture can be seen in Figure 3.30, 3.31 and 3.32, respectively. Similar trend observed in total phenolic content graphs can be seen in antioxidant activity graphs. Similar to total phenolic content, 1:30 solid to solvent ratio gave the highest antioxidant activity.

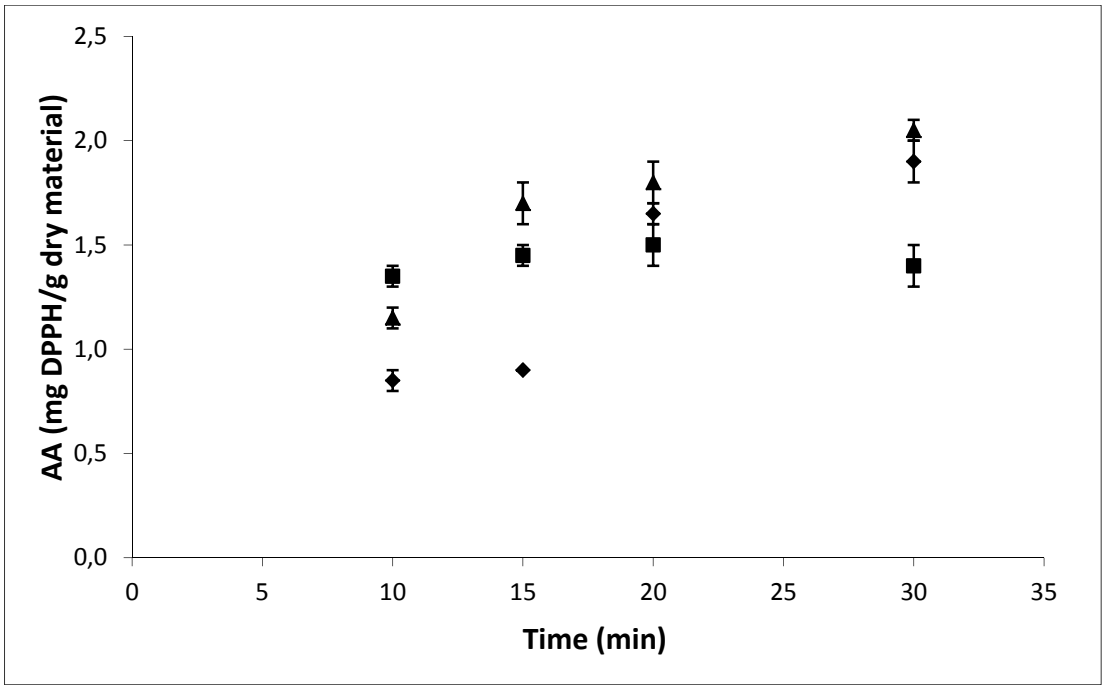

Figure 3.30. Effects of different solid to solvent ratios on antioxidant activity of oleaster extracts obtained by microwave extraction with water at 400 W

♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

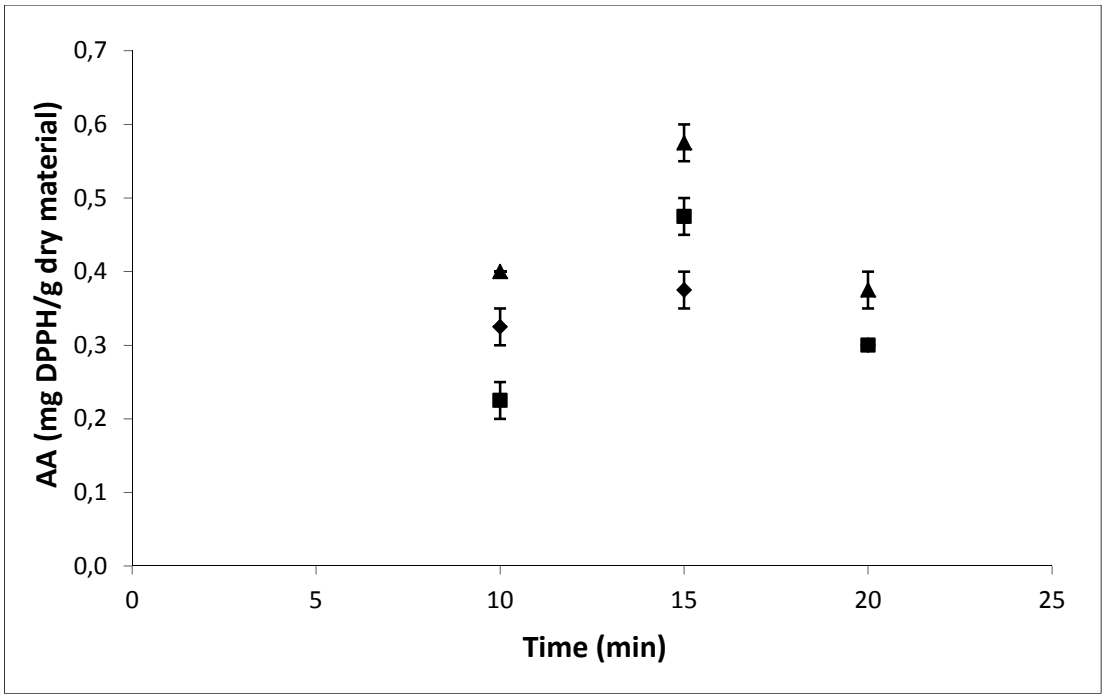

Figure 3.31. Effects of different solid to solvent ratios on antioxidant activity of oleaster extracts obtained by microwave extraction with ethanol at 400 W

♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

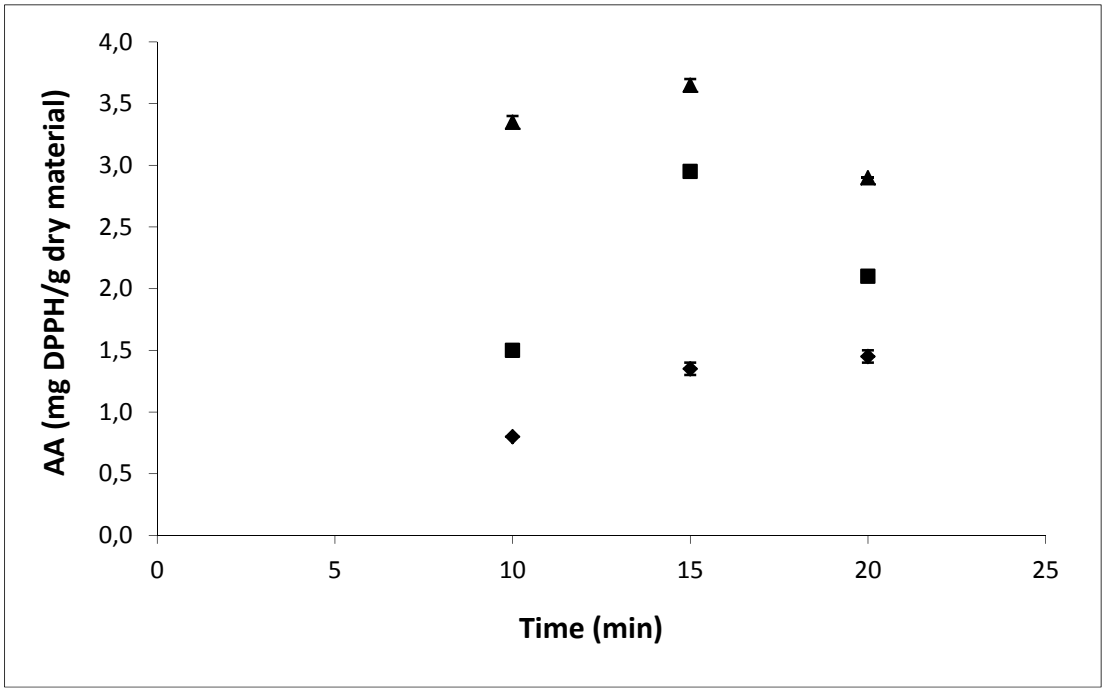

Figure 3.32. Effects of different solid to solvent ratios on antioxidant activity of oleaster extracts obtained by microwave extraction with 50 % ethanol-water mixture at 400W ♦ 1:10[c] ■ 1:20[b] ▲1:30[a]

3.2.4. Comparison of Microwave and Conventional Extraction in terms of Antioxidant Activity

Figure 3.33 and 3.34 show the change in antioxidant activity of oleaster extracts with respect to time when water and ethanol-water mixture was used as solvent and conventional extraction method was used. When the solvent was water, the highest antioxidant activity was reached in 4 h while it was 6 h when ethanol-water mixture was used as solvent by conventional extraction method (Table D.12).

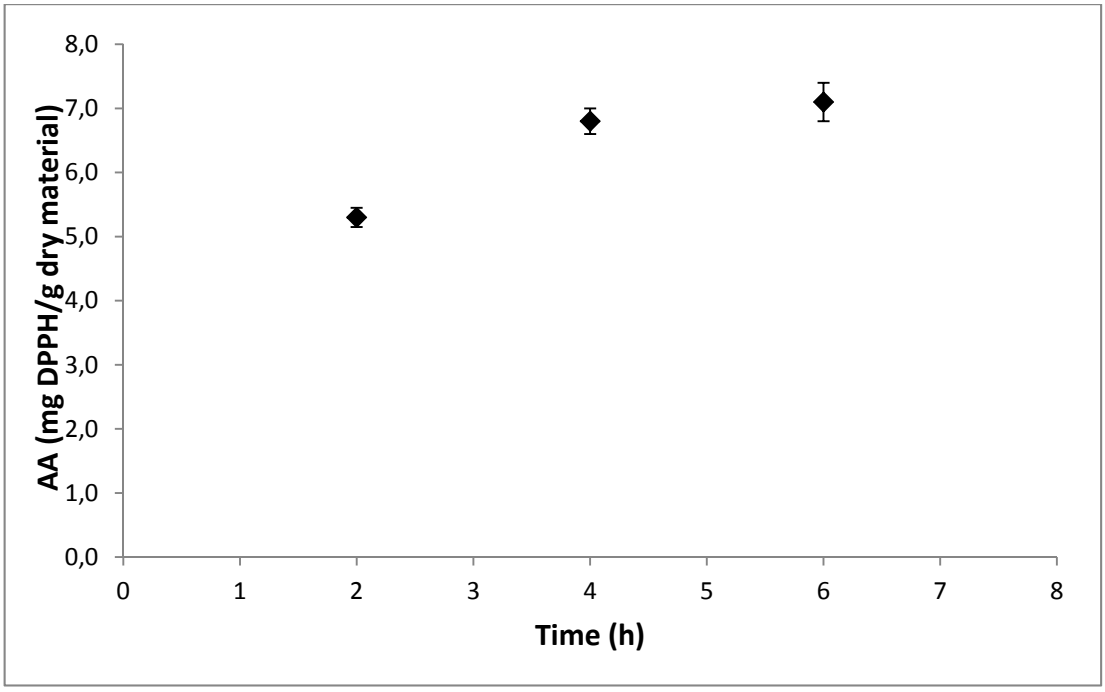

Figure 3.33. Change in antioxidant activity of oleaster extracts obtained by conventional extraction in water with respect to time

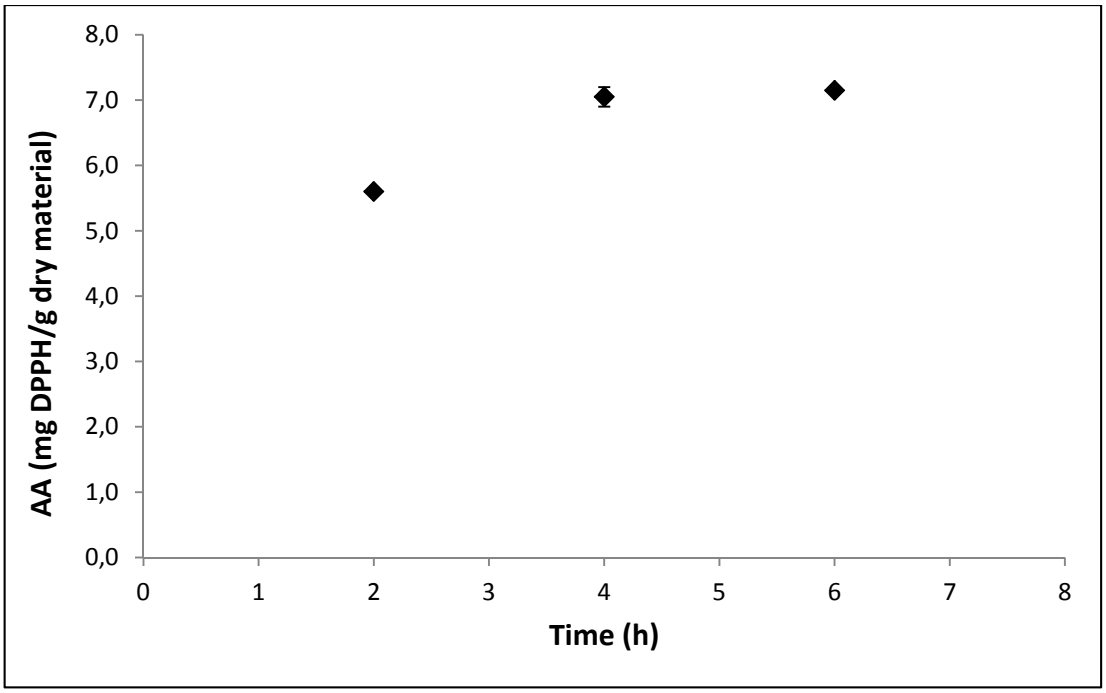

Figure 3.34. Change in antioxidant activity of oleaster extracts obtained by conventional extraction in 50 % ethanol-water mixture with respect to time

3.2.5. Comparison of Microwave and Conventional Extraction in terms of Concentration of Phenolic Acids

In High Pressure Liquid Chromatography, the extracts which gave the highest total phenolic compounds in microwave method (1:30 solid to solvent ratio; 400 W microwave power; 20 min extraction with water, 15 min extraction with 50 % ethanol-water mixture) and conventional method (1:30 solid to solvent ratio; 4 h extraction with water, 6 h extraction with 50% ethanol-water mixture) were analyzed. Since oleasters extracted with ethanol gave lower total phenolic content, they were not investigated in HPLC.

Table 3.2 shows the concentrations of 13 kinds of phenolic compounds that were found in oleaster extracts, namely 4-hydroxybenzoic acid, rutin, protocatechuic(3,4-dihydroxybenzoic)acid, vanillin (vanillic acid), kaempferol, gallic acid, benzoic acid, m-coumaric acid (trans-3-hydroxycinnamic acid), p-coumaric acid, caffeic acid,

ferulic acid, sinapic acid and chlorogenic acid. The amounts are expressed as mg/g dry material.

Table 3.2. Concentration of phenolic compounds of oleaster extracts in different solvents for the best parameters

Extraction Method	Microwave (400 W, 1:30 solid to solvent ratio)		Conventional (1:30 solid to solvent ratio)	
Solvent Type	Water	Ethanol-water Mixture	Water	Ethanol-water Mixture
4-hydroxybenzoic acid	0.6	0.6	0.8	0.8
rutin	1.7	1.8	2.5	2.3
protocatechuic acid	0.01	0.02	0.03	0.02
vanillic acid	0.2	0.2	0.2	0.2
kaempferol	2.3	2.4	3.3	3.0
gallic acid	1.3	1.2	0.9	1.0
benzoic acid	0.7	0.8	1.0	0.9
m-coumaric acid	0.6	0.6	0.7	0.7
p-coumaric acid	0.7	0.7	0.9	0.8
caffeic acid	0.3	0.3	0.4	0.4
ferulic acid	0.3	0.3	0.3	0.3
sinapic acid	0.3	0.3	-	-
chlorogenic acid	-	0.2	-	-

As can be seen from Table 3.2, sinapic acid was only detected in samples that were extracted with microwave. This may be because of destruction of this compound during long extraction process that were carried out with conventional method; 4 h with water, 6 h with ethanol-water mixture. Moreover, chlorogenic acid was only detected in samples extracted with microwave in ethanol-water mixture. This may be related to the solubility of the chlorogenic acid in the presence of ethanol or the synergistic effect of ethanol-water mixture together with the rapid extraction advantage of microwave. In general, there is no distinctive difference in phenolic concentrations between microwave and conventional extraction.

Vanillin is an aromatic compound that is used widely in cakes, ice creams and other aromatic foods. It has a specific flavor and it gives a pleasant taste. In this study it is found that all of the oleaster extracts had 0.2 mg vanillin/g dry weight.

The most abundant phenolic compound in oleaster extracts was found as kaempferol followed by rutin which was just the opposite in the results of caper extracts.

Ayaz and Bertoft (2001) investigated the phenolic acid composition of oleaster fruits, and found that benzoic acid was 11.6±1.20 mg/ 100 g dry weight, which is equivalent to between 0.104 and 0.128 mg/g dry weight. The other phenolic acids found in their study were vanillic, 4-hydroxybenzoic, ferulic and caffeic acids; and their average amounts were 0.15, 0.46, 0.023 and 0.32 mg/g dry weight, respectively. These results were similar or a little lower than the results found in this study. The difference may be due to differences in maturity of extracted oleasters, solvents used or extraction methods.

Chromatograms prepared at different wavelengths can be seen in Appendix C.2.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, caper and oleaster plants were extracted by using microwave. Total phenolic content, antioxidant activity and concentration of phenolic contents were analyzed. The results were compared with that of conventional extraction.

Solvent type and solid to solvent ratio were found to be the significant factors in affecting microwave extraction of phenolic compounds from caper and oleaster. The increase in solvent amount increased total phenolic content. Using ethanol with water in equal proportions as solvent increased the total phenolic content and antioxidant activity of both plant extracts.

The highest total phenolic content and antioxidant activity values were obtained when 50% ethanol-water mixture, 1:30 solid to solvent ratio and 400 W microwave power were used. The main phenolic constituents were found as rutin and kaempferol for both caper and oleaster. Concentration of phenolic compounds changed with different solvent types due to the difference in solubility of these phenolic compounds in different solvents.

In caper, there was no significant difference between microwave and conventional extraction in terms of total phenolic content, antioxidant activity and concentration of phenolics. In oleaster, concentration of phenolics was not affected by extraction method either. On the other hand, microwave decreased extraction time significantly which has an important role in retention of phenolic compounds and reduction of the extraction cost in industry. Therefore, microwave extraction can be considered as an alternative method for the extraction of phenolic compounds from caper and oleaster.

Further studies can be focused on the effect of maturity degree of caper and oleaster on total phenolic content and antioxidant activity. These plants can also be extracted under pressure by using close microwave extraction system in order to determine how pressure affects the microwave extraction.

