DETERMINATION OF MORPHINE AND TOTAL PHENOLIC CONTENT IN POPPY SEED OF TURKISH ORIGIN

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ABSTRACT

DETERMINATION OF MORPHINE AND TOTAL PHENOLIC CONTENT IN POPPY SEED OF TURKISH ORIGIN

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Turkey is important major licid opium poppy (*Papaver Somniferum*) producer for medicinal and scientific purposes in the world and one of the two traditional producer country. The poppy seeds which are unique part of the opium poppy are used for food purpose. About 25.000 ton/year poppy seeds were produced for food purpose. Recent years morphine content of commercially available poppy seeds were speculated. Some studies have been reported that poppy seeds from different origins contain a wide variation of morphine (2 – 294) μg/g content. They have been used also different sample preparation method (extraction and derivatization) and different instruments (GC-MS) during analyses. In this study morphine content was determined in Turkish origin which were white, yellow and blue poppy seeds colors by using high pressure liquid chromatography (HPLC). Different

sample preparation method (without derivatization) was used. Morphine was eluted from poppy seeds by acidic water through stationary phase activated aluminum oxide. Poppy seed samples were collected from 13 provinces and 35 different sowing fields of Turkey. The determined morphine contents in poppy seeds were ranged between (9.73 to 37.46) µg/g. Any study was not found in the literatures on determination of total phenolic in poppy seed. In this study, the optimum condition for extraction of polyphenols in poppy seeds was determined and then, total phenolic values were obtained in three different colours seeds by spectrophotometric method. At the end of experiments the total phenolic contents in white, yellow and blue colours poppy seed were found to be 4.44, 3.05 and 3.67 mg/g respectively.

Key Words: *Papaver somniferum* L., poppy seed, morphine, HPLC, phenolic, total polyphenol

ÖZ

TÜRK MENŞEYLİ HAŞHAŞ TOHUMUNDAKİ MORFİN VE TOPLAM FENOLİK IÇERİĞİNE BAKILMASI

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Türkiye, dünyada tıbbi ve bilimsel amaçlı başta gelen yasal haşhaş (*Papaver somniferum*) üreticisi olup ayrıca iki geleneksel üretici ülkeden birisidir. Haşhaşın tohumu bitkinin gıda amaçlı kullanılan tek kısmıdır. Yılda yaklaşık 25.000 ton gıda amaçlı haşhaş tohumu üretilmektedir. Son yıllarda ticari haşhaş tohumunda morfin içeriği tartışılmaya başlanmıştır. Bazı çalışmalarda farklı menşeyli haşhaş tohumlarında geniş farklılık gösteren morfin miktarları (2 – 294) µg/g rapor edilmiştir. Çalışmalarda, analizden önce farklı numune hazırlama metodu, (ekstraksiyon ve türevleme) ve farklı cihazlar (GC-MS) kullanılmıştır. Bu çalışmada, morfin içeriği beyaz, sarı ve mavi renkli Türk menşeyli haşhaş tohumunda ve yüksek basınçlı sıvı kromatografi (HPLC) cihazı kullanılarak morfin içeriği tayin edilmiştir. Analiz sırasında farklı

numune hazırlama metodu (türevleme olmadan) kullanılmıştır. Morfin, haşhaş tohumundan aktive edilmiş aluminyum oksit içeren kolondan su ile geçirilerek ekstre edilmiştir. Haşhaş tohumu numuneleri Türkiyenin 13 il ve 35 farklı ekim alanlarından temin edilmiştir. Haşhaş tohumunda tespit edilen morfin miktarları (9.73 - 37.46) µg/g arasında değişmektedir. Yapılan literatür araştırmalarında haşhaş tohumunda toplam fenolik madde analizine rastlanmamıştır. Bu çalışmada haşhaş tohumundaki fenolik maddelerin özütlenmesi için optimum koşullar belirlenmiş ve üç farklı renkteki tohumlarda toplam fenolik değerler spektrofotometrik metod ile elde edilmiştir. Deneylerin sonucunda beyaz, sarı ve mavi renkli haşhaş tohumlarında fenolik içerikler sırasıyla 4.44, 3.05 ve 3.67 mg/g olarak bulunmuştur.

Anahtar kelimeler: *Papaver somniferum* L, haşhaş tohumu, morfin, HPLC, fenolik, toplam polifenol

To my wife…

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LIST OF SYMBOLS AND ABBREVIATION

ASTM	American Society for Testing and Materials
Cse	Concentration of morphine in sample extract
DAD	Diode-array detector
ECOSOC	Economic and Social Council
INCB	International Narcotics Control Board
GA	Gallic Acid
GAE	Gallic acid equivalent
Ha	Hectar
HPLC	High Performance Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of quantitation
RSD	Relative standard deviation
RT	Retention time
SD	Standard deviation
THF	Tetrahydrofuran
TMO	Turkish Grain Board
TFA	Triflouroacetic acid
UK	United Kingdom
UN	United Nations
TP	Total phenolic
USA	United States of America
USD	United States Dollar
USP	United States Pharmacopy
Vse	Volume of sample extract
Ws	Weight of sample
GC/MS	Gas chromatography/mass spectrometry

CHAPTER I

INTRODUCTION

Turkey is traditional producer one of the two country accepted by United Nations (UN) with India (Resolution 51/9). The only seed part of the opium poppy is used for food purpose. Although poppy seed is not included in Schedules of the 1961 single convention, UN called upon Member States to take some measures to fight the international trade in opium poppy seeds from countries not permitting the cultivation of opium poppy (Report 2008, Resolution 51/15). Because of those reasons Turkey has about 50% of world total opium poppy sowing areas. The poppy seed is one of the important export goods. Recent years income from poppy seed exportation was increased (TMO, 2009).