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APPENDIX A

CALIBRATION CURVES

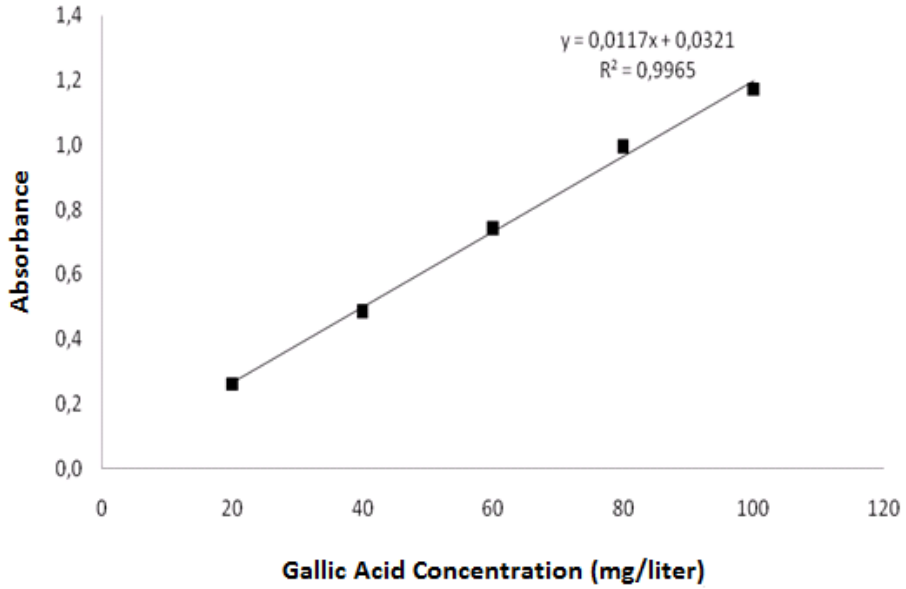

Figure A.1 Calibration curve prepared with water

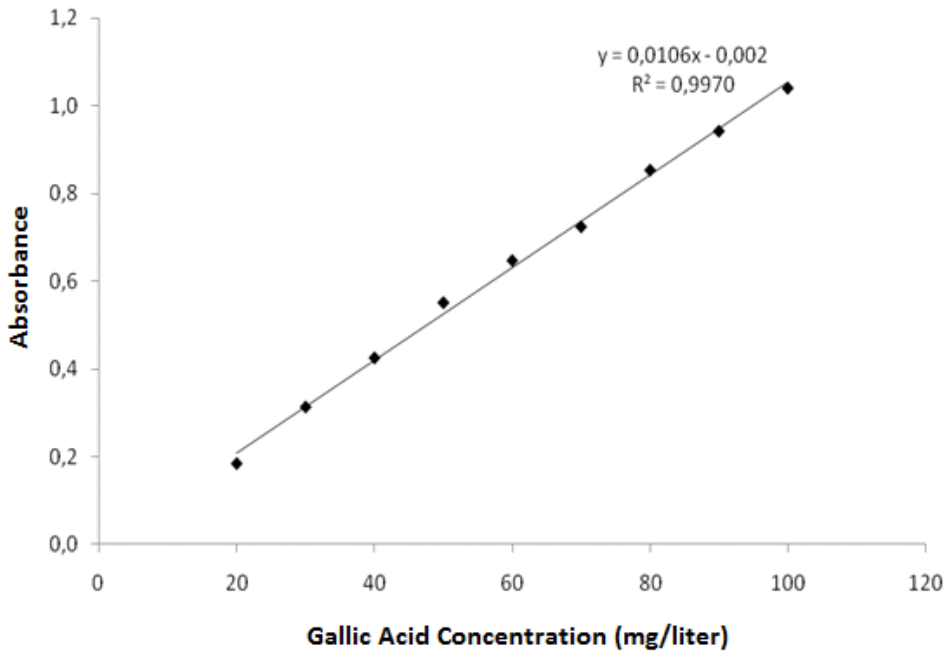

Figure A.2 Calibration curve prepared with ethanol

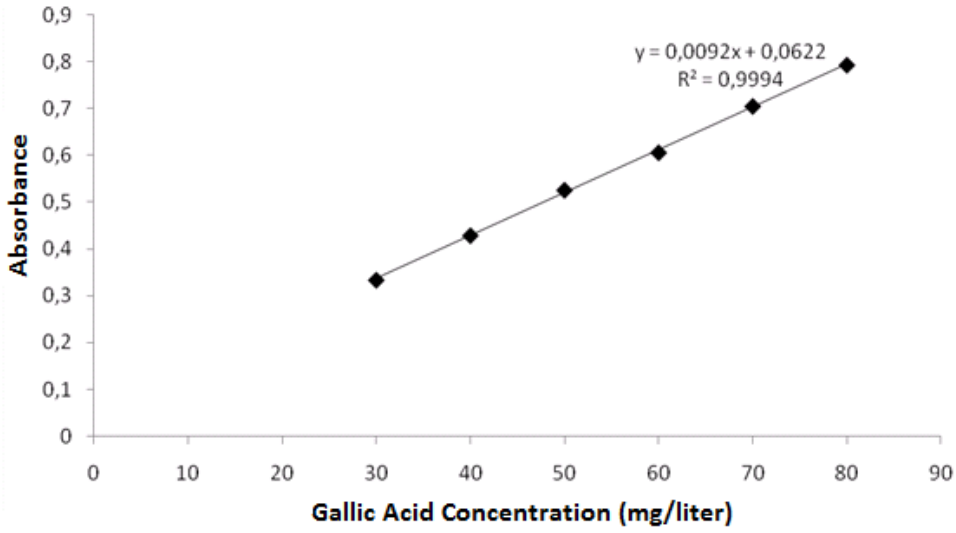

Figure A.3 Calibration curve prepared with ethanol: water mixture of 25:75 ratio

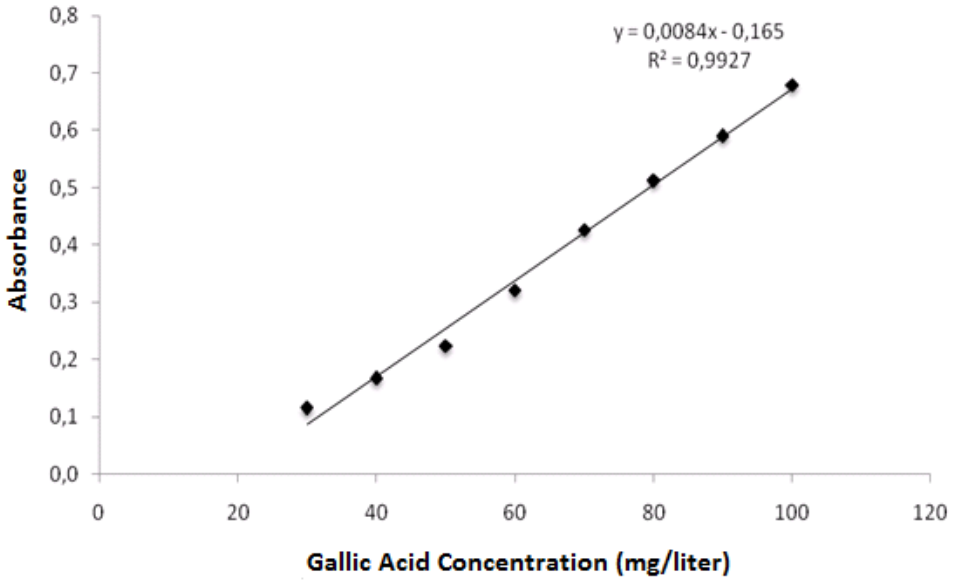

Figure A.4 Calibration curve prepared with ethanol: water mixture of 50:50 ratio

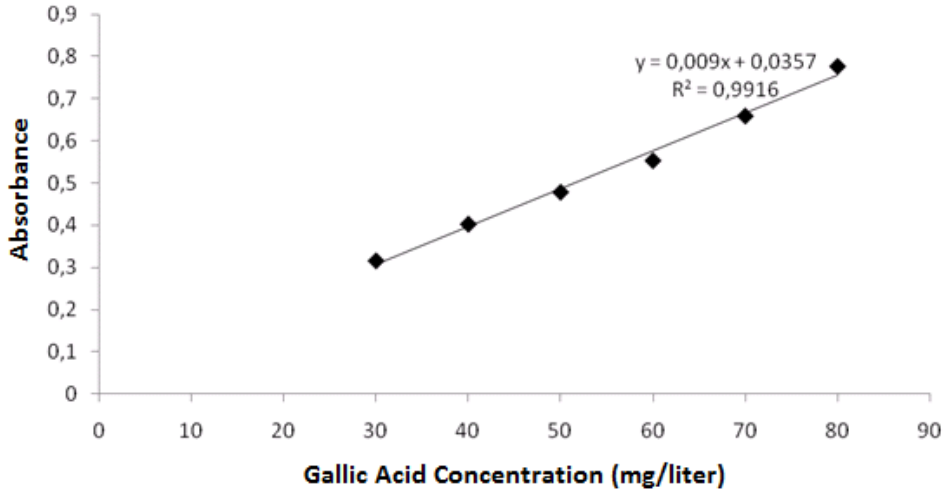

Figure A.5 Calibration curve prepared with ethanol: water mixture of 75:25 ratio

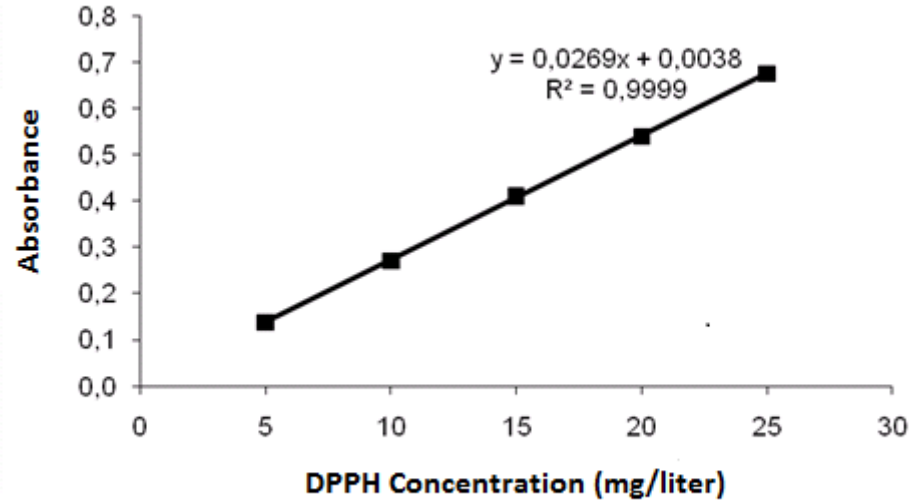

Figure A.6 Calibration curve prepared with DPPH

APPENDIX B

EXPERIMENTAL DATA

Table B.1 Experimental data of total phenolic content and antioxidant activity of caper extracted by using microwave

Microwave Power (W)	Solvent Type	Solid to Solvent Ratio (g/ml)	Time (min)	Total Phenolic Content (mg GAE/g dry material)		Antioxidant Activity (mg DPPH/g dry material)	
				RUN1	RUN2	RUN1	RUN2
700	water	1/10	5	6.0	5.9	0.5	0.6
700	water	1/10	10	5.3	5.0	0.5	0.6
700	water	1/10	15	5.9	5.5	0.5	0.5
700	water	1/20	5	10.1	10.6	0.9	0.9
700	water	1/20	10	11.0	11.8	0.9	1.0
700	water	1/20	15	9.9	9.9	0.8	0.9
700	water	1/30	5	11.0	12.0	1.1	1.2
700	water	1/30	10	11.7	12.5	1.2	1.2

Table B.1 Experimental data of total phenolic content and antioxidant activity of caper extracted by using microwave (cont'd)

700	water	1/30	15	10.8	11.9	1.2	1.1
700	ethanol	1/10	5	6.3	7.0	0.6	0.6
700	ethanol	1/10	10	6.5	7.2	0.9	0.9
700	ethanol	1/10	15	6.1	6.6	0.7	0.8
700	ethanol	1/20	5	11.0	12.0	0.8	0.7
700	ethanol	1/20	10	9.6	8.9	0.9	0.9
700	ethanol	1/20	15	11.1	10.6	0.8	0.8
700	ethanol	1/30	5	13.9	14.5	1.2	1.2
700	ethanol	1/30	10	13.6	13.8	1.2	1.2
700	ethanol	1/30	15	15.2	16.4	1.3	1.3
700	water-ethanol mixture	1/10	5	25.7	27.5	1.0	0.9
700	water-ethanol mixture	1/10	10	14.0	12.8	0.7	0.8
700	water-ethanol mixture	1/10	15	12.4	11.7	0.7	0.7
700	water-ethanol mixture	1/20	5	27.9	27.1	1.0	1.0

Table B.1 Experimental data of total phenolic content and antioxidant activity of caper extracted by using microwave (cont'd)

700	water-ethanol mixture	1/20	10	16.6	17.4	1.1	1.1
700	water-ethanol mixture	1/20	15	19.1	20.0	1.0	0.9
700	water-ethanol mixture	1/30	5	49.3	50.6	1.2	1.3
700	water-ethanol mixture	1/30	10	49.5	45.9	1.8	1.9
700	water-ethanol mixture	1/30	15	50.3	54.6	1.9	1.8
400	water	1/10	5	7.0	7.1	0.4	0.5
400	water	1/10	10	6.1	7.0	0.7	0.6
400	water	1/10	15	5.3	5.8	0.6	0.7
400	water	1/20	5	10.0	11.1	0.9	1.0
400	water	1/20	10	10.2	9.6	0.9	0.9
400	water	1/20	15	9.9	11.0	0.8	0.9
400	water	1/30	5	14.0	13.1	1.0	1.0
400	water	1/30	10	14.0	15.2	1.0	1.0
400	water	1/30	15	14.6	14.6	1.1	1.1

Table B.1 Experimental data of total phenolic content and antioxidant activity of caper extracted by using microwave (cont'd)

400	ethanol	1/10	5	6.0	6.4	0.7	0.6
400	ethanol	1/10	10	6.2	6.7	0.6	0.5
400	ethanol	1/10	15	7.1	7.8	0.6	0.6
400	ethanol	1/20	5	11.5	11.6	0.8	0.7
400	ethanol	1/20	10	13.0	14.2	0.7	0.8
400	ethanol	1/20	15	14.0	13.3	0.7	0.7
400	ethanol	1/30	5	13.5	13.2	1.2	1.1
400	ethanol	1/30	10	15.8	14.2	1.2	1.2
400	ethanol	1/30	15	16.2	16.1	1.1	1.2
400	water-ethanol mixture	1/10	5	20.0	21.7	1.2	1.1
400	water-ethanol mixture	1/10	10	21.2	19.5	0.9	0.9
400	water-ethanol mixture	1/10	15	15.0	15.0	0.8	0.9
400	water-ethanol mixture	1/20	5	28.5	26.2	1.3	1.4
400	water-ethanol mixture	1/20	10	33.9	36.8	0.9	0.8

Table B.1 Experimental data of total phenolic content and antioxidant activity of caper extracted by using microwave (cont'd)

400	water-ethanol mixture	1/20	15	36.1	38.3	0.8	0.9
400	water-ethanol mixture	1/30	5	30.1	35.1	2.0	1.9
400	water-ethanol mixture	1/30	10	46.4	50.0	1.7	1.6
400	water-ethanol mixture	1/30	15	47.7	52.4	1.7	1.6

Table B.2 Experimental data of total phenolic content and antioxidant activity of caper extracted by using conventional method

Solvent Type	**Time**	**Total Phenolic Content (mg GAE/g dry material)**		**Antioxidant Activity (mg DPPH/g dry material)**	
		RUN1	RUN2	RUN1	RUN2
water	1 h	14.6	13.6	1.1	1.3
water	2 h	15.7	14.0	1.1	1.1
water	6 h	13.7	12.3	1.1	1.1
water	10 h	12.4	13.4	0.3	0.3
ethanol	2 h	19.2	20.6	0.8	0.9
ethanol	4 h	23.7	23.1	0.8	0.9
ethanol	6 h	20.4	18.6	0.8	0.8
water-ethanol mixture	1 h	25.1	26.3	2.6	2.8
water-ethanol mixture	2 h	34.0	32.2	2.7	2.9
water-ethanol mixture	4 h	42.5	39.7	2.3	2.3
water-ethanol mixture	6 h	40.9	38.8	1.2	1.3

Table B.3 Experimental data of total phenolic content and antioxidant activity of oleaster extracted by using microwave

Microwave Power (W)	Solvent Type	Solid to Solvent Ratio (g/ml)	Time (min)	Total Phenolic Content (mg GAE/g dry material)		Antioxidant Activity (mg DPPH/g dry material)	
				RUN1	RUN2	RUN1	RUN2
400	water	1/10	10	9.6	8.7	0.9	0.8
400	water	1/10	15	11.3	10.4	0.9	0.9
400	water	1/10	20	11.0	12.1	1.7	1.6
400	water	1/10	30	12.4	12.1	2.0	1.8
400	water	1/20	10	11.0	10.1	1.4	1.3
400	water	1/20	15	10.3	11.0	1.4	1.5
400	water	1/20	20	14.8	13.6	1.4	1.6
400	water	1/20	30	16.1	15.2	1.3	1.5
400	water	1/30	10	13.0	12.7	1.1	1.2
400	water	1/30	15	17.4	15.6	1.6	1.8
400	water	1/30	20	18.8	17.0	1.9	1.7
400	water	1/30	30	17.1	17.1	2.1	2.0

Table B.3 Experimental data of total phenolic content and antioxidant activity of oleaster extracted by using microwave (cont'd)

400	ethanol	1/10	10	3.0	2.9	0.3	0.4
400	ethanol	1/10	15	3.1	3.0	0.4	0.4
400	ethanol	1/10	20	3.4	3.3	0.3	0.3
400	ethanol	1/20	10	3.3	3.2	0.3	0.2
400	ethanol	1/20	15	4.6	4.6	0.5	0.5
400	ethanol	1/20	20	4.9	4.7	0.3	0.3
400	ethanol	1/30	10	4.2	4.3	0.4	0.4
400	ethanol	1/30	15	5.5	5.7	0.6	0.6
400	ethanol	1/30	20	5.0	5.1	0.4	0.4
400	water-ethanol mixture	1/10	10	12.7	12.4	0.8	0.8
400	water-ethanol mixture	1/10	15	14.2	14.3	1.3	1.4
400	water-ethanol mixture	1/10	20	15.6	15.2	1.5	1.4
400	water-ethanol mixture	1/20	10	29.4	29.0	1.5	1.5
400	water-ethanol mixture	1/20	15	38.8	36.5	3.0	2.9

Table B.3 Experimental data of total phenolic content and antioxidant activity of oleaster extracted by using microwave (cont'd)

400	water-ethanol mixture	1/20	20	29.5	29.8	2.1	2.1
400	water-ethanol mixture	1/30	10	39.6	40.4	3.3	3.4
400	water-ethanol mixture	1/30	15	43.0	41.1	3.7	3.6
400	water-ethanol mixture	1/30	20	37.6	34.2	2.9	2.9

Table B.4 Experimental data of total phenolic content and antioxidant activity of oleaster extracted by using conventional method

Solvent Type	**Time**	**Total Phenolic Content (mg GAE/g dry material)**		**Antioxidant Activity (mg DPPH/g dry material)**	
		RUN1	RUN2	RUN1	RUN2
water	2 h	26.3	28.1	5.1	5.4
water	4 h	34.5	33.8	7.0	6.6
water	6 h	35.6	34.0	6.8	7.4
water-ethanol mixture	2 h	45.7	48.0	5.6	5.6
water-ethanol mixture	4 h	49.8	48.4	6.9	7.2
water-ethanol mixture	6 h	51.4	52.2	7.1	7.2

Table B.5 Experimental data of total phenolic content of mature oleaster extracted by using microwave

Microwave Power (W)	Solvent Type	Solid to Solvent Ratio (g/ml)	Time (min)	Total Phenolic Content (mg GAE/g dry material)	
				RUN1	RUN2
700	water	1/10	10	6.0	5.9
700	water	1/10	15	6.7	6.8
700	water	1/10	20	8.4	7.9
700	water	1/20	10	8.0	8.2
700	water	1/20	15	7.9	8.4
700	water	1/20	20	9.7	8.9
700	water	1/30	10	7.6	7.6
700	water	1/30	15	9.0	9.3
700	water	1/30	20	11.7	11.5
700	ethanol	1/10	10	2.5	2.3
700	ethanol	1/10	15	2.4	2.6
700	ethanol	1/10	20	1.3	1.2

Table B.5 Experimental data of total phenolic content of mature oleaster extracted by using microwave (cont'd)

700	ethanol	1/20	10	2.7	2.6
700	ethanol	1/20	15	2.6	2.8
700	ethanol	1/20	20	3.1	2.8
700	ethanol	1/30	10	3.1	3.2
700	ethanol	1/30	15	3.8	4.0
700	ethanol	1/30	20	4.0	4.0
400	water	1/10	10	5.8	5.5
400	water	1/10	15	5.1	5.6
400	water	1/10	20	7.9	7.0
400	water	1/20	10	8.1	7.6
400	water	1/20	15	8.5	8.1
400	water	1/20	20	10.1	9.5
400	water	1/30	10	7.5	7.5
400	water	1/30	15	13.2	13.7

Table B.5 Experimental data of total phenolic content of mature oleaster extracted by using microwave (cont'd)

400	water	1/30	20	11.1	11.0
400	ethanol	1/10	10	2.0	2.0
400	ethanol	1/10	15	2.2	2.4
400	ethanol	1/10	20	1.8	1.9
400	ethanol	1/20	10	2.6	2.7
400	ethanol	1/20	15	2.8	2.9
400	ethanol	1/20	20	2.8	2.7
400	ethanol	1/30	10	3.6	3.2
400	ethanol	1/30	15	3.9	4.0
400	ethanol	1/30	20	3.9	3.7

APPENDIX C

HPLC CHROMATOGRAMS

C.1. HPLC Chromatograms of caper

1 quercetin

2 rutin

3 kaempferol

4 gallic acid

5 guaiacol

6 thymol

7 vanillin (vanillic acid)

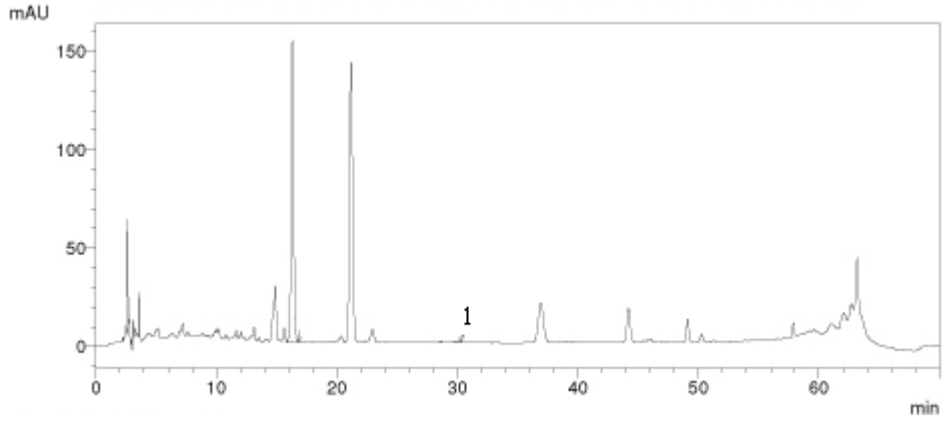

Figure C.1 HPLC chromatogram at 255 nm for caper in ethanol extracted by conventional method

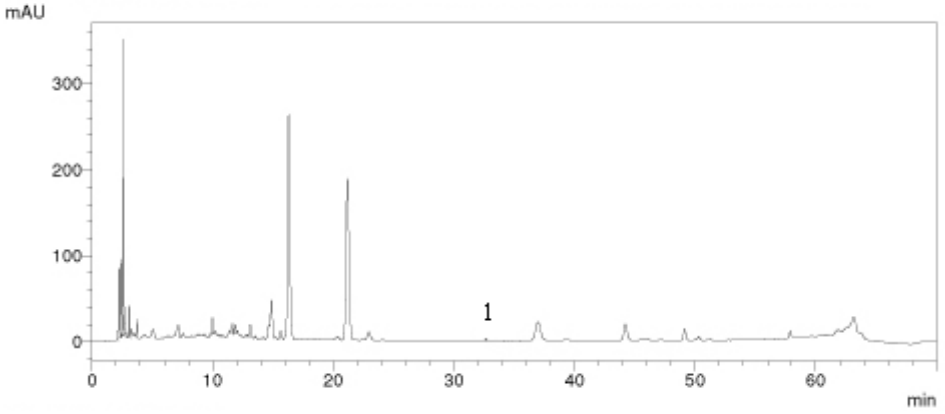

Figure C.2 HPLC chromatogram at 255 nm for caper in ethanol and water mixture extracted by conventional method

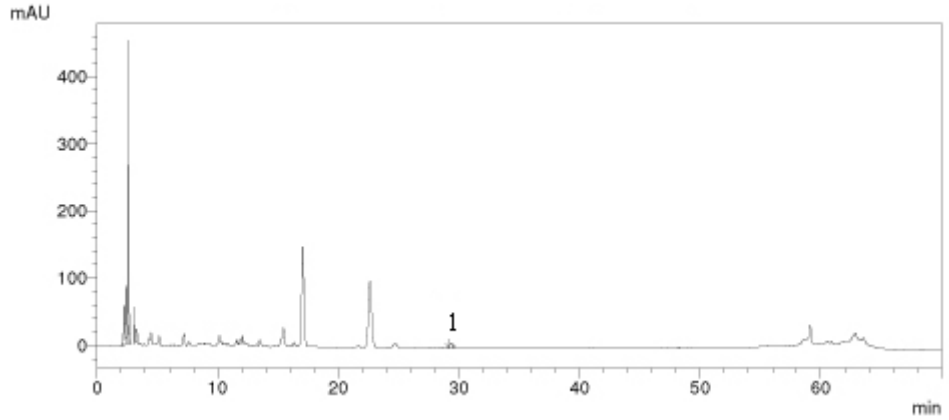

Figure C.3 HPLC chromatogram at 255 nm for caper in water extracted by microwave

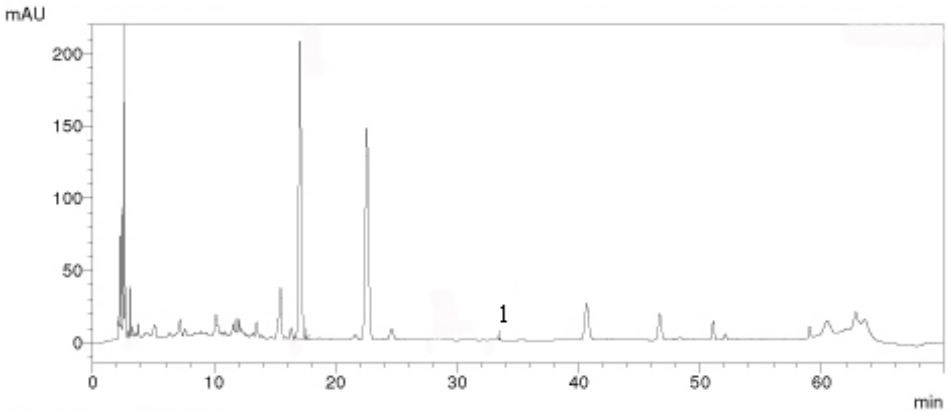

Figure C.4 HPLC chromatogram at 255 nm for caper in ethanol and water mixture extracted by microwave

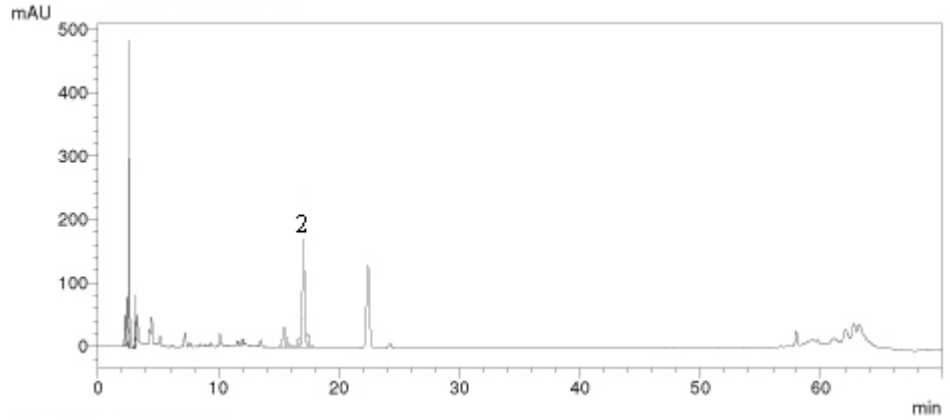

Figure C.5 HPLC chromatogram at 256 nm for caper in water extracted by conventional method

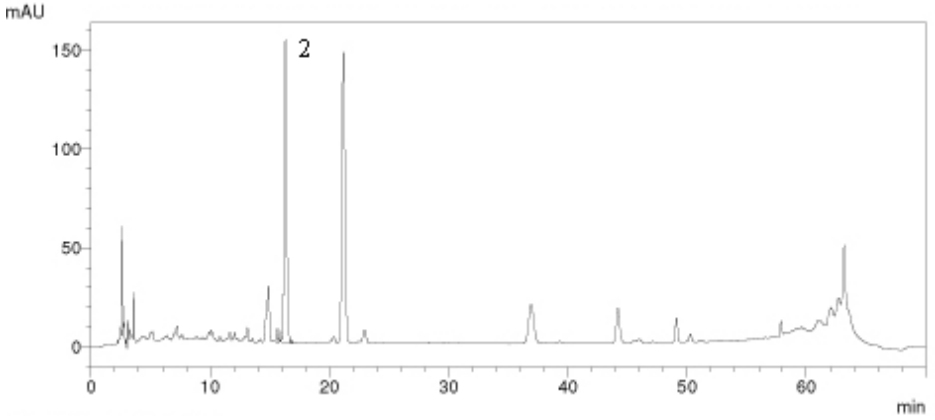

Figure C.6 HPLC chromatogram at 256 nm for caper in ethanol extracted by conventional method

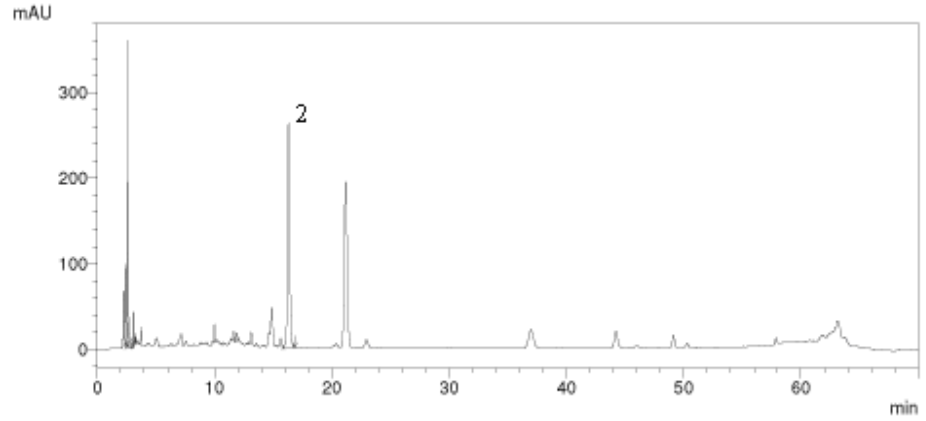

Figure C.7 HPLC chromatogram at 256 nm for caper in ethanol and water mixture extracted by conventional method

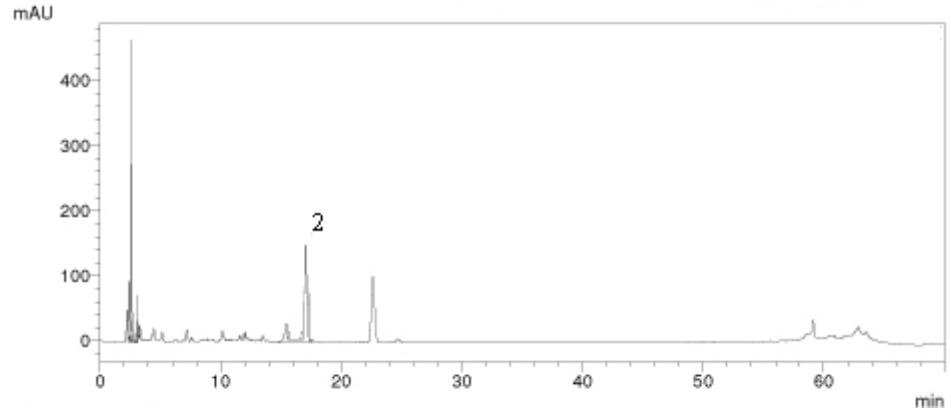

Figure C.8 HPLC chromatogram at 256 nm for caper in water extracted by microwave

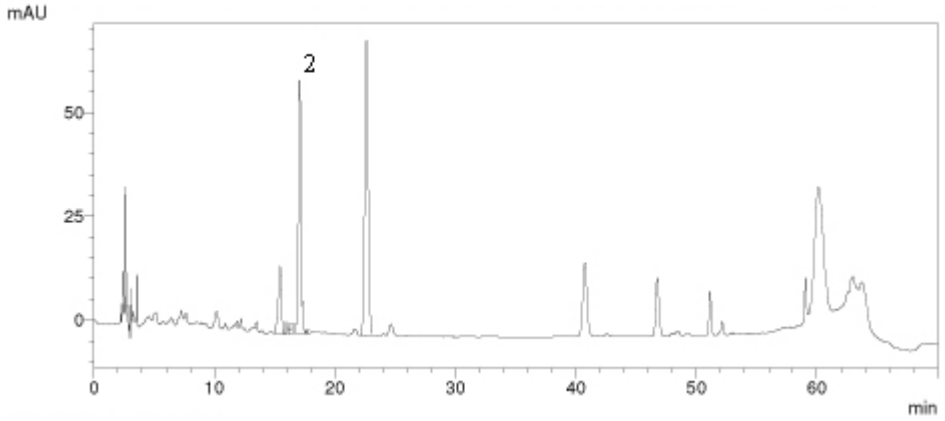

Figure C.9 HPLC chromatogram at 256 nm for caper in ethanol extracted by microwave

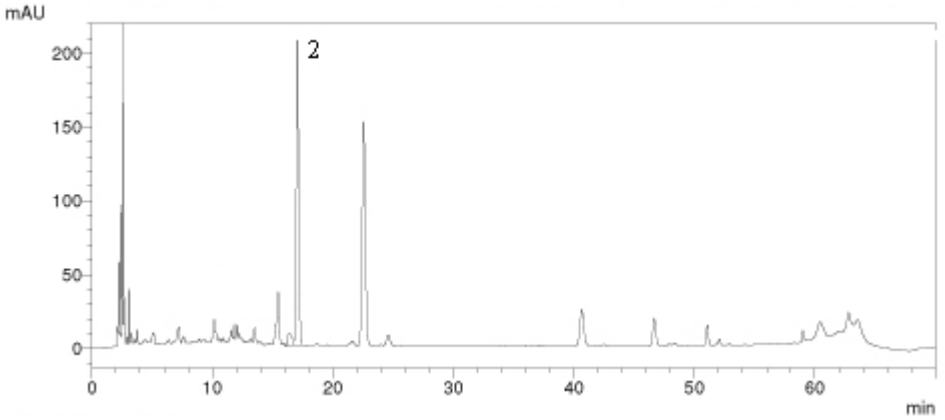

Figure C.10 HPLC chromatogram at 256 nm for caper in ethanol and water mixture extracted by microwave

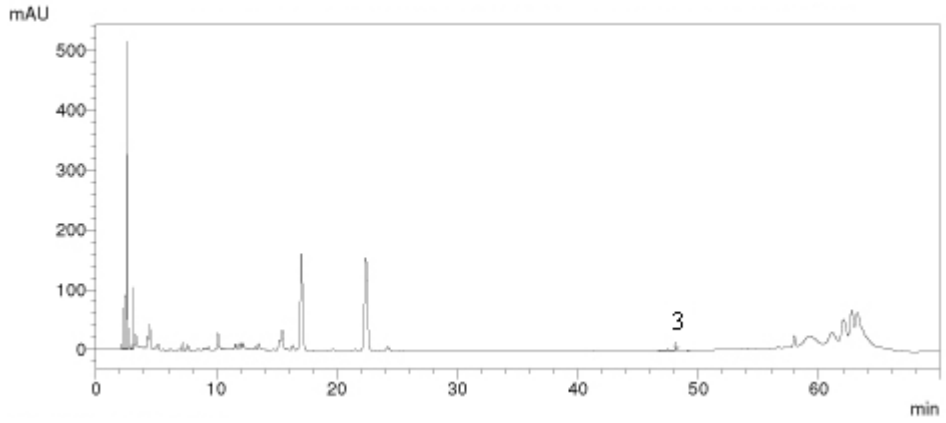

Figure C.11 HPLC chromatogram at 264 nm for caper in water extracted by conventional method

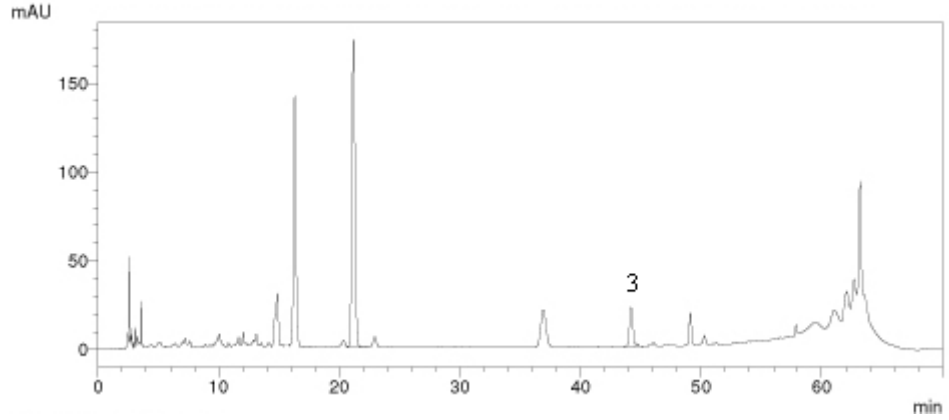

Figure C.12 HPLC chromatogram at 264 nm for caper in ethanol extracted by conventional method

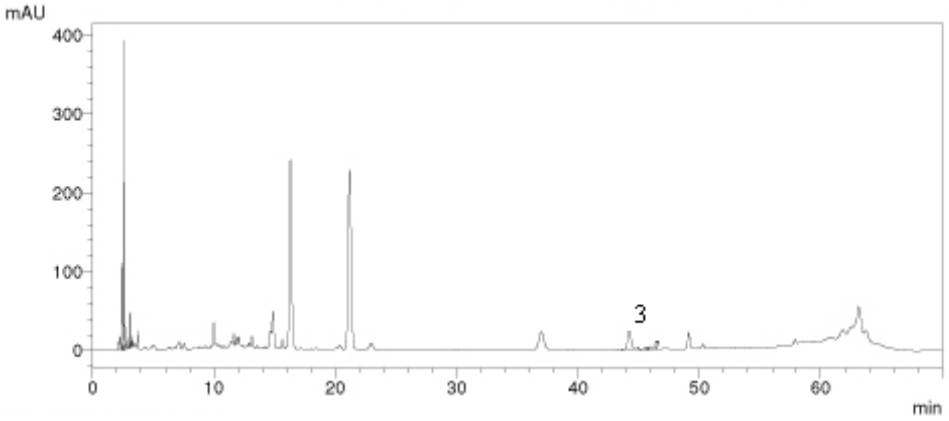

Figure C.13 HPLC chromatogram at 264 nm for caper in ethanol and water mixture extracted by conventional method

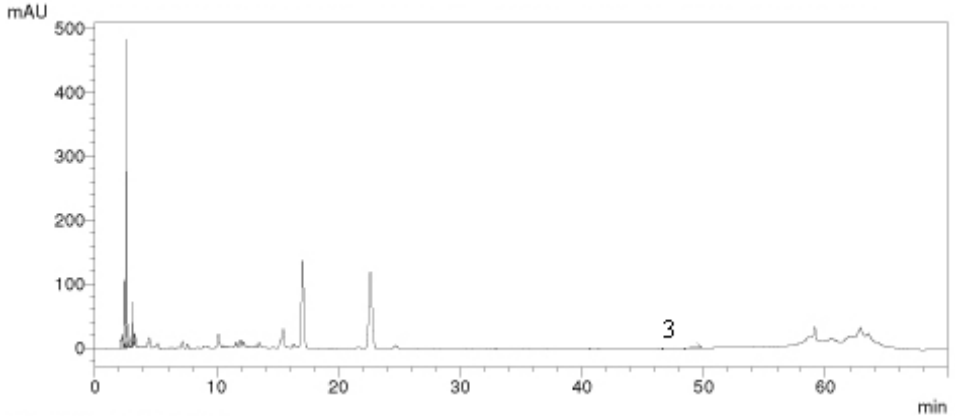

Figure C.14 HPLC chromatogram at 264 nm for caper in water extracted by microwave