Although poppy seed itself does not contain latex and some botanical literature describes the seeds as alkaloid-free (Mika 1955), a change in harvesting technology was speculated as an explanation for high morphine contents in the last 20 years (Moeller et al 2004). Some studies have been reported that poppy seeds from different origins contain a wide variation of morphine (2 – 294 µg/g) content. Such large variations in opiate alkaloid concentrations may arise due to variations in the climate, soil composition, seed quality, the year of harvest and the variety of *Papaver somniferum* cultivated (Pelders et al 1996). Soaking poppy seeds in water for five minutes was found to remove about 45 % of their free morphine (Lo et al 1992). Some studies were confirmed the observations that poppy seed morphine originates predominantly from external contaminations (Sproll et al 2006).

1.1 Poppy plant

The poppy belongs to a family of plants called "Papaveraceae". There are about 200 species that belong to this family of plants. The Papaveraceae are dicotyledonous, dialypetalous superovaried plants (Bulletin of Narcotics, 1950). The most famous, naturally, is the species *Papaver somniferum*, also known as the Opium Poppy which produces Opium and poppy straw from, which alkaloids such as morphine, thebaine, codeine, oripavine are extracted for use as an analgesic narcotic in the treatment of mild to severe pain (E/INCB/2008/1) and noscapine and papaverine are extracted for medicinal use.

1.2 Opium poppy

Opium Poppy (Papaver somniferum), is a hardy annual that can grow almost anywhere in North America, Europe, Asia, and Australia. Sowing, production, control, and trading, of opium poppy are controlled under the UN Single Convention on Narcotic Drugs, 1961.

1.3 History

The opium poppy has been grown in Anatolia since 2000 B.C. during Hitit civilization. Poppy word was defined as “haşşika” by Hitits. So it is very interesting similarity on Turkish definition as “haşhaş”. This word means to sleep or to relax in Hitit language (Baytop, 1963; Ertem, 1974). In 1st centry, poppy species which where used for medicinal purpose, opium production from plant, analgesic and narcotic effects were reported by Dioscorides (Gunther, 1968). During the Ottoman Empire opium was one of the important export material at 1858, to UK and USA 41.130 kg and 40.800 kg opium were exported. This plant is why the opium wars started between Great Britan and China in around 1840, and why Hong Kong belonged to Great Britian for about 150 years. One of the evidences for history of poppy plant and wheat figures at coin which was found in Turkey was shown in Figure 1.1

Figure 1.1 The coin with poppy fıgure for II. century B.C. (Afyonkarahisar museum)

In Turkey, up to 1933 poppy cultivation was free from any limitation. In 1933 Narcotics Monopoly Administration was founded and poppy cultivation was made in the restricted areas and under the control. While making opium production by lancing poppy capsules, until 1971, poppy cultivation had banned in 1971. In 1974 unlanced method of poppy cultivation has started in 7 provinces as a plot under the license and control. In 1974, an opium alkaloids factory project for production of opium alkaloids from unlanced poppy straw was planned. The opium alkaloids plant was founded and started to production in 1981 in Bolvadin.

As it is already known, Turkey is one of the two countries who are accepted as traditional poppy producing countries by United Nations. According to the statutes of the Law and Regulations no 3298, which has arranged within the requirements of 1961 Single Convention that has also signed by Turkey controlled poppy cultivation against licence has being carried out by Turkish Grain Board (TMO) which is a State Organization.

1.4 Classification

In taxonomy, *Papaver somniferum* L. is classified by Karaca as follows

Kingdom : Plantae
Subkingdom : Tracheobionta . Vascular plants
Division : Magnoliophyta - Flowering plants
Subdivision : Spermatophyta - Seed plants
Class : Magnoliopsida - Dicotyledons
Subclass : Spermatophyta - Seed plants
Order : Ranunculales
Family : Papaveraceae - Dicotyledons
Genus : *Papaver* - poppy
Species : *Papaver somniferum* L.

In Turkey, there are about 30 species of *papaver* genus. An attempt has also been made to classify poppies according to the colour of the seeds, and two main varieties have been established: *Papaver somniferum* L. album, with white or light-coloured seeds and smooth, glabrous leaves; and Papaver somniferum L. nigrum, with black or dark-coloured seeds, and red flowers. Turkish poppy-seeds are usually white, yellow, grey, coffee-coloured, blue, or dark blue but black and all other colours are also found.

The opium poppy, *Papaver somniferum L*. is a plant which is often self-pollinated, sometimes cross-pollinated. For that reason its morphological features are not stable. Cross-breeding between different varieties, however, is sometimes rather difficult. The poppies cultivated in Turkey form a "population" composed of various strains whose characteristics are constantly changing (Bulletin of Narcotics, 1950). Attempts to obtain varieties with stable characteristics and a high yield have not yet given satisfactory results. In spite of the presence of different strains, however, the poppies of a given region or area display common characteristics under the influence of climate and soil and, taken together, they form a more or less definite type.

1.5 Varieties of the plant (Bulletin of Narcotics, 1950)

Varieties of the poppy plant were described before. The fact that from the botanical point of view it is difficult to distinguish sub-species or varieties within the species *Papaver somniferum* is immaterial to the agriculturalist.