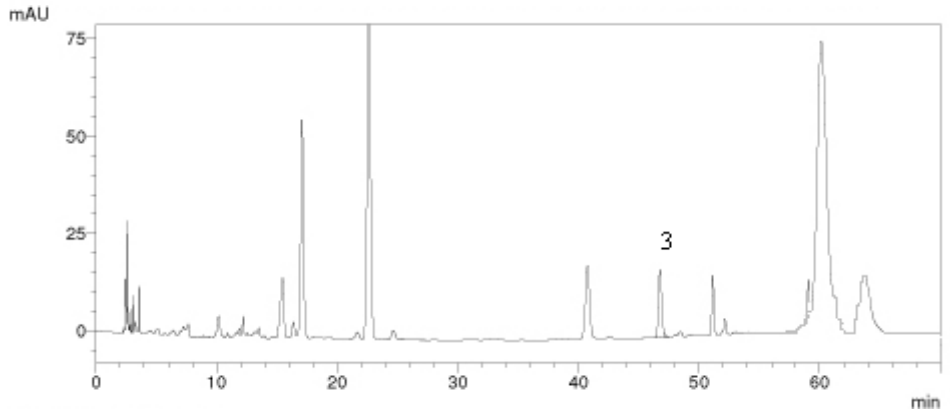

Figure C.15 HPLC chromatogram at 264 nm for caper in ethanol extracted by microwave

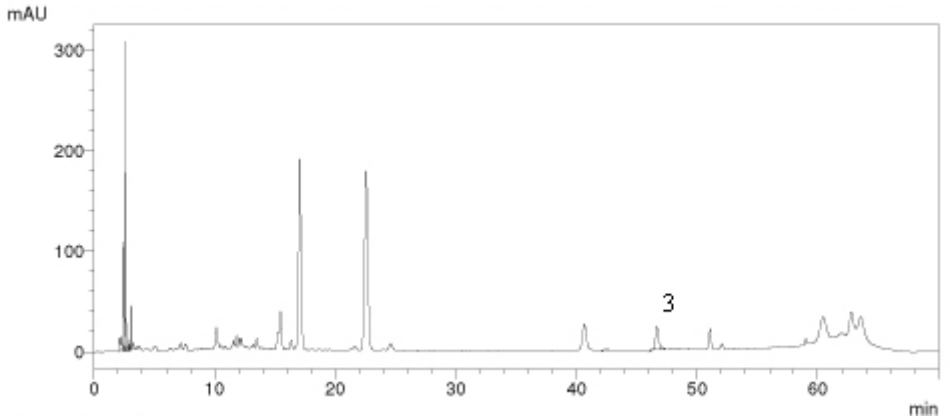

Figure C.16 HPLC chromatogram at 264 nm for caper in ethanol and water mixture extracted by microwave

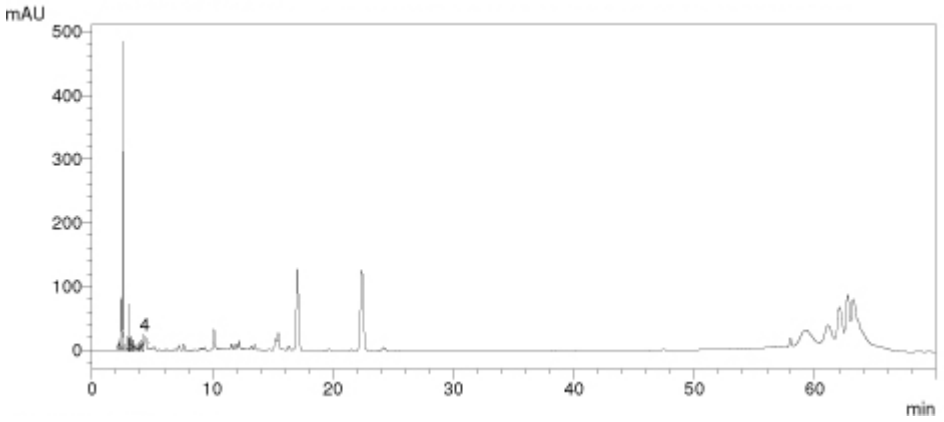

Figure C.17 HPLC chromatogram at 270 nm for caper in water extracted by conventional method

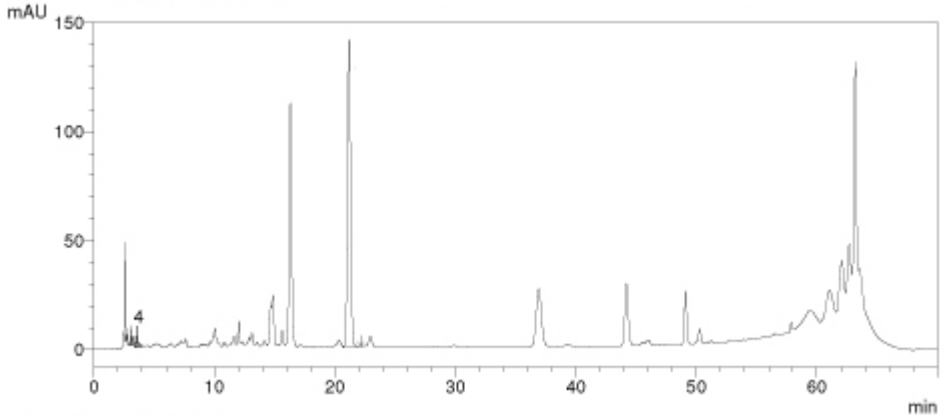

Figure C.18 HPLC chromatogram at 270 nm for caper in ethanol extracted by conventional method

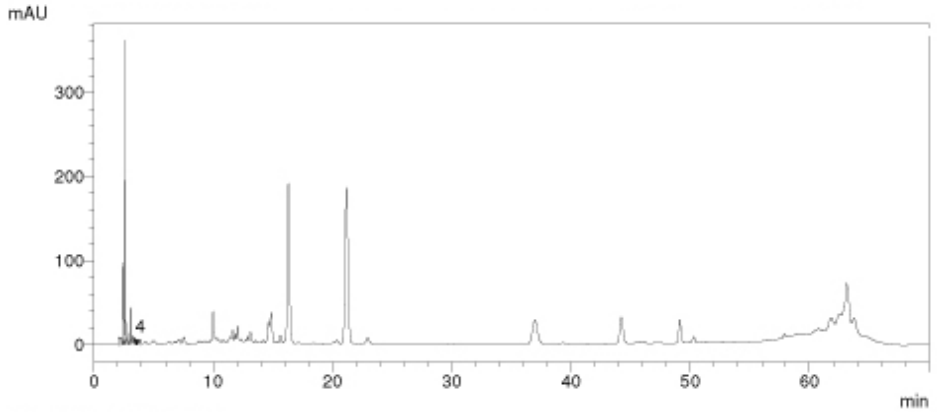

Figure C.19 HPLC chromatogram at 270 nm for caper in ethanol and water mixture extracted by conventional method

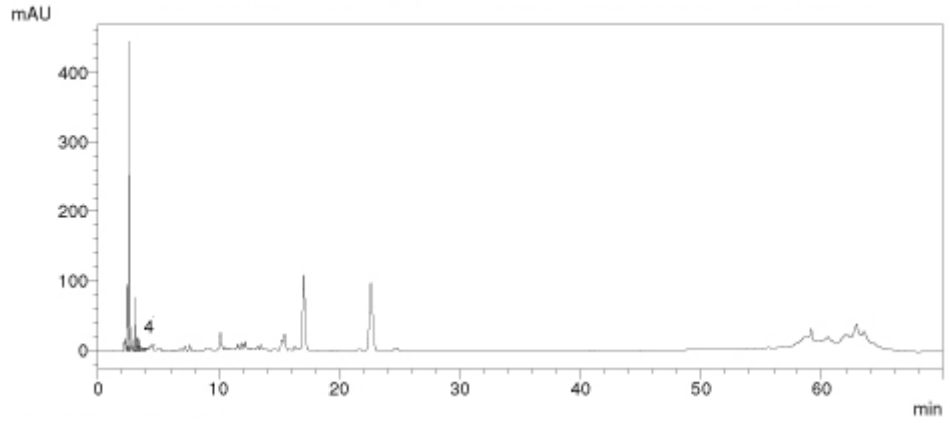

Figure C.20 HPLC chromatogram at 270 nm for caper in water extracted by microwave

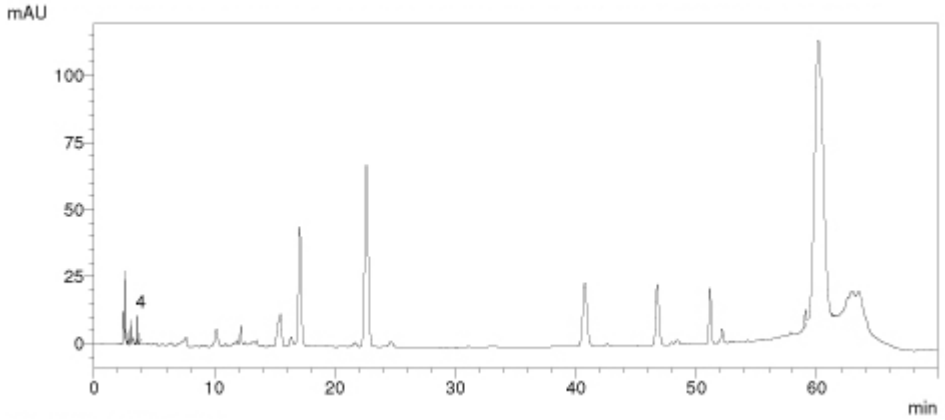

Figure C.21 HPLC chromatogram at 270 nm for caper in ethanol extracted by microwave

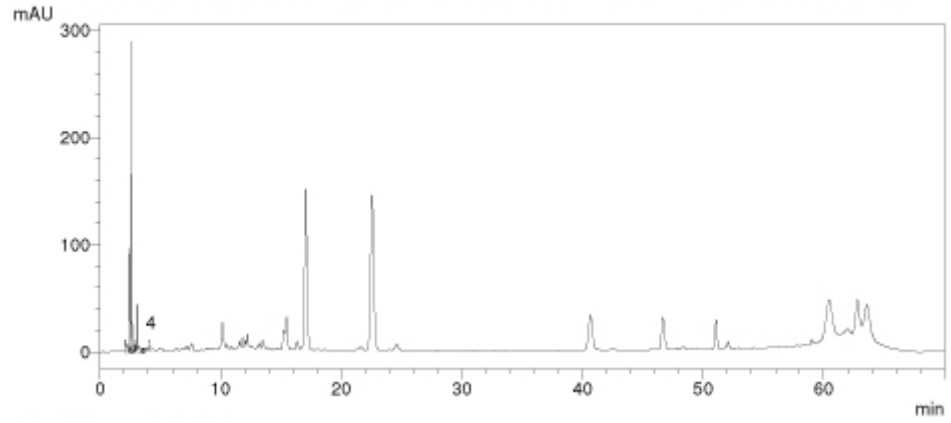

Figure C.22 HPLC chromatogram at 270 nm for caper in ethanol and water mixture extracted by microwave

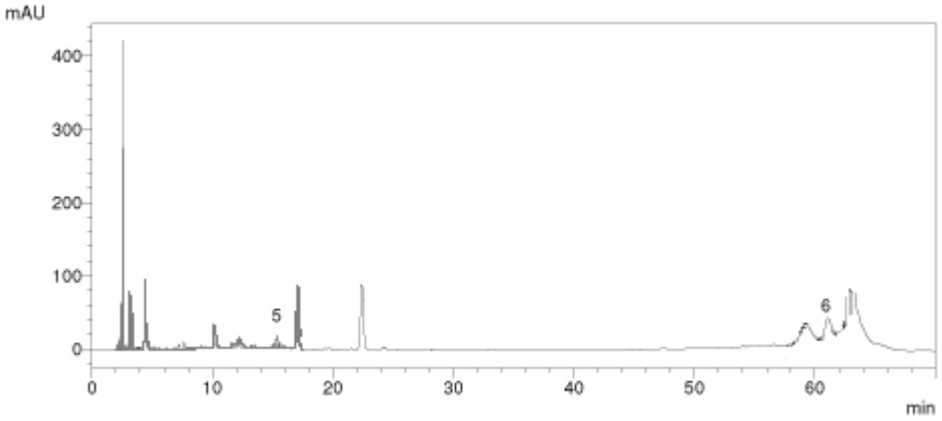

Figure C.23 HPLC chromatogram at 275 nm for caper in water extracted by conventional method

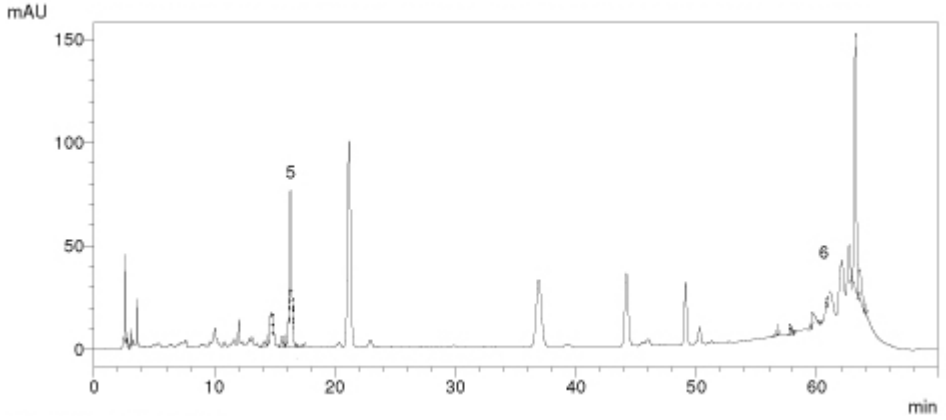

Figure C.24 HPLC chromatogram at 275 nm for caper in ethanol extracted by conventional method

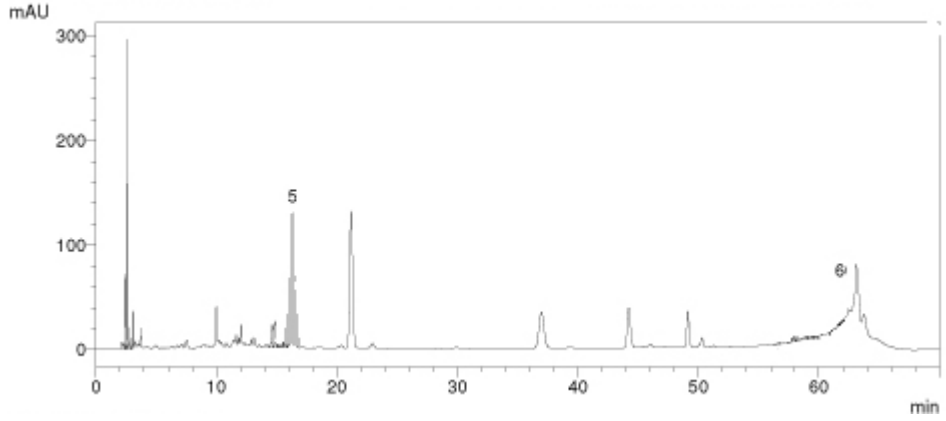

Figure C.25 HPLC chromatogram at 275 nm for caper in ethanol and water mixture extracted by conventional method

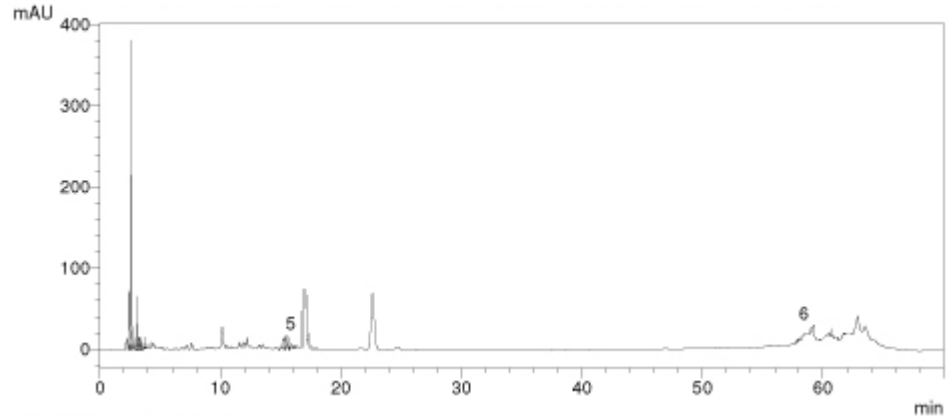

Figure C.26 HPLC chromatogram at 275 nm for caper in water extracted by microwave

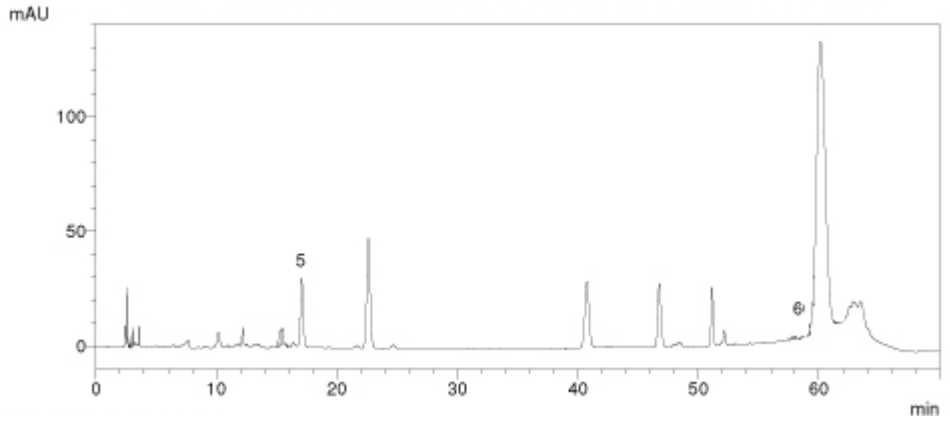

Figure C.27 HPLC chromatogram at 275 nm for caper in ethanol extracted by microwave

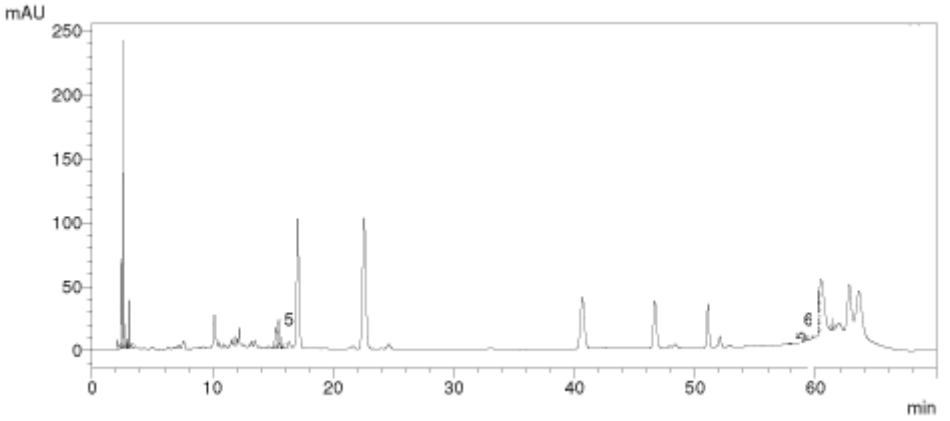

Figure C.28 HPLC chromatogram at 275 nm for caper in ethanol and water mixture extracted by microwave