1. *Variation in the flower:* The flower may be single or double, with considerable variation of shape, arrangement and colour of petals (white, pink, red, purple, crimson or variegated).

2. *Variation in the seed:* Here again contradictory assertions and classifications, attributing particular effects on the plant's alkaloid content to certain colours of seed, are made. The fact is that there are white, yellow, coffee-coloured, black, grey, blue, etc., seeds, and there appears to be virtually no relationship between the colour of the flower and that of the seed (white and yellow seed is white flower, blue seed is violet flower). Turkish poppy-seeds are usually white, yellow, grey, coffee-coloured, blue, or dark blue but black and all other colours are also found.

3. *Variation in the capsule:* The capsules may be of different shapes (elongated, globular, oblate, etc.) as shown ın Figure 1.2 and no relationship can be detected between the shape and the alkaloid content. The capsules may also be closed or open, and there may be two, three or more capsules on each plant.

4. *Other variations:* The poppy may vary as to height (30 to 150 centimetres or more), stem appearance (glabrous or hairy), and leaf, which may be of many different shapes.

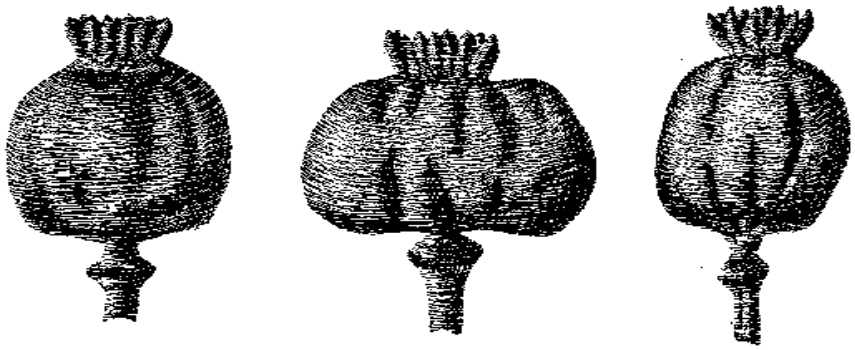

Figure 1.2 The poppy capsules which are different shapes in Turkey (Meadwey at al, 1998)

1.6 Poppy cultivation in the world

In the world, cultivation, production, importation and exportation of opiate and opiate raw materials are executed within the frame of 1961 Single Convention on Narcotic Drugs and 1972 Protocol amending 1961 Single Convention

Turkey, India, Australya, France, Spain, Hungary (Table: 1.1) are main licid opium poppy producers in the World under the control of United Nations. Other producer countries are Czech Republic and UK. Turkey and India were accepted traditional opium poppy producer by UN, other countries are commercially producers. Licid opium poppy harvesting area of main producer countries in the period 1999-2009 were described in Table 1.1.

Table 1.1 Licid opium poppy harvesting area (ha) of main producer countries

Years	Turkey	India	Australya	France	Spain	Hungary	Total
2000	27.554	32.085	15.166	5.914	5.698	2.789	89.206
2001	45.836	18.087	8.925	5.402	5.536	6.961	90.747
2002	50.741	18.447	11.701	6.451	7.912	9.924	105.176
2003	99.430	12.320	9.811	7.919	5.732	2.937	138.149
2004	30.343	18.591	6.644	8.312	5.986	7.084	76.960
2005	25.335	7.833	6.599	8.841	4.802	5.106	58.516
2006	42.023	6.976	3.457	6.632	2.146	4.322	65.556
2007	24.603	5.913	4.661	3.198	5.606	3.269	47.250
2008	20.043	2.653	3.336	3.705	5.507	2.267	37.506
2009(*)	48.893	11.262	10.506	7.500	8.830	15.500	102.491

(TMO, 2009)

(*) Estimation

1.7 Poppy cultivation in Turkey

The poppy is usually grown in Turkey at a height of between 300 and 700 metres, although it is found at 60 metres and also at 1,300 metres. As it is already known, Turkey is one of the two countries who are accepted as traditional poppy producing countries by United Nations.

According to the statutes of the Law and Regulations which were arranged within the requirements of 1961 Single Convention that has also signed by Turkey; controlled poppy cultivation against licence has being carried out by TMO which is a State Organization. Poppy cultivation and production of unlanced poppy capsules are being made legally in 13 provinces and at 70.000 ha total annualy under the planning and control of TMO on the restricted areas, being determined and permitted for poppy cultivation every year by the Council of Ministers (Figure 1.3).

According to the Law and Regulation, permission certificates for cultivation of poppy and production of unlanced poppy capsules on the legal cultivation areas are being delivered by TMO and it is being controlled as organized.
The controlling of poppy cultivation on the permitted areas, being determined and permitted for poppy cultivation by Council of Ministers are being carried out by planning on the town basis.

Figure 1.3 Poppy cultivation area map in Turkey (white coloured provinces) (TMO, 2009)

Poppy harvesting areas and number of farmers in Turkey between 2000 - 2009 are shown in Table 1.2.