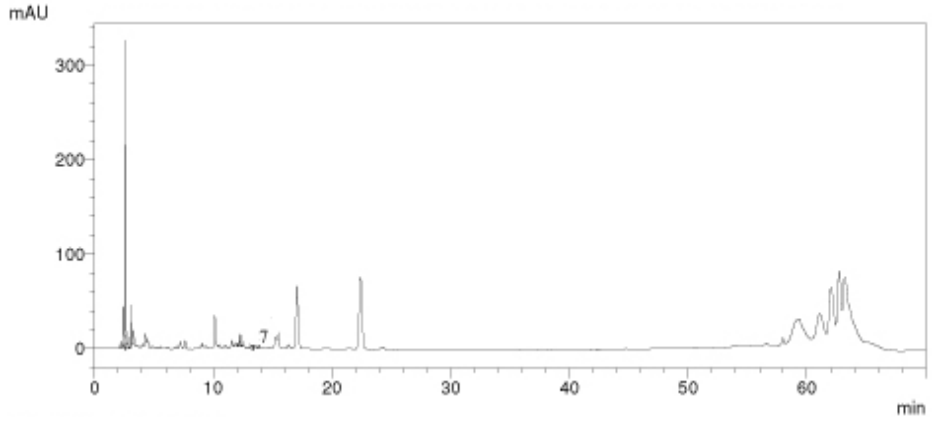

Figure C.29 HPLC chromatogram at 281 nm for caper in water extracted by conventional method

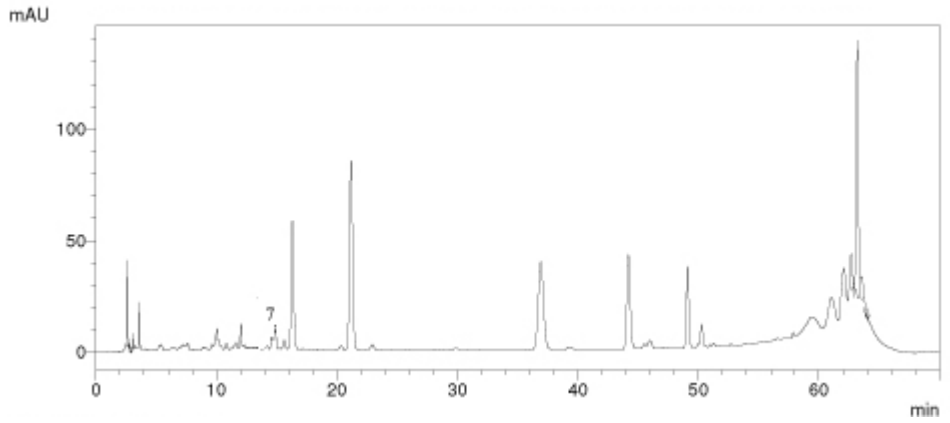

Figure C.30 HPLC chromatogram at 281 nm for caper in ethanol extracted by conventional method

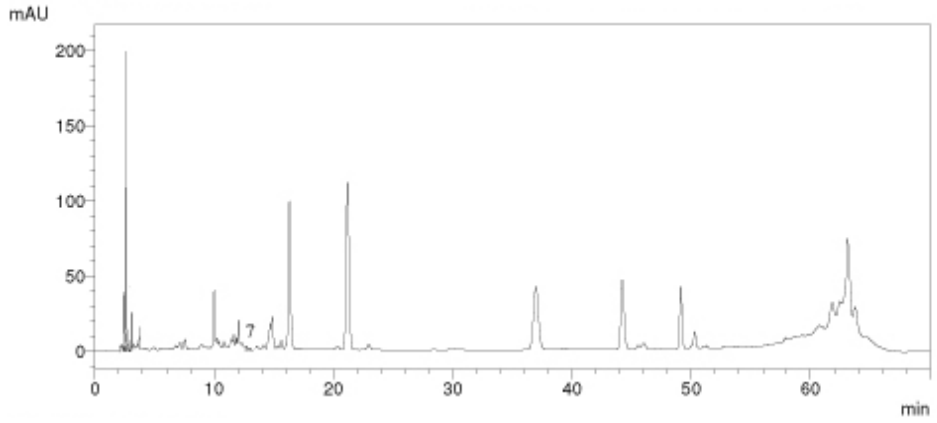

Figure C.31 HPLC chromatogram at 281 nm for caper in ethanol and water mixture extracted by conventional method

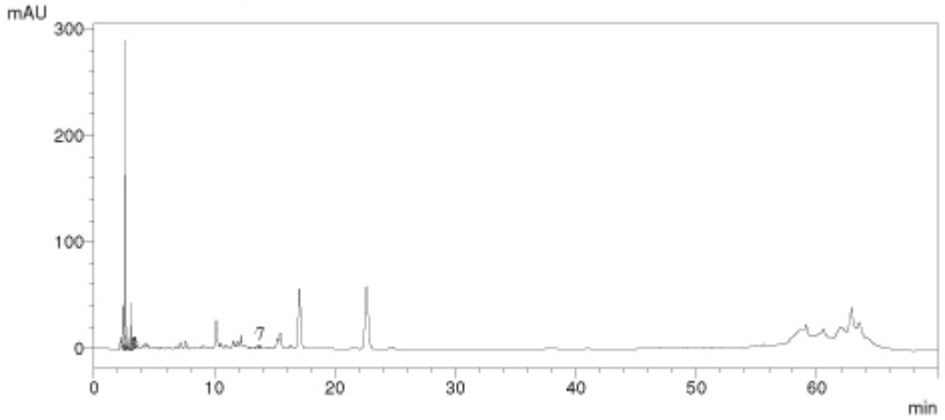

Figure C.32 HPLC chromatogram at 281 nm for caper in water extracted by microwave

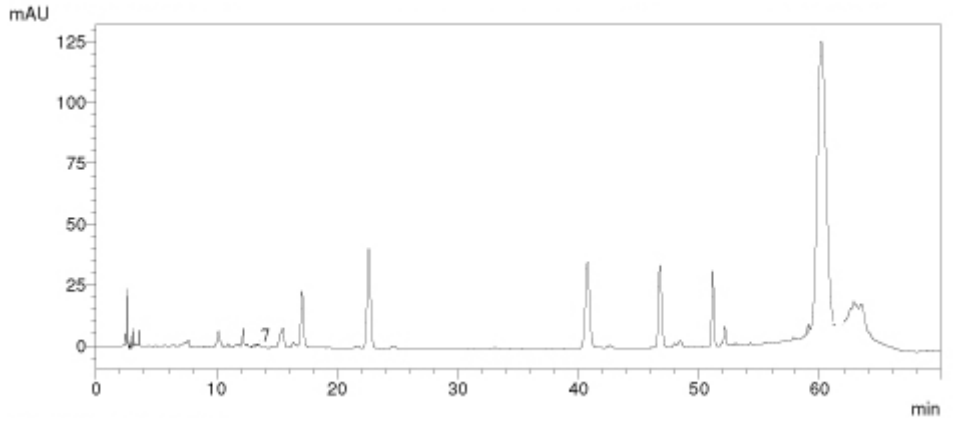

Figure C.33 HPLC chromatogram at 281 nm for caper in ethanol extracted by microwave

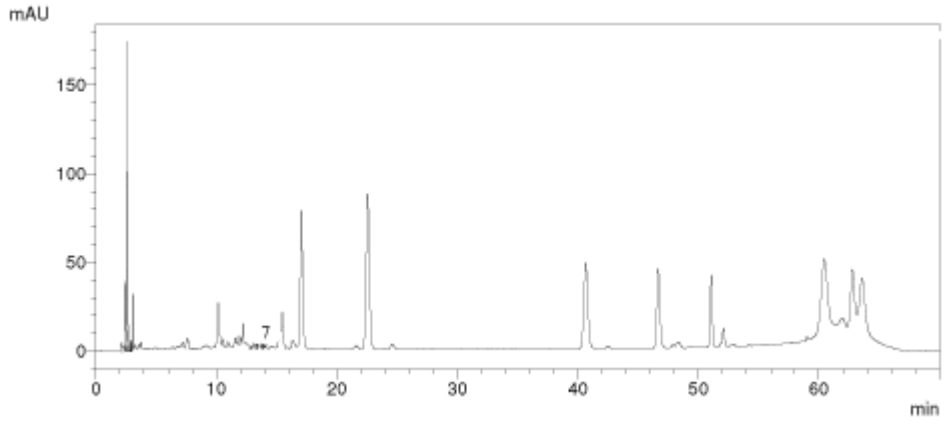

Figure C.34 HPLC chromatogram at 281 nm for caper in ethanol and water mixture extracted by microwave

C.2. HPLC Chromatograms of oleaster

1 4-hydroxybenzoic acid
2 rutin
3 protocatechuic acid
4 vanillin (vanillic acid)
5 kaempferol
6 gallic acid
7 benzoic acid
8 m-coumaric acid (trans-3-hydroxycinnamic acid)
9 p-coumaric acid
10 caffeic acid
11 ferulic acid
12 sinapic acid
13 chlorogenic acid

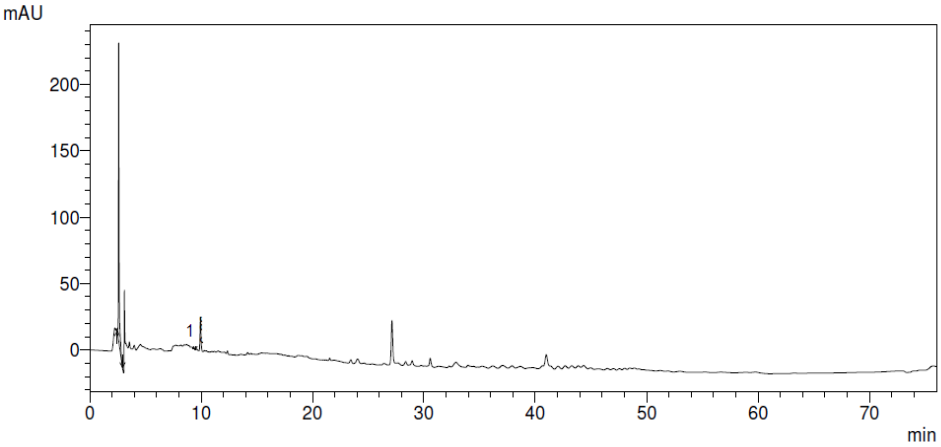

Figure C.35 HPLC chromatogram at 252 nm for oleaster in water extracted by conventional method

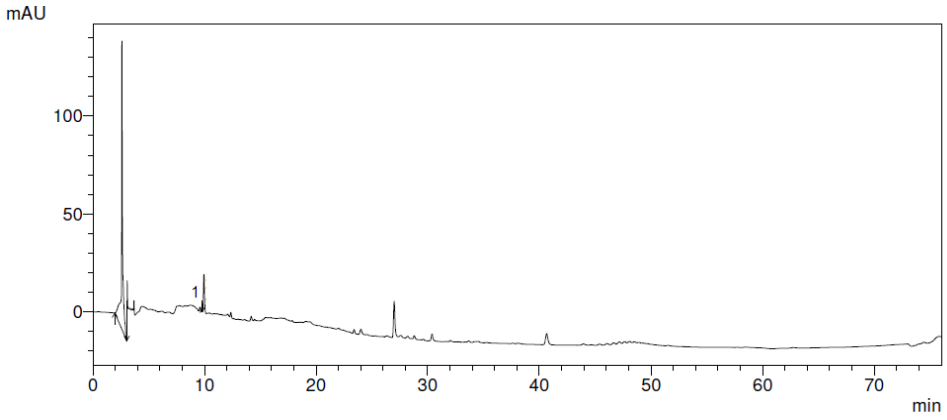

Figure C.36 HPLC chromatogram at 252 nm for oleaster in ethanol and water mixture extracted by conventional method

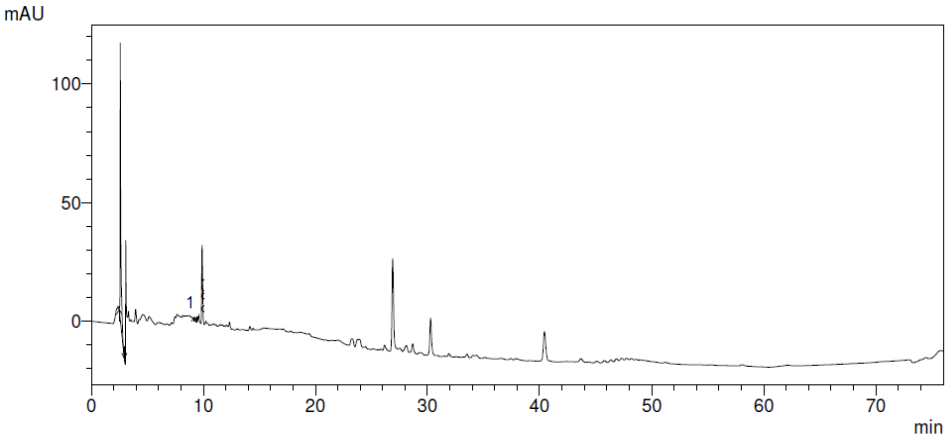

Figure C.37 HPLC chromatogram at 252 nm for oleaster in water extracted by microwave

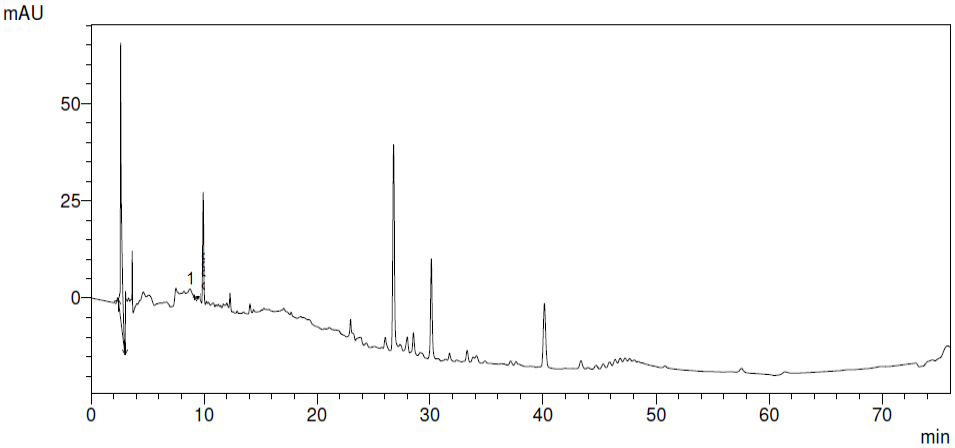

Figure C.38 HPLC chromatogram at 252 nm for oleaster in ethanol and water mixture extracted by microwave

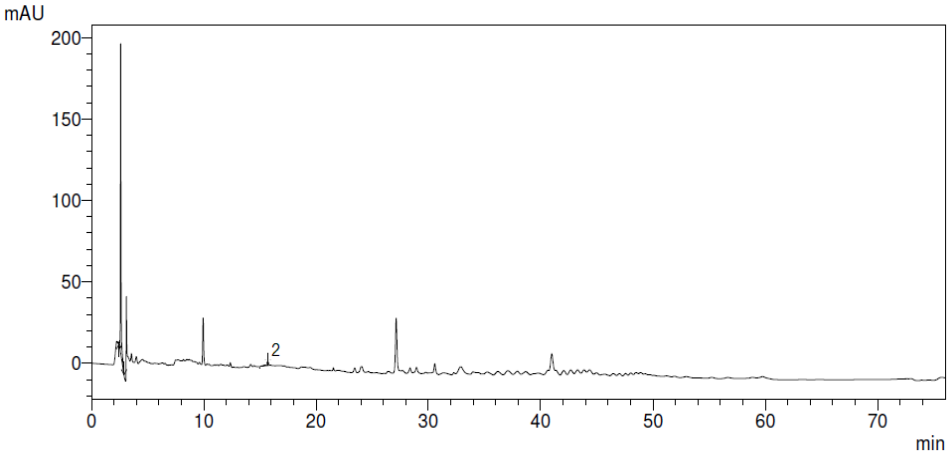

Figure C.39 chromatogram at 254 nm for oleaster in water extracted by conventional method

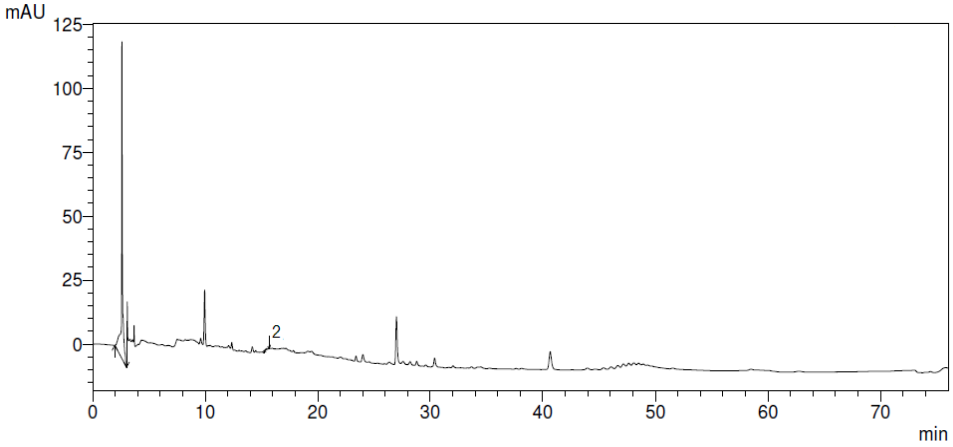

Figure C.40 HPLC chromatogram at 254 nm for oleaster in ethanol and water mixture extracted by conventional method

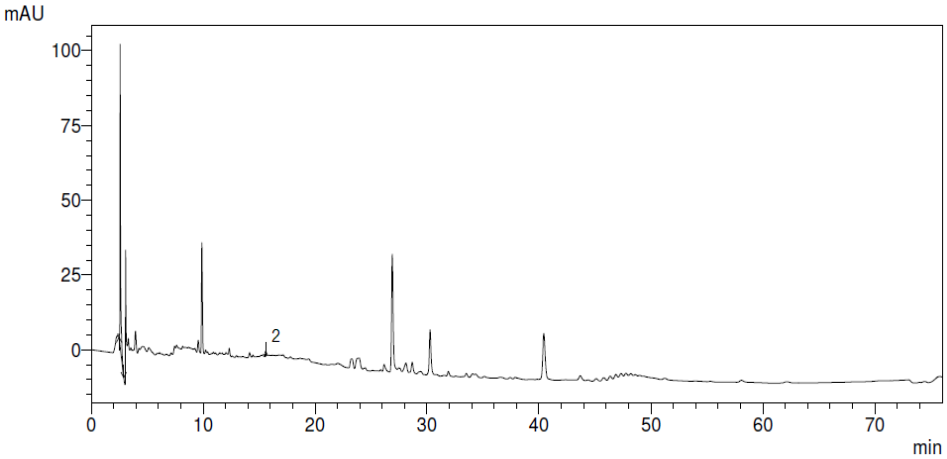

Figure C.41 HPLC chromatogram at 254 nm for oleaster in water extracted by microwave