Table 1.2 Poppy harvesting areas and number of farmers (TMO, 2009)

Years	Harvesting area (Ha)	Number of farmers
2000	27.554	66.090
2001	45.836	96.338
2002	50.741	93.486
2003	99.430	167.648
2004	30.331	86.209
2005	25.635	67.119
2006	42.023	102.681
2007	24.603	44.780
2008	20.042	35.079
2009	48.893	79.152

1.7.1 The cultivation and harvesting (Bulletin of Narcotics, 1950)

The poppy is a hardy plant; it will grow in varying climates but cannot endure extreme cold. In a cold climate, its opium yield is greatly diminished: climatic conditions, particularly humidity, affect the yield more than any other factor. Because of the climate conditions, there ıs about 50% difference between the areas given licence and the areas after the control.

In damp climates the poppy is attacked by some plant diseases. Moreover, during much rain, the plants grow very tall and may be beaten down when they are ripe. Heavy rain at the time of harvesting washes away some of the latex and may even wash it off completely and reduce the yield and the morphine content. Thus the ideal conditions are snow in winter, rain in spring and dry weather while the plant is mature.

The poppy plants are very sensitive to wind, particularly when it is maturing. The ideal is an average soil treated with natural or chemical fertilizers, for the poppy plant impoverishes the soil.

The poppy is an annual and may be sown in autumn or spring: according to soil temperarure and climate. The most important point is the alternation of rainy and dry seasons, which determines the opium yield. In poppy-growing areas, which are generally warm temperate zones, the sowing time may therefore begin in September and continue to as late as April.

Germination lasts two to three weeks; about a month later the first four leaves appear as shown in Figure 1.4; and two or three weeks later the stem begins to form as shown in Figure 1.5. The plant reaches full development in about two months. The flowering time (Figure 1.6) varies according to climate, soil properties and sowing date. The plant flowers by day and the flower lasts thirty to forty hours after the petals fall the capsule part continues to grow and is ripe in about two weeks.

Figure 1.4 Poppy plants after sowing in October (TMO presentation, 2008)

Figure 1.5 Poppy plants in early spring in March (TMO presentation, 2008)

Figure 1.6 Opium poppy flower in May (blue seed) (TMO presentation, 2008)

1.7.2 Harvesting

When the capsules become maturity (dry) (Figure1.7) for harvest, the permission certificate for harvesting is given to the farmer, and than the poppy capsules are harvested by hand. Harvested capsules after separating from the seeds from straw by farmers are delivered to the local organisations of TMO, who is the unique buyer. The remained seeds are sieved coarsely and keeped by putting in sacks as packages according to colours of seeds to sell free market Trade of poppy seed is free and the producer personally appraises his seeds. The yield in Turkey averages about 500 kg of poppy capsules and about 550 kg poppy seed per hectare (TMO, 2009).

Figure 1.7 Matured poppy capsules in July (TMO presentation, 2008)

1.8 Use of the opium poppy

The opium poppy has a number of uses of great economic importance. It is used for medicinal, decorative and food purposes.

1.8.1 Medicinal purposes

Morphine, codeine, thebaine, noscapine, oripavine, papaverine and narceine are natural opium alkaloids contained in opium poppy plant (Figure 1.4) and dihydrocodeine, ethylmorphine hydrocodone, hydromorphine, oxicodone, pholcodine and heroine are semi-synthetic alkaloids are derivatives of natural opiates (INCB Report, 2009) . Those natural and semi-synthetic alkaloids are used widely for medicinal purpose. The seeds are also used in pharmacy for making emulsions

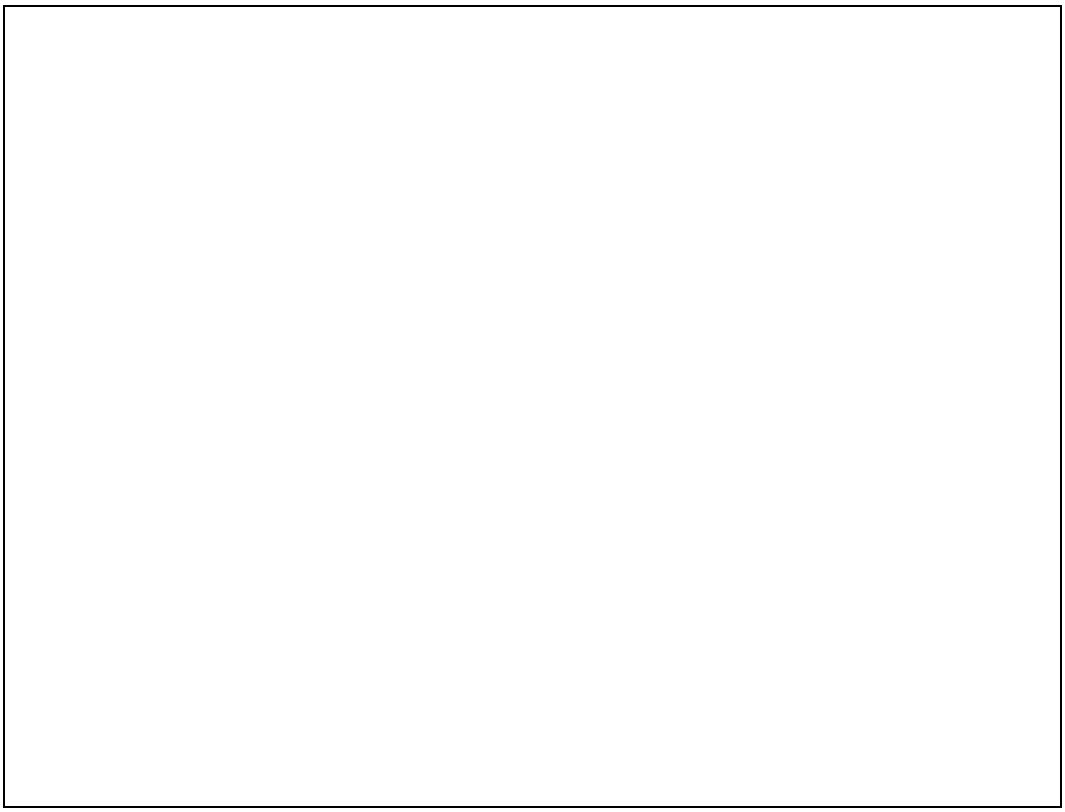

Figure 1.8 Chemical structures of major opium alkaloid

From a clinical point of wiew, opioids may be classified according to their actions compares with those of morphine: similar affinity (agonist), competitive (antagonist) or mixed (agonist/antagonist) for the same reseptor sites (the so called opioid receptors) in the central and peripheral nervous system (INCB Report, 2009).

Morphine is the prototype of natural opiates and many opioids and, because of its strong analgesic potency. It is used as a reference parameter for comparative purposes (Table 1.5).

Table 1.3 Analgesic potencies of some opiate and opioids (Bryant, 1988)

Alkaloids	**Relative potency**
Morfin	1.0
Kodein	0.1
Dihidrokodein	0.15
Nalbufin	0.15
Diamorfin (heroin)	2.5
Hidromorfon	4.4
Buprenorfin	38
Oksimorfon	10
Etorfin	10,000

1.8.2 Decorative purposes

Because of the beauty of its flowers, the opium poppy is used throughout the world as an ornamental plant. Dried poppy pods are used in flower arrangements.

1.8.3 Food purposes

The only seed part of the opium poppy is used for food purpose. Although poppy seed is not included in Schedules of the 1961 single convention, The UN Economic and Social Council (ECOSOC) in its resolutions 1999/32 called upon Member States to take some measures to fight the international trade in opium poppy seeds from countries not permitting the cultivation of opium poppy (INCB Report for 2008, Resolution 51/15).

Poppy seeds are edible, and are used, especially in Central Europe but also throughout the rest of the world for the preparation of pastry, nougat; etc. The seeds of the poppy plant can be found in and on a variety of different food items such as bagels, muffins and cakes. Poppy seeds can be pressed to create poppy seed oil, which is very useful in cooking or as a carrier for oil-based paints. In Turkey, Iran, India and many other countries worldwide

poppy seeds are considered a greatly nutritious food item which is mostly added into dough for baking bread or desserts.

1.8.3.1 Nutrition value of poppy seeds

The mineral contents of oil-bearing seeds and kernels are presented in Table 1.4. Mineral values were established to vary widely depending on the different seeds and kernels. According to table 1.4 poppy seed has valuable mineral composition for Ca, Mg, P, etc. minerals

Table 1.4: Comparing mineral content of some seeds or kernel (mg/kg) (Özcan, 2006)

Seeds (mg/kg)	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
Poppy	13195.6	14.9	75.9	6468.8	2343.5	58.6	844.6	7945.0	58.1
Hazelnut	1192.6	10.6	41.6	5310.3	1284.4	26.1	674.2	3800.3	16.9
Sesam oil	723.5	11.5	64.4	4295.7	2191.4	ND	1138.8	8308.9	28.1
Soybean	1867.2	8.7	79.6	20895.8	1762.7	4.6	757.8	5972.2	23.3
Walnut	511.1	3.7	24.8	3379.2	1321.1	11.7	650.4	4380.9	13.8
Sunflower	464.8	18.1	49.6	8753.8	2251.3	6.9	1026.7	9607.9	36.5

The oil yields, fatty acid compositions, and total protein contents of three varieties of Turkish poppy were investigated. Solvent extraction of yellow seed gave the highest oil yield (49.2%), while white seed (36.8%) and blue seed (33.6) showed considerably lower yields (Azcan et al, 2004) Poppy seed oil is rich about oleic, linoleic and palmitic acid as seen in Table 1.5.

Table 1.5 Comparision of percentage of fatty acid compositions in different seeds oil (Teblig-2001/29)

Fatty acid	Poopy seed oil	Hazelnut oil	Sesam oil	Olive oil	Grape seed oil	Sunflower oil
Oleic C 18:1	16 - 22	71.0 –91.0	35.9- 42.3	56 - 85	12 - 28	14 - 39.4
Linoleic C 18:2	62 - 76	5.7- 22.2	41.5- 47.9	3,5 - 20	58 - 78	48.3 - 74.0
Linolenic C 18:3	0.1- 0.3	Max 1	0.3 - 0.4	Max1,2	Max 1	ND
Palmitic C 14:0	6 - 12	4.3-8.8	7.9 - 10.2	7,5 - 2	5.5 - 11	5 - 7.6
Stearic C 18:0	1- 2.1	Max 2.7	4.8 - 6.1	0,5 - 5	3 - 6.5	2.7 - 6.5

ND: Non-detected

Poppy seeds are one of the valuable food items and mostly added into dough for baking bread or desserts in Turkey. It is important export material for Turkish farmers. Exported poppy seed amounts and total income figures are shown in Table 1.6.