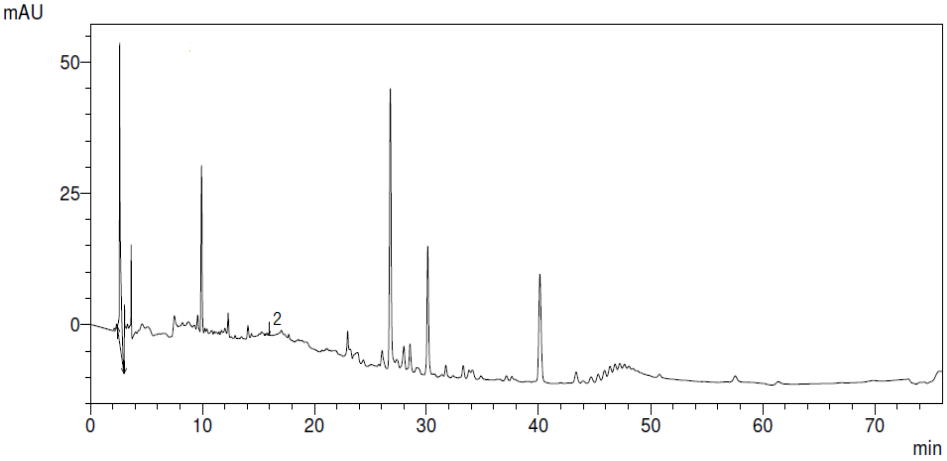

Figure C.42 HPLC chromatogram at 254 nm for oleaster in ethanol and water mixture extracted by microwave

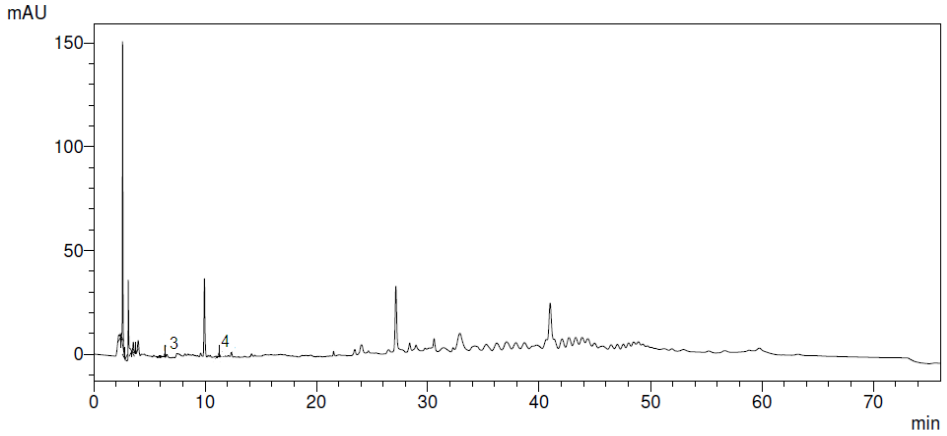

Figure C.43 HPLC chromatogram at 260 nm for oleaster in water extracted by conventional method

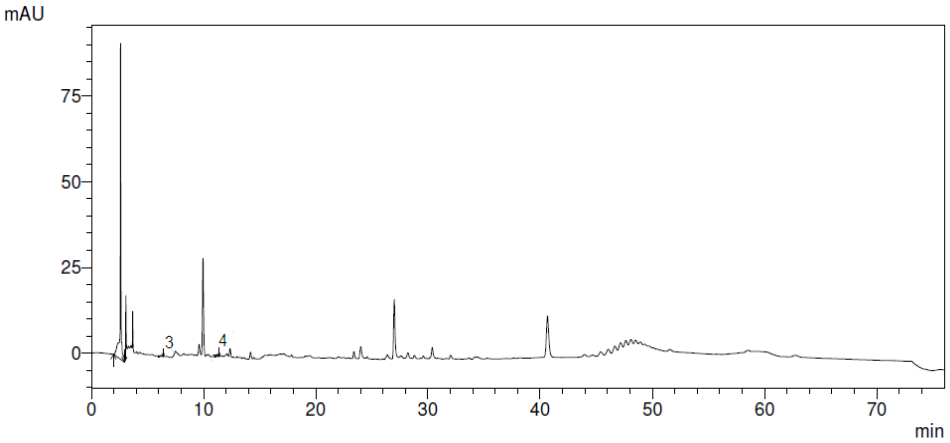

Figure C.44 HPLC chromatogram at 260 nm for oleaster in ethanol and water mixture extracted by conventional method

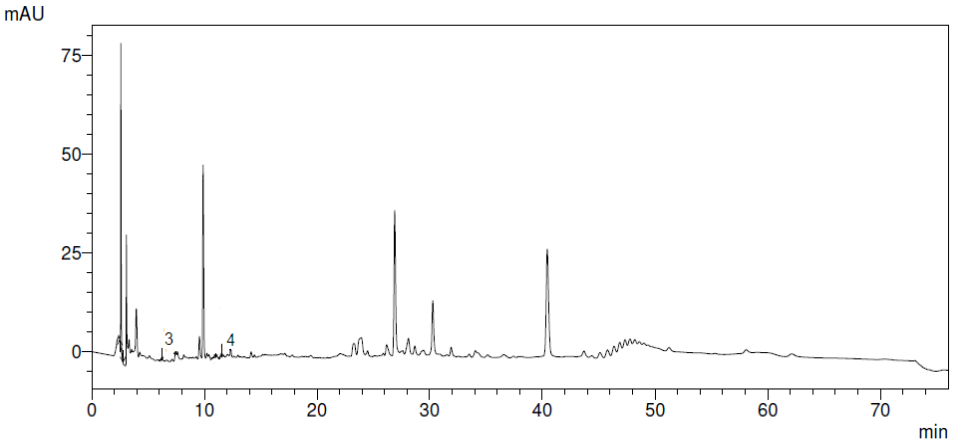

Figure C.45 HPLC chromatogram at 260 nm for oleaster in water extracted by microwave

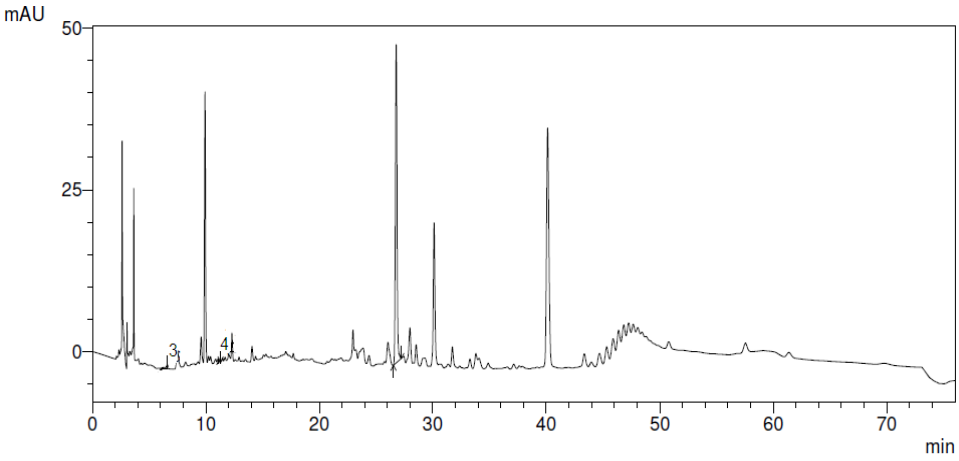

Figure C.46 HPLC chromatogram at 260 nm for oleaster in ethanol and water mixture extracted by microwave

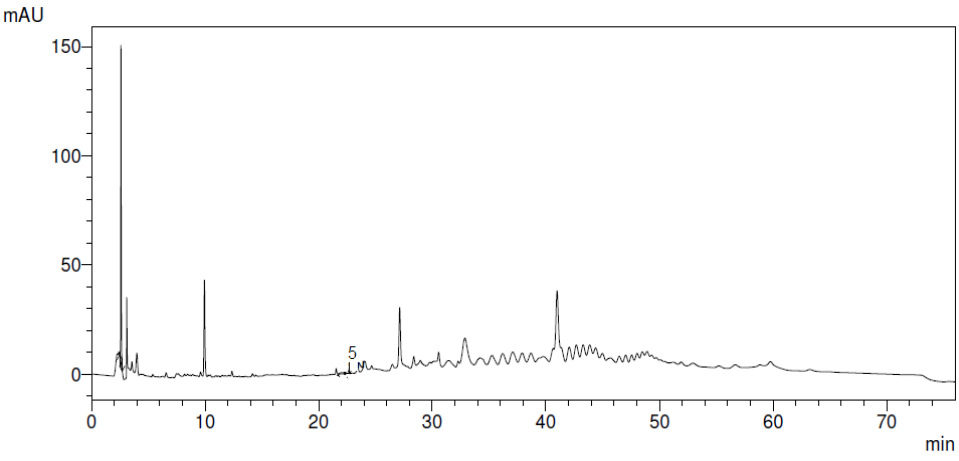

Figure C.47 HPLC chromatogram at 264 nm for oleaster in water extracted by conventional method

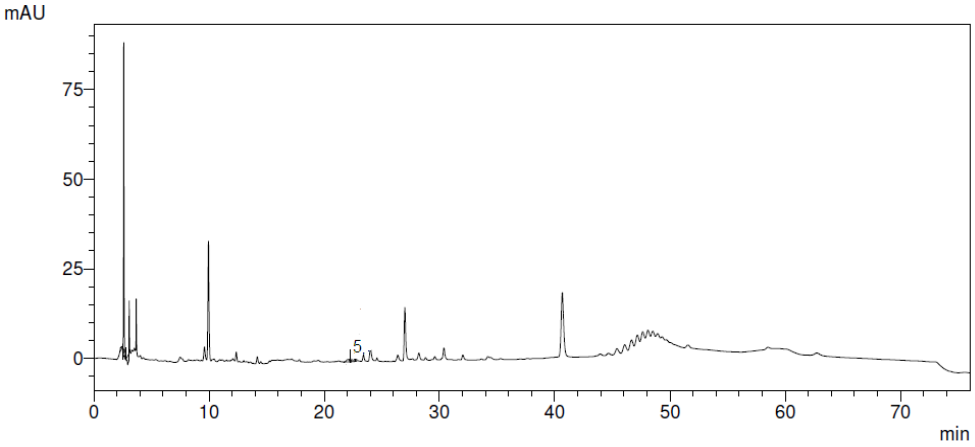

Figure C.48 HPLC chromatogram at 264 nm for oleaster in ethanol and water mixture extracted by conventional method

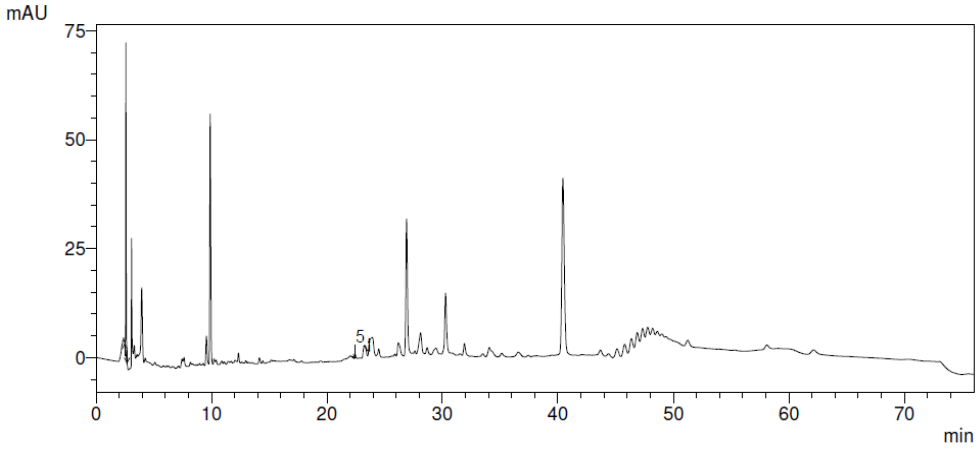

Figure C.49 HPLC chromatogram at 264 nm for oleaster in water extracted by microwave

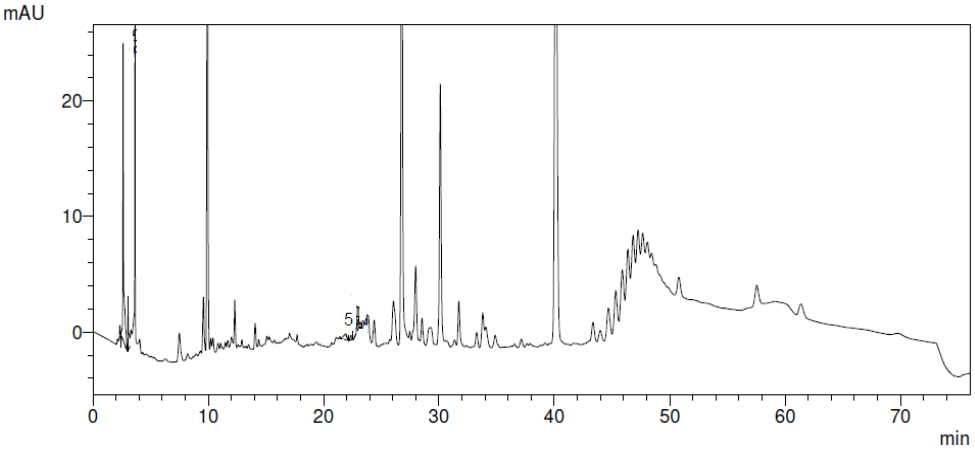

Figure C.50 HPLC chromatogram at 264 nm for oleaster in ethanol and water mixture extracted by microwave

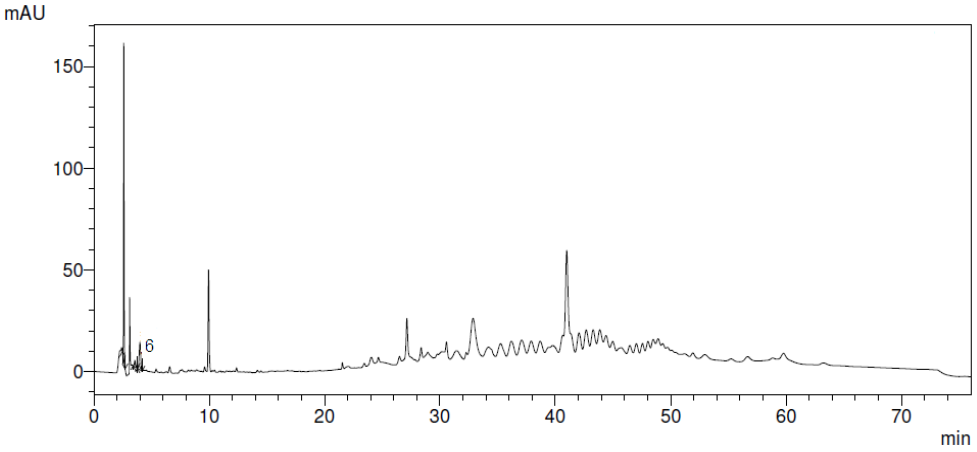

Figure C.51 HPLC chromatogram at 270 nm for oleaster in water extracted by conventional method

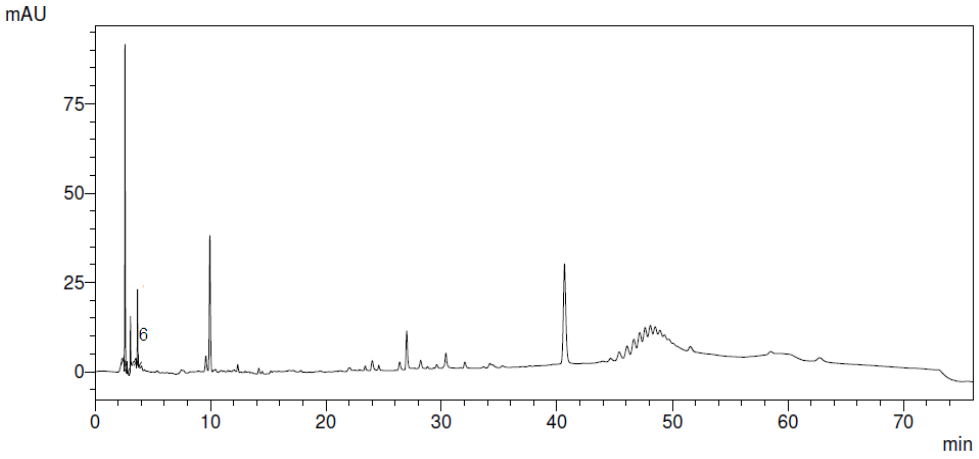

Figure C.52 HPLC chromatogram at 270 nm for oleaster in ethanol and water mixture extracted by conventional method

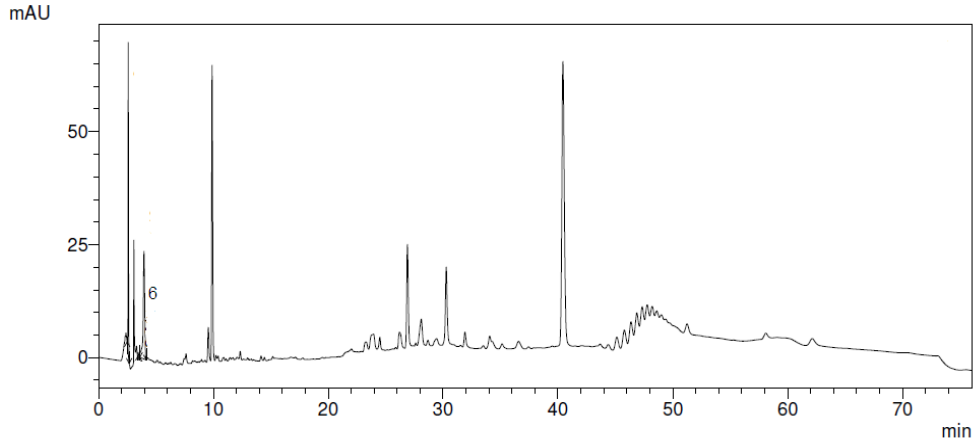

Figure C.53 HPLC chromatogram at 270 nm for oleaster in water extracted by microwave

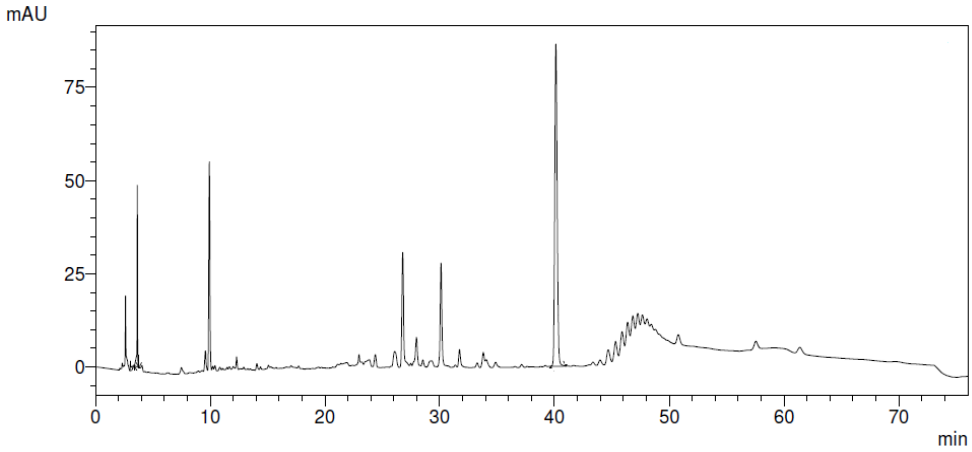

Figure C.54 HPLC chromatogram at 270 nm for oleaster in ethanol and water mixture extracted by microwave

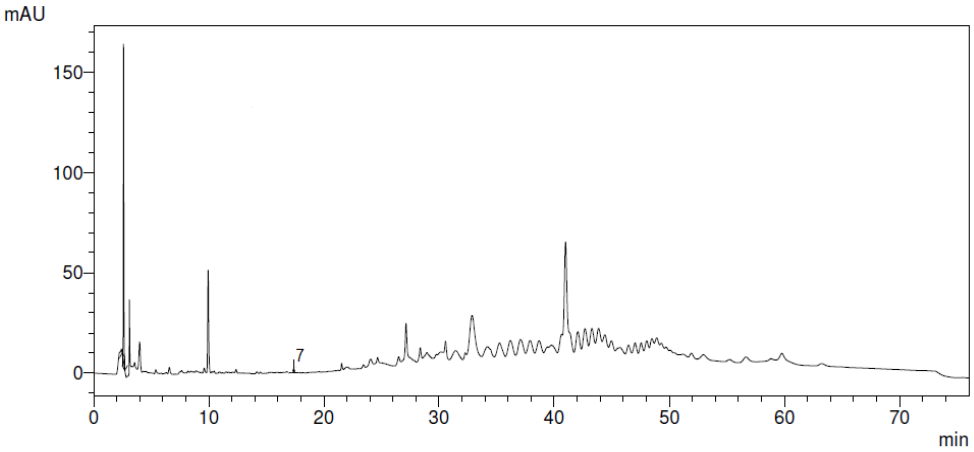

Figure C.55 HPLC chromatogram at 272 nm for oleaster in water extracted by conventional method