Table: 1.6 Poppy seeds exportation figures for Turkey (TMO, 2009)

Years	Amount (Tons)	Average Price (USD/Tons)	Total (Tons)
2000	12 855	930	11,950,683
2001	24,711	764	18,887,109
2002	17,661	904	15,968,886
2003	33,778	976	32,974381
2004	23,859	1,349	32,190,483
2005	14,052	2,032	28,550,831
2006	22,468	1,910	42,931,322
2007	14,335	3,396	48,744,534
2008	10,085	5,389	54,345,530
2009	13,751	3,416	46,976,487

1.9 Phenolic compounds

Phenolic compounds are synthesized as secondary metabolites in plants. They posses biological properities such as: anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, antioxidant, antiapoptosis, cardiovascular protection, improvement of the endothelialfunction, as well as inhibition of cell proliferation activity and angiogenesis (Han et al, 2007). Phenolic compounds are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years.

1.9.1 Total phenolic content

Solid – liquid extraction can performed using a solvent like water, methanol, methanol/formic acid, methanol/water/acetic or formic acid during extraction of polyphenols. Other techniques are heat reflux extraction, ultrasonic extraction, microwave assisted extraction. The extraction conditions (temperature, extraction time, ratio of solvent to raw material, solvent and concentrations) have to be optimized.

Mainly found in the fruit skins and seeds, high levels of polyphenols may reflect only the measured extractable polyphenol content of a fruit which may also contain non-extractable polyphenols (Arranz et al, 2009) Currently, not much data exist on polyphenols in poppy seed or poppy seed oil. Trace amounts of lignans as matairesinol and lariciresinol 0.01mg/100g for each have been reported in poppy seed after hydrolysis (Milder et al, 2005).

Some methods for quantification of total polyphenol content are based on colorimetric measurements. Some tests are relatively specific to polyphenols (for instance the Porter's assay). Total phenols (or antioxidant effect) can be

measured using the Folin-Ciocalteu reaction. Results are typically expressed as gallic acid equivalents. Polyphenols are seldom evaluated by antibodies technologies (Mello L., 2003)

The total phenolic content of extracts was determined using to the Folin-Ciocalteu method (Singelton & Rossi, 1965; Singelton et al. 1999). The extracts were oxidized with Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 750 nm after 60 min. Using galic acid as standard total phenolic content (standard curve was prepared using concentratons 2,5-50 mg/L) was expresed as mg gallic acid equivalent (GAE)/L of extract. Data reported of three replications.

1.10 Aim of the study

The seeds of the opium poppy (*Papaver somniferum* L.) are commonly used in dishes, pastries and in some other foods in the world. In this study, because of important export and domestic consumption material for Turkish farmers, we tried to determine morphine and total polyphenol content in Turkish origin poppy seeds by using HPLC and colorimertic methods. The aim was investigation of some nutritional properties of representing material for all poppy harvesting areas. Three different colours of poppy seeds (white, yellow and blue) were studied. During experimental works, new effected extraction methods were developed and used.

CHAPTER II

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

Active aluminium oxide (Al_2O_3), HPLC grade acetonitrile, methanol, glacial acetic acid and triflouroacetic acid (TFA) where purchased from Merck (Darmstadt, Germany), and analytical grade demineralized water (Elix Millipore S.A.S) was used in mobile phase. Morphine monohydrate USP reference as a standard. Disposable syringe filter (pore size:0.22 and 0.45, Diameter: 4 mm) was perchased from Millipore Co. (Bedford, MA, USA) Gallic acid and sodium carbonate (Na_2CO_3), were perchased from Sigma Chemical Company (St. Louis, MO, USA).

Folin Ciocalteu's reagent were perchased from Merck (Darmstadt, Germany).

2.1.2 Apparatus

HPLC was performed Agilent 1100 series HPLC system equipped with binary pump (HP G1312A), degasser (HP G1322A), autosampler (HP G1329), column compartment (HP G1330A) and diode array detector (DAD) (HP G1315A). The column was Eurospher-100 C18 , 120×4mm ID, 5 µm particle (Knauer, Berlin, Germany).

Glass column 2.5 x 15 cm ID (Ildam, Ankara, Turkey). 500 micron sieve (ASTM), Spectroscopic data where obtained in Cary 50 Bio UV-VIS spectrophotometer (Varian). Ultrasonic bath was performed with a Bendalin

Sonorex. Incubator shaker was Optic Ivymen System. Blender was Waring model 32BL80 (New Hartford, CT, USA). Cenrifuge was Hettich, Universal 32 R model (Tuttingen, Germany). Vortex was performed with a Velp Scientifica (Italy). Balances were performed with Sartorius, BL 1500 model (d: 0.1g) (Göttingen, Germany) and Precissa, XB 220A model, (d: 0.0001g) (Swiss).

2.2 METHODS

2.2.1 Collection and preparation of materials

Poppy seed samples are collected from 13 provinces (Afyonkarahisar, Amasya, Balıkesir, Burdur, Çorum, Denizli, Eskisehir, Isparta, Konya, Kütahya, Manisa, Tokat and Uşak) which are decided to produce opium poppy by Concil of Ministers and 39 samples from different farmer fields. Samples were collected by TMO officers who were expert in poppy plants. These experts examined the poppy seed content by eye for uniform colours. Sampling were composed of multiple container items as follows:

If there are less than 10 packages, all packages were sampled.

If there are 10-100 packages, 10 packages were randomly selected and sampled.

The seed samples were selected from three different colour varieties which have been used traditionally to produce poppy by the farmers. Poppy seed samples were picked about 1 kg from each packages. Then, samples were mixed and kept in well closed cotton bags. Seeds which were obtained from farmers were cleaned from their fine capsule particles by sieving through a 500 micron sieve. Later, a complete separation from the dust and capsule reminders is ensured via a blow of air. Cleaning procedure is repeated until the seeds were completely cleaned up to a 99.9 % of purity.

2.2.2 Determination of morphine by HPLC method

Aplication method for separation and quantitation by HPLC was made according to Szucs Z. et al, (2002). This method has good separation and high resolution between the alkaloids and minor components of the poppy seed samples.

2.2.2.1 Preparation of column for morphine extraction

Some cotton is put into the cylindrical glass column (2.5 x 15 cm). Cotton is compressed at the bottom of the column, than it was filled with 25 ml deionized (DI) water and 8 grams of activated acidic Al_2O_3 was added under permanent stirring with a glass stick. It was waited until the Al_2O_3 was settled, then the valve was opened and water was removed from the column. The column was refilled with deionized water again for washing the aluminum oxide. Then valve was closed and column filled with 5 mL of water.

2.2.2.2 Extraction of poppy seeds

2.0 g of poppy seed (99.9 %) sample was taken into a mortar, than mixed and crushed with 2.0 g activated acidic Al_2O_3 about for 30 minutes. Crushed mixture was wet with 7 ml of distilled water and remained for 60 minute, and then transferred into prepared column (2.2.2.1) and air bubles were removed by carefull stirring. Elution of morphine continued by addition of water in portions of 5 mL with a flow-rate of 10 drops per minute (0.5 mL/min).

Elution was completed when the volume of the collected eluant reaches to 50 mL. Then, sample solution was filtered into vials through a 0.22 μm syringe filter before the injection to HPLC column. All determinations were performed in triplicate.

2.2.2.3 HPLC method

Analysis was performed on following conditions:

Stationary phase: Eurospher-100 C18, 120×4mm ID, 5 µm particle RP column(Knauer, Berlin, Germany)

The column owen temperature: 40ºC

Flow rate: 1 mL/min

Injection volume: 10 µL

Wavelength: 288 nm

Mobile Phase:

Eluent A: 1% TFA in water

Eluent B: 1% TFA in water – acetonitrile - methanol (46: 40: 14, v/v)

Gradient program:

Time (min)	A%	B%
0.0	82.0	18.0
2.5	64.0	36.0
3.8	48.0	52.0
13.0	48.0	52.0
16.5	0.0	100

2.2.3 Determination of total polyphenol content

The total phenolic content of extracts was determined by using the Folin-Ciocalteu reagent according to Singleton et al, (1999) method. This method involved some modifications. Since the assay measures all phenolics, the choice of gallic acid as standard is based on the availability of a stable and pure substance. The stability of gallic acid standard solutions were tested and they lose less than 5% of their value over two weeks when refrigerated and kept tightly closed (Waterhouse, A.L, 2001).

2.2.3.1 Preparation of sample solutions for total polyphenol

The poppy seeds (2.2.1) were defatted by cold press from each colour before preparation of sample. 50 g defatted poppy seeds were ground by Waring (model 32BL80) commercial blender at high speed for at least 3 minutes. Then the ground sample crushed well in a porcelain mortar. The crushed poppy seeds samples for 30 g were taken into 500 mL amber glass bottles and added 200 mL demineralized water. Then the mixture were extracted in shaker incubator for 4 hours at 25 ºC. The solution was centrifuged for 5 min at 320 rpm to settel poppy seed residue and upper liquid solutions was taken into the amber coloured bottles and kept in refrigerator at 4 ºC for test.

2.2.3.2 Assay for determination of total polyphenol

0.1 mL of each sample, 7 mL DI water and 0.5 mL of Folin-Ciocalteu reagent were added to a 10 mL volumetric tube. The contents were mixed and allowed to stand for 5-8 min at room temperature. Next, 1 mL of saturated Na_2CO_3 solution was added, followed by the addition of DI water filled to volume. Solutions were vortexed vigorously and allowed to stand at room temperature for 1 h for incubation in dark. These steps were repeated for blank (water) and standart gallic acid solutions (0.05-0.5 mg/mL). After 1 h, the absorbance of the sample was measured at 750 nm against a blank by spectrofotometer. Total polyphenols content was calculated against gallic acid calibration curve and expressed as milligrams per grams of gallic acid equivalents (GAE). All determinations were performed in triplicate.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Identification of morphine extraction methods

2.0 g poppy seed sample was taken into a porcelain mortar. 2.0 g activated Al_2O_3 and 5 mL distilled water are added. After they were mixed and crushed for 30 minutes in the mortar, 5 ml distilled water was added and waited for 60 minute. The prepared mixture is transferred into the extraction column which was prepared before. The flow rate of the extract from the column was adjusted as 10 drops per minute (0.5 mL/min).

When the volume of collected eluant reaches 10 ml, 20 ml, 30 ml, 40 ml and 50 ml, analysis samples were taken into vials. Morphine concentrations were determined by HPLC method. Then a curve was obtained as collected volumes (mL) vs morphine concentrations (µg/mL) and the last elution volume was determined to extract (Figure 5).