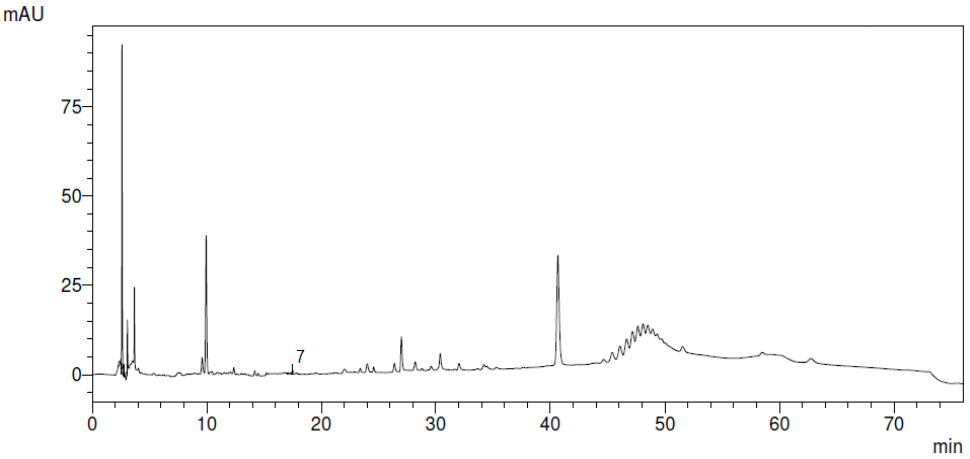

Figure C.56 HPLC chromatogram at 272 nm for oleaster in ethanol and water mixture extracted by conventional method

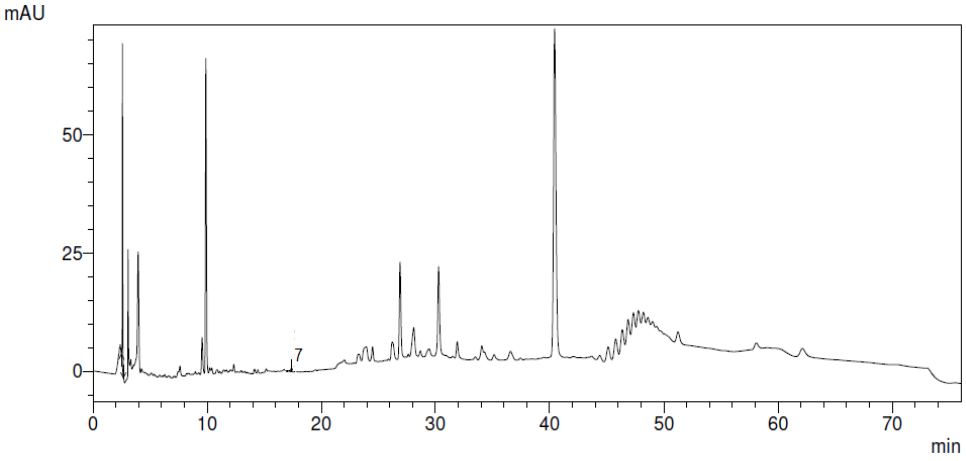

Figure C.57 HPLC chromatogram at 272 nm for oleaster in water extracted by microwave

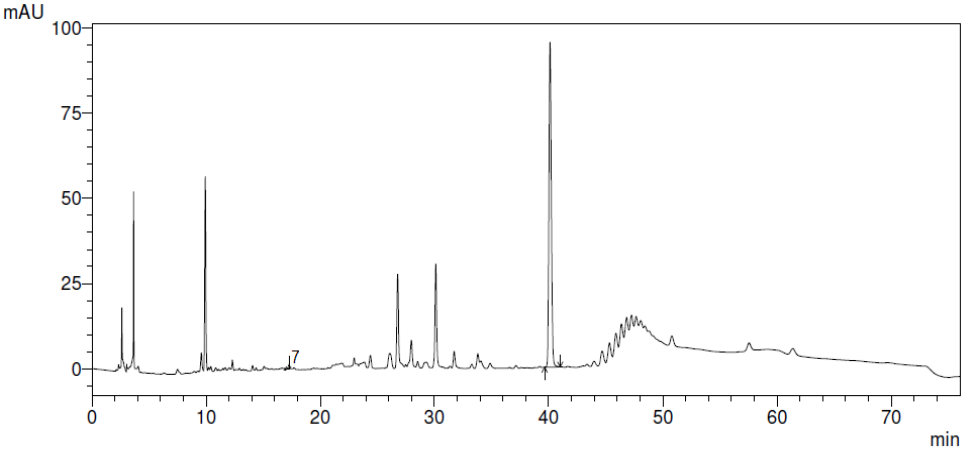

Figure C.58 HPLC chromatogram at 272 nm for oleaster in ethanol and water mixture extracted by microwave

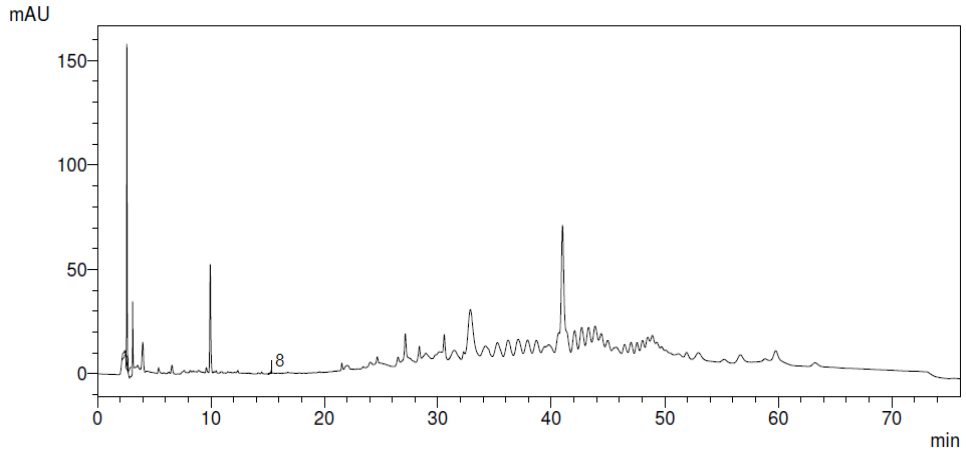

Figure C.59 HPLC chromatogram at 278 nm for oleaster in water extracted by conventional method

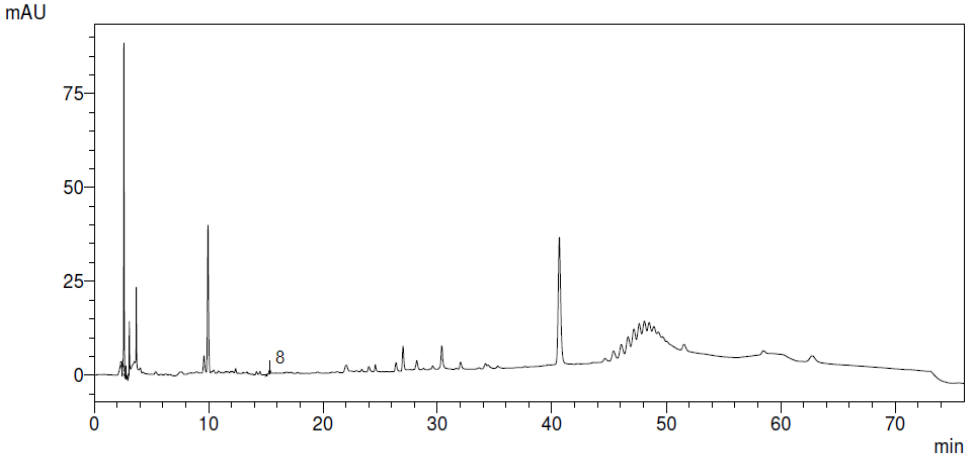

Figure C.60 HPLC chromatogram at 278 nm for oleaster in ethanol and water mixture extracted by conventional method

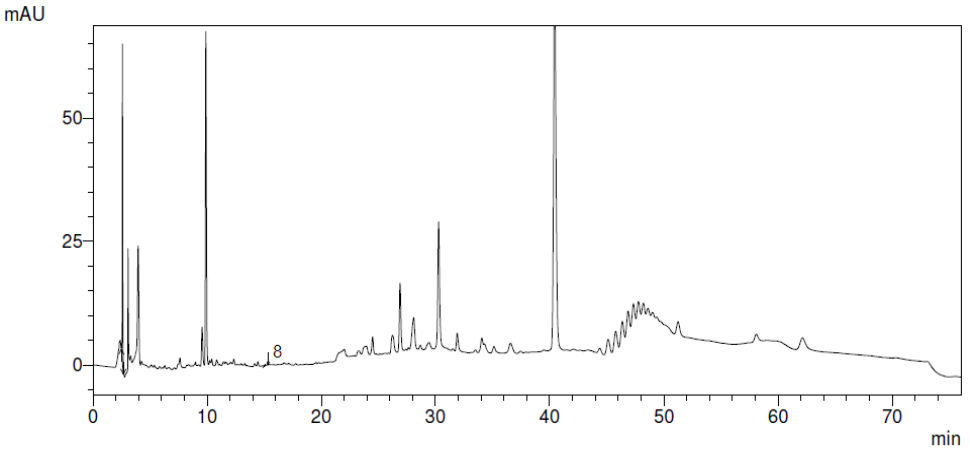

Figure C.61 HPLC chromatogram at 278 nm for oleaster in water extracted by microwave

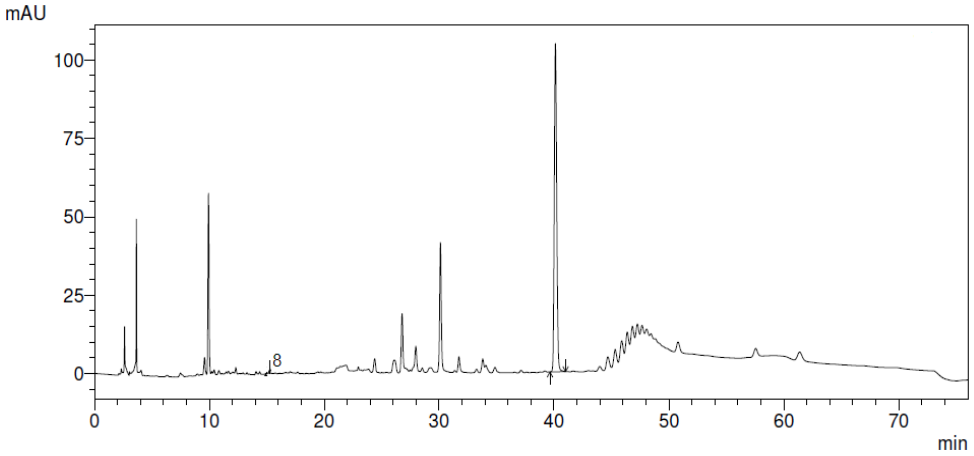

Figure C.62 HPLC chromatogram at 278 nm for oleaster in ethanol and water mixture extracted by microwave

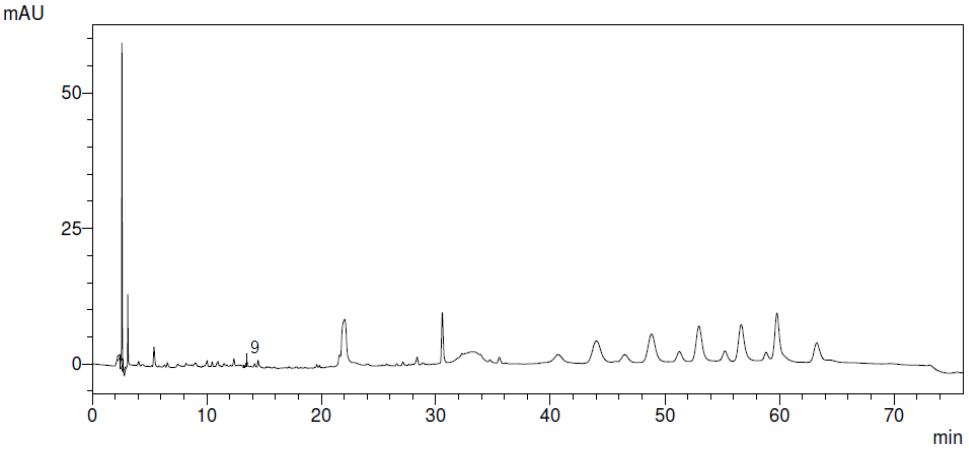

Figure C.63 HPLC chromatogram at 308 nm for oleaster in water extracted by conventional method

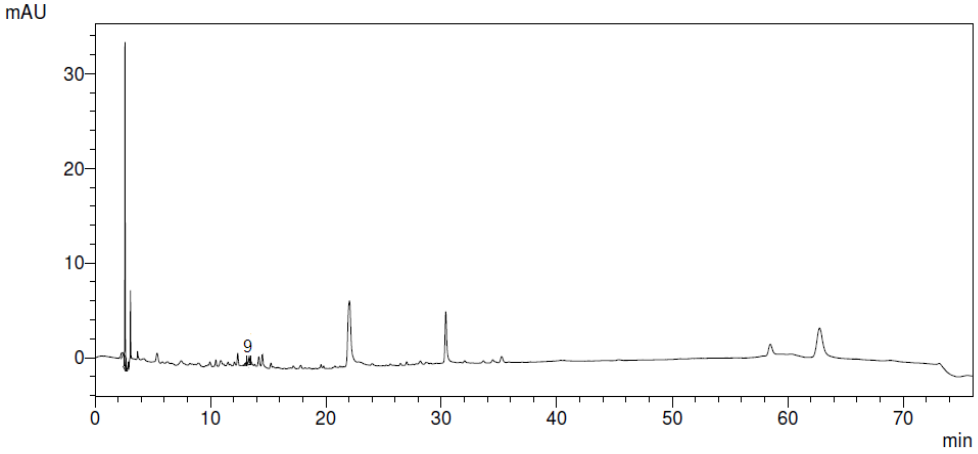

Figure C.64 HPLC chromatogram at 308 nm for oleaster in ethanol and water mixture extracted by conventional method

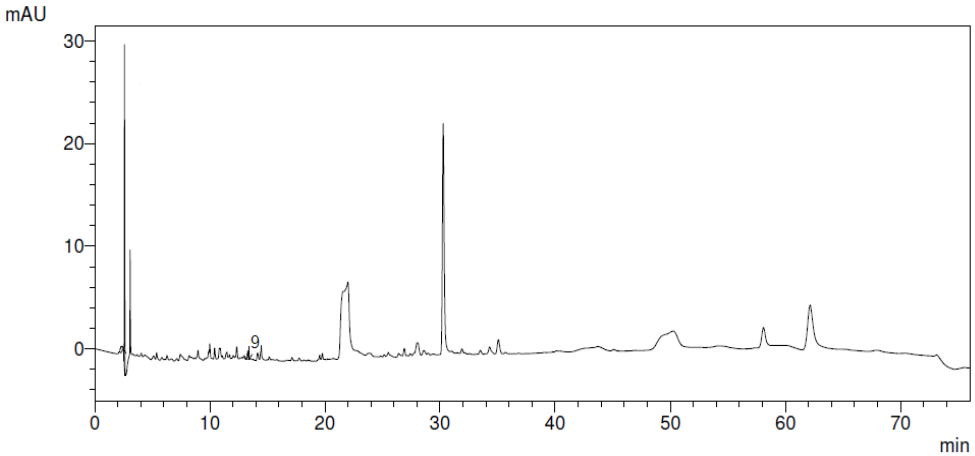

Figure C.65 HPLC chromatogram at 308 nm for oleaster in water extracted by microwave

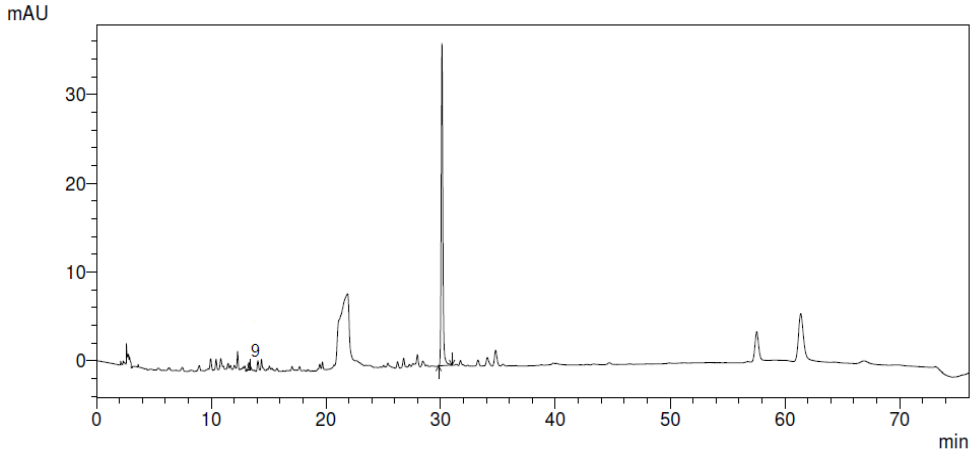

Figure C.66 HPLC chromatogram at 308 nm for oleaster in ethanol and water mixture extracted by microwave

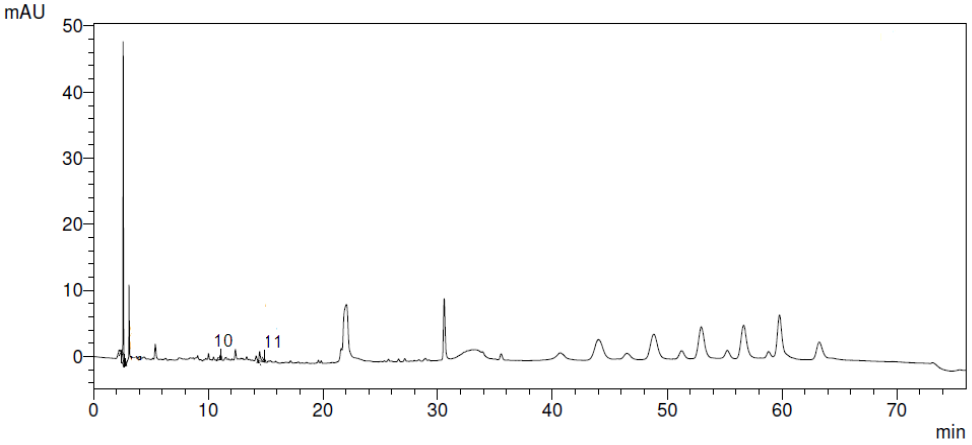

Figure C.67 HPLC chromatogram at 322 nm for oleaster in water extracted by conventional method

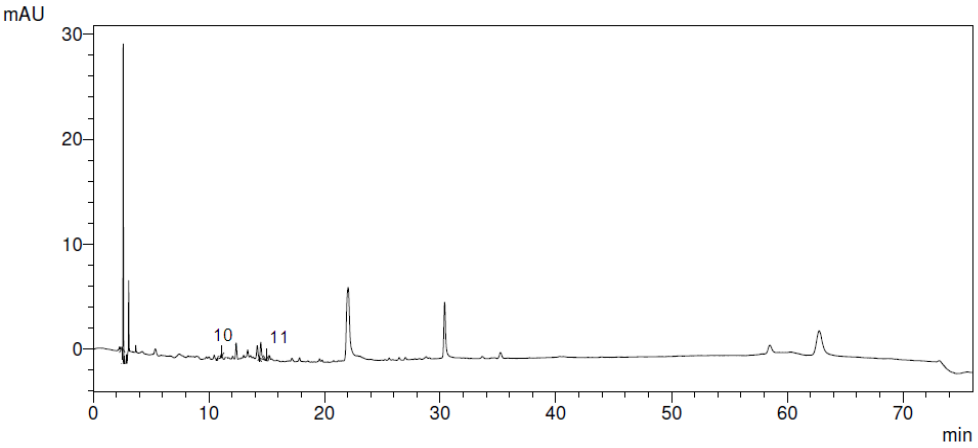

Figure C.68 HPLC chromatogram at 322 nm for oleaster in ethanol and water mixture extracted by conventional method

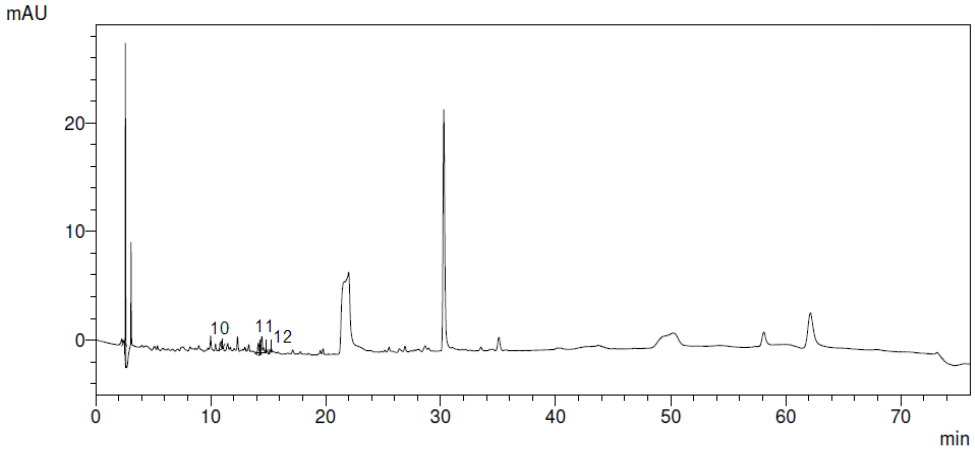

Figure C.69 HPLC chromatogram at 322 nm for oleaster in water extracted by microwave

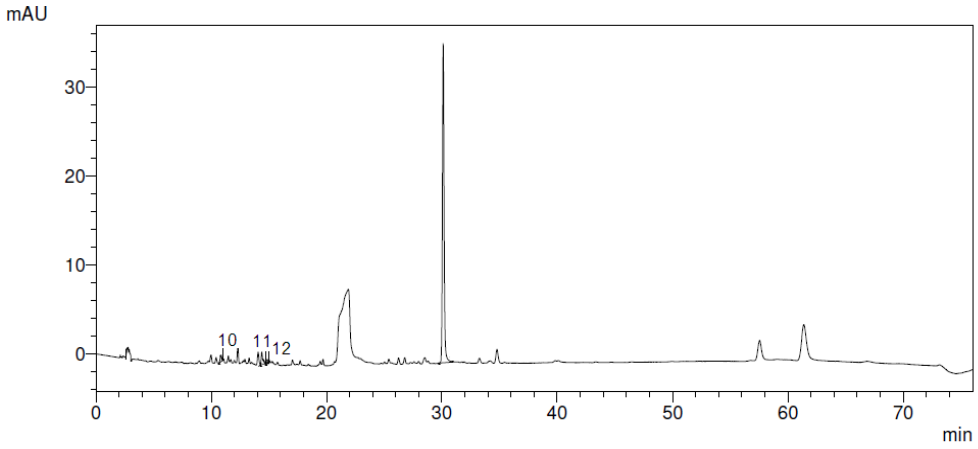

Figure C.70 HPLC chromatogram at 322 nm for oleaster in ethanol and water mixture extracted by microwave

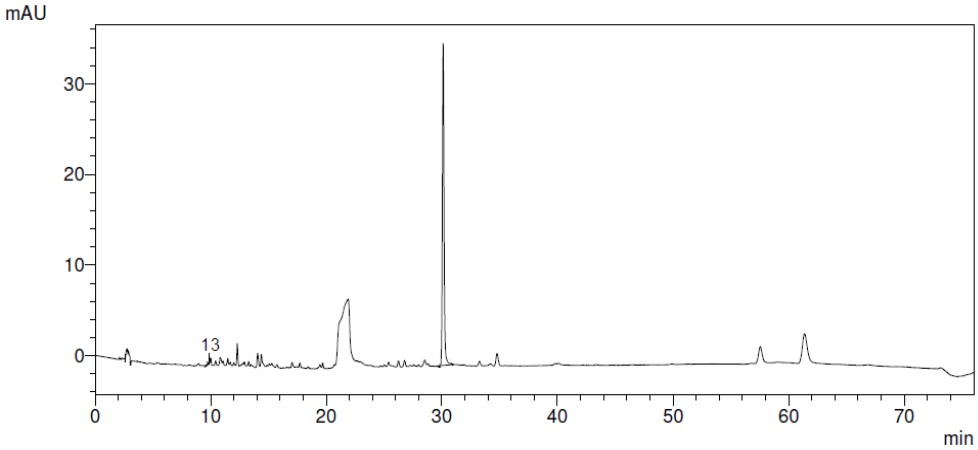

Figure C.71 HPLC chromatogram at 326 nm for oleaster in ethanol and water mixture extracted by microwave

APPENDIX D

STATISTICAL ANALYSIS RESULTS

Table D.1. Microwave Extraction of total phenolic content from caper for optimum independent variables

X1 microwave power (1, 700 W; 2, 400 W)

X2 solvent type (1, water; 2, ethanol; 3, ethanol-water mixture)

X3 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30)

X4 extraction time (1, 5 min; 2, 10 min; 3, 15 min)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3
X3	3	1 2 3
X4	3	1 2 3

Number of Observations Read	108
Number of Observations Used	108

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	13856.28944	1979.46992	55.29	<.0001
Error	100	3580.44056	35.80441		
Corrected Total	107	17436.73000			

R-Square	Coeff Var	Root MSE	Y Mean
0.794661	34.82257	5.983678	17.18333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	54.613333	54.613333	1.53	0.2197
X2	2	9938.221667	4969.110833	138.78	<.0001
X3	2	3859.587222	1929.793611	53.90	<.0001
X4	2	3.867222	1.933611	0.05	0.9475

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	54.613333	54.613333	1.53	0.2197
X2	2	9938.221667	4969.110833	138.78	<.0001
X3	2	3859.587222	1929.793611	53.90	<.0001
X4	2	3.867222	1.933611	0.05	0.9475

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	100
Error Mean Square	35.80441

Number of Means	2
Critical Range	2.285

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	17.894	54	2
A			
A	16.472	54	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	100
Error Mean Square	35.80441

Number of Means	2	3
Critical Range	2.798	2.945

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	30.731	36	3
B	11.031	36	2
B			
B	9.789	36	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	100
Error Mean Square	35.80441

Number of Means	2	3
Critical Range	2.798	2.945

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	24.825	36	3
B	16.494	36	2
C	10.231	36	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	100
Error Mean Square	35.80441

Number of Means	2	3
Critical Range	2.798	2.945

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X4
A	17.450	36	3
A			
A	17.069	36	1
A			
A	17.031	36	2

Table D.2. Microwave Extraction of total phenolic content from caper for the optimum solid to solvent ratio

X1 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30; 4, 1:40; 5, 1:50)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	5	1 2 3 4 5

Number of Observations Read	10
Number of Observations Used	10

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1360.066000	340.016500	23.64	0.0019
Error	5	71.910000	14.382000		
Corrected Total	9	1431.976000			

R-Square	Coeff Var	Root MSE	Y Mean
0.949783	10.01151	3.792361	37.88000

Source	DF	Type I SS	Mean Square	F Value	Pr > F

X1	4	1360.066000	340.016500	23.64	0.0019

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	4	1360.066000	340.016500	23.64	0.0019

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	14.382

Number of Means	2	3	4	5
Critical Range	9.75	10.05	10.18	10.23

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	51.200	2	5
A			
A	45.400	2	4
A			
A	44.600	2	3
B	27.350	2	2
B			
B	20.850	2	1

Table D.3. Conventional Extraction of total phenolic content from caper with ethanol and water mixture for optimum extraction time

X1 extraction time (1, 1 h; 2, 2 h; 3, 4 h; 4, 6 h)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	4	1 2 3 4

Number of Observations Read	8
Number of Observations Used	8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	301.6337500	100.5445833	47.51	0.0014
Error	4	8.4650000	2.1162500		
Corrected Total	7	310.0987500			

R-Square	Coeff Var	Root MSE	Y Mean
0.972702	4.163817	1.454734	34.93750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	3	301.6337500	100.5445833	47.51	0.0014
Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	3	301.6337500	100.5445833	47.51	0.0014

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	4
Error Mean Square	2.11625

Number of Means	2	3	4
Critical Range	4.039	4.127	4.149

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	41.100	2	3
A			
A	39.850	2	4
B	33.100	2	2
C	25.700	2	1

Table D.4. Microwave Extraction of antioxidant activity from caper for optimum independent variables

X1 microwave power (1, 700 W; 2, 400 W)

X2 solvent type (1, water; 2, ethanol; 3, ethanol-water mixture)

X3 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30)

X4 extraction time (1, 5 min; 2, 10 min; 3, 15 min)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3
X3	3	1 2 3
X4	3	1 2 3

Number of Observations Read	54
Number of Observations Used	54

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	5.08236111	0.72605159	26.25	<.0001
Error	46	1.27231481	0.02765902		
Corrected Total	53	6.35467593			

R-Square	Coeff Var	Root MSE	Y Mean
0.799783	16.83363	0.166310	0.987963

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.00041667	0.00041667	0.02	0.9028
X2	2	1.35009259	0.67504630	24.41	<.0001
X3	2	3.72453704	1.86226852	67.33	<.0001
X4	2	0.00731481	0.00365741	0.13	0.8765

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.00041667	0.00041667	0.02	0.9028
X2	2	1.35009259	0.67504630	24.41	<.0001
X3	2	3.72453704	1.86226852	67.33	<.0001
X4	2	0.00731481	0.00365741	0.13	0.8765

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	46
Error Mean Square	0.027659

Number of Means	2
Critical Range	.09111

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	0.99074	27	1
A			
A	0.98519	27	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	46
Error Mean Square	0.027659

Number of Means	2	3
Critical Range	.1116	.1174

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	1.21111	18	3
B	0.88889	18	2
B			
B	0.86389	18	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	46
Error Mean Square	0.027659

Number of Means	2	3
Critical Range	.1116	.1174

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	1.34444	18	3
B	0.90000	18	2
C	0.71944	18	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	46
Error Mean Square	0.027659

Number of Means	2	3
Critical Range	.1116	.1174

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X4
A	1.00000	18	1
A			
A	0.99167	18	2
A			
A	0.97222	18	3

Table D.5. Comparison of conventional and microwave extraction of antioxidant activity from caper

X1 extraction method (1, conventional; 2, microwave)

X2 solvent type (1, water; 2, ethanol; 3, ethanol-water mixture)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.69916667	1.56638889	17.17	0.0008
Error	8	0.73000000	0.09125000		
Corrected Total	11	5.42916667			

R-Square	Coeff Var	Root MSE	Y Mean
0.865541	20.02715	0.302076	1.508333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.18750000	0.18750000	2.05	0.1896
X2	2	4.51166667	2.25583333	24.72	0.0004

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.18750000	0.18750000	2.05	0.1896
X2	2	4.51166667	2.25583333	24.72	0.0004

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.09125

Number of Means	2
Critical Range	.4022

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	1.6333	6	1
A			
A	1.3833	6	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.09125

Number of Means	2	3
Critical Range	.4926	.5133

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	2.3750	4	3
B	1.1000	4	1
B			
B	1.0500	4	2

Table D.6. Microwave extraction of total phenolic content from oleaster in ethanol-water mixture for optimum independent variables

X1 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30)

X2 extraction time (1, 10 min; 2, 15 min; 3, 20 min)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3
X2	3	1 2 3

Number of Observations Read	18
Number of Observations Used	18

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	2103.363333	525.840833	87.77	<.0001
Error	13	77.881667	5.990897		
Corrected Total	17	2181.245000			

R-Square	Coeff Var	Root MSE	Y Mean
0.964295	8.583160	2.447631	28.51667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	2032.590000	1016.295000	169.64	<.0001
X2	2	70.773333	35.386667	5.91	0.0150

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	2032.590000	1016.295000	169.64	<.0001
X2	2	70.773333	35.386667	5.91	0.0150

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	5.990897

Number of Means	2	3
Critical Range	3.053	3.197

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	39.317	6	3
B	32.167	6	2
C	14.067	6	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	13
Error Mean Square	5.990897

Number of Means	2	3
Critical Range	3.053	3.197

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	31.317	6	2
B	27.250	6	1
B			
B	26.983	6	3

Table D.7. Microwave extraction of total phenolic content from oleaster in water for optimum independent variables

X1 microwave power (1, 700 W; 2, 400 W)

X2 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30)

X3 extraction time (1, 10 min; 2, 15 min; 3, 20 min; 4, 30 min)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3
X3	4	1 2 3 4

Number of Observations Read	46
Number of Observations Used	46

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	355.5370487	59.2561748	29.28	<.0001
Error	39	78.9194730	2.0235762		
Corrected Total	45	434.4565217			

R-Square	Coeff Var	Root MSE	Y Mean
0.818349	10.53044	1.422525	13.50870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	2.9395520	2.9395520	1.45	0.2354
X2	2	262.0345634	131.0172817	64.75	<.0001
X3	3	90.5629332	30.1876444	14.92	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	4.0700061	4.0700061	2.01	0.1641
X2	2	260.9648252	130.4824126	64.48	<.0001
X3	3	90.5629332	30.1876444	14.92	<.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	39
Error Mean Square	2.023576

Number of Means	2
Critical Range	.8493

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	13.7727	22	1
A			
A	13.2667	24	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	39
Error Mean Square	2.023576

Number of Means	2	3
Critical Range	1.041	1.095

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	16.4938	16	3
B	13.2071	14	2
C	10.7875	16	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	39
Error Mean Square	2.023576

Number of Means	2	3	4
Critical Range	1.204	1.266	1.306

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	14.8750	12	3
A			
A	14.7100	10	4
B	13.2333	12	2
C	11.4167	12	1

Table D.8. Microwave extraction of total phenolic content from oleaster in ethanol for optimum independent variables

X1 microwave power (1, 700 W; 2, 400 W)

X2 solid: solvent ratio (1, 1:10; 2, 1:20; 3, 1:30)

X3 extraction time (1, 10 min; 2, 15 min; 3, 20 min)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3
X3	3	1 2 3

Number of Observations Read	36
Number of Observations Used	36

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	30.38888889	6.07777778	22.08	<.0001
Error	30	8.25666667	0.27522222		
Corrected Total	35	38.64555556			

R-Square	Coeff Var	Root MSE	Y Mean
0.786349	12.67529	0.524616	4.138889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	0.05444444	0.05444444	0.20	0.6597
X2	2	29.74388889	14.87194444	54.04	<.0001
X3	2	0.59055556	0.29527778	1.07	0.3548

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	0.05444444	0.05444444	0.20	0.6597
X2	2	29.74388889	14.87194444	54.04	<.0001
X3	2	0.59055556	0.29527778	1.07	0.3548

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	0.275222

Number of Means	2
Critical Range	.3571

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	4.1778	18	1
A			
A	4.1000	18	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	0.275222

Number of Means	2	3
Critical Range	.4374	.4597

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	5.2750	12	3
B	4.0917	12	2
C	3.0500	12	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	0.275222

Number of Means	2	3
Critical Range	.4374	.4597

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X3
A	4.2417	12	3
A			
A	4.2167	12	2
A			
A	3.9583	12	1

Table D.9. Conventional extraction of total phenolic content from oleaster in water for determination of optimum extraction time

X1 extraction time (1, 2 h; 2, 4 h; 3, 6 h)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	70.99000000	35.49500000	33.86	0.0087
Error	3	3.14500000	1.04833333		
Corrected Total	5	74.13500000			

R-Square	Coeff Var	Root MSE	Y Mean
0.957577	3.194638	1.023882	32.05000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	70.99000000	35.49500000	33.86	0.0087

Source	DF	Type III SS	Mean Square	F Value	Pr > F

X1	2	70.99000000	35.49500000	33.86	0.0087

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	1.048333

Number of Means	2	3
Critical Range	3.258	3.269

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	34.800	2	3
A			
A	34.150	2	2
B	27.200	2	1

Table D.10. Conventional extraction of total phenolic content from oleaster for optimum independent variables

X1 solvent type (1, water; 2, ethanol-water mixture)

X2 extraction time (1, 2 h; 2, 4 h; 3, 6 h)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	3	1 2 3

Number of Observations Read	12
Number of Observations Used	12

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1216.525000	405.508333	72.64	<.0001
Error	8	44.661667	5.582708		
Corrected Total	11	1261.186667			

R-Square	Coeff Var	Root MSE	Y Mean
0.964588	5.730256	2.362776	41.23333

Source	DF	Type I SS	Mean Square	F Value	Pr > F

X1	1	1086.803333	1086.803333	194.67	<.0001
X2	2	129.721667	64.860833	11.62	0.0043

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	1086.803333	1086.803333	194.67	<.0001
X2	2	129.721667	64.860833	11.62	0.0043

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	5.582708

Number of Means	2
Critical Range	3.146

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	50.750	6	2
B	31.717	6	1

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	5.582708

Number of Means	2	3
Critical Range	3.853	4.015

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	45.050	4	3
A			
A	41.625	4	2
B	37.025	4	1

Table D.11. Comparison of conventional and microwave extraction of total phenolic content from oleaster

X1 extraction method (1, conventional; 2, microwave)

X2 solvent type (1, water; 2, ethanol-water mixture)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	2	1 2
X2	2	1 2

Number of Observations Read	8
Number of Observations Used	8

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	909.2200000	454.6100000	76.14	0.0002
Error	5	29.8550000	5.9710000		
Corrected Total	7	939.0750000			

R-Square	Coeff Var	Root MSE	Y Mean
0.968208	6.726946	2.443563	36.32500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	1	224.7200000	224.7200000	37.64	0.0017
X2	1	684.5000000	684.5000000	114.64	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	1	224.7200000	224.7200000	37.64	0.0017
X2	1	684.5000000	684.5000000	114.64	0.0001

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	5.971

Number of Means	2
Critical Range	4.442

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	41.625	4	1
B	31.025	4	2

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	5
Error Mean Square	5.971

Number of Means	2
Critical Range	4.442

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X2
A	45.575	4	2
B	27.075	4	1

Table D.12. Conventional extraction of total antioxidant activity from oleaster in water for determination of optimum extraction time

X1 extraction time (1, 2 h; 2, 4 h; 3, 6 h)

The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
X1	3	1 2 3

Number of Observations Read	6
Number of Observations Used	6

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	3.94333333	1.97166667	19.39	0.0192
Error	3	0.30500000	0.10166667		
Corrected Total	5	4.24833333			

R-Square	Coeff Var	Root MSE	Y Mean
0.928207	4.995072	0.318852	6.383333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
X1	2	3.94333333	1.97166667	19.39	0.0192

Source	DF	Type III SS	Mean Square	F Value	Pr > F
X1	2	3.94333333	1.97166667	19.39	0.0192

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	3
Error Mean Square	0.101667

Number of Means	2	3
Critical Range	1.015	1.018

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	X1
A	7.1000	2	3
A			
A	6.8000	2	2
B	5.2500	2	1