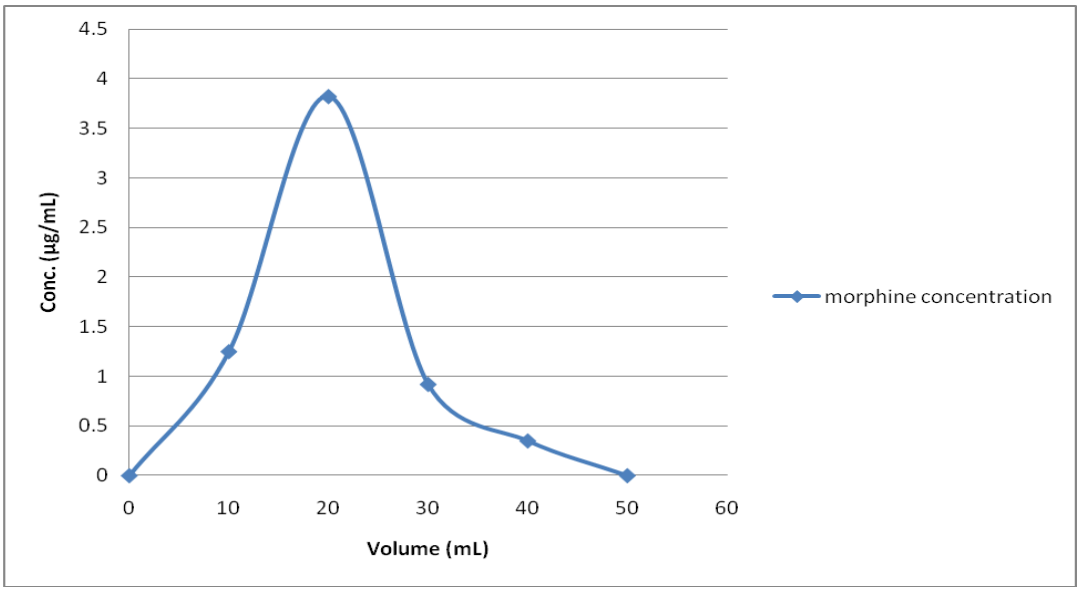

Figure 3.1 Curve for extraction of morphine from poppy seed (eluent volume vs. morphine conc.)

3.1.1 Preparation of standard solutions for morphine

106.3 mg morphine monohydrate was accurately weighed and dissolved in 100 mL of 10% acetic acid to prepare a stock solution at a final concentration of 1.0 mg/ml. Than, 0.5, 0.3, 0.25, 0.2, 0.15 and 0.1 mL of stock solution were taken into 100mL volumetric flask and diluted to the volume with 10% acetic acid solution to prepare 5.0, 3.0, 2.5, 2.0, 1.5 and 1.0 µg/mL of standard solutions. The standard solutions were stored at 4°C and remained stable for at least one month.

3.1.2 Calculation of Morphine Content in Poppy Seed Sample

The calibration curve (Figure 3.2) was created with prepared standart solutions (3.2) as peak area vs. concentration to determine the morphine levels in the samples. Regression coefficient had been calculated by the instrument and it was found as 0.9992.

The concentration of samples were obtaind as µg/mL by corresponding the peak area to concentration in the calibration curve or calculated by instrument. As an example, the chromatograms for poppy seeds sample (Figure 3.3) and Standard solution (Figure 3.4) which were obtained from

HPLC method (2.1.2). Than, µg of morphine content in g of poppy seed samples was calculated (Table 3.1) from the following equation:

Morphine content (µg/g) = (Cse x Vse) / Ws

Cse = Concentration of morphine in sample extract (µg/ml)
Vse = Volume of sample extract (ml)
Ws = Weight of sample (g)

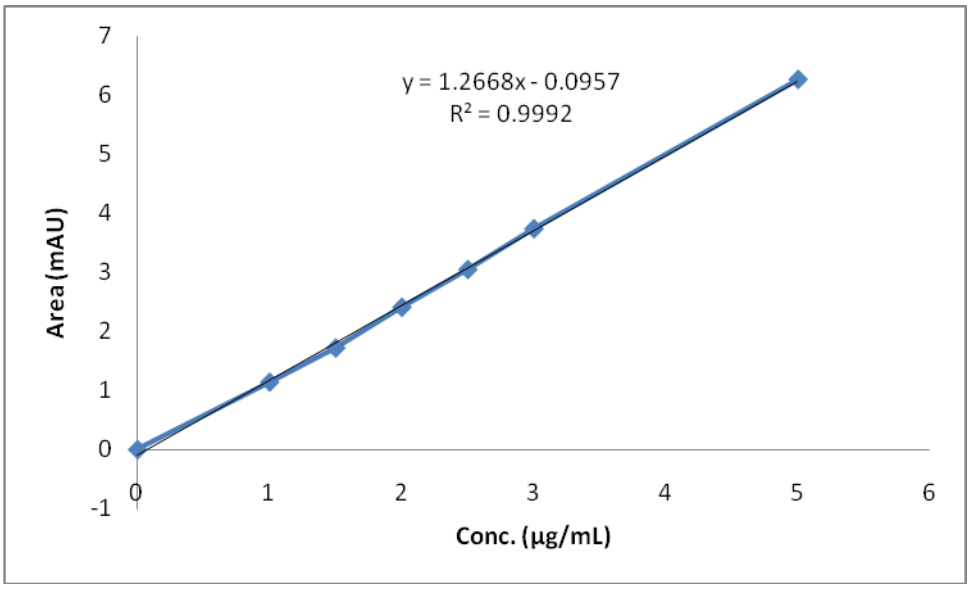

Figure 3.2 Calibration curve of morphine standard concentration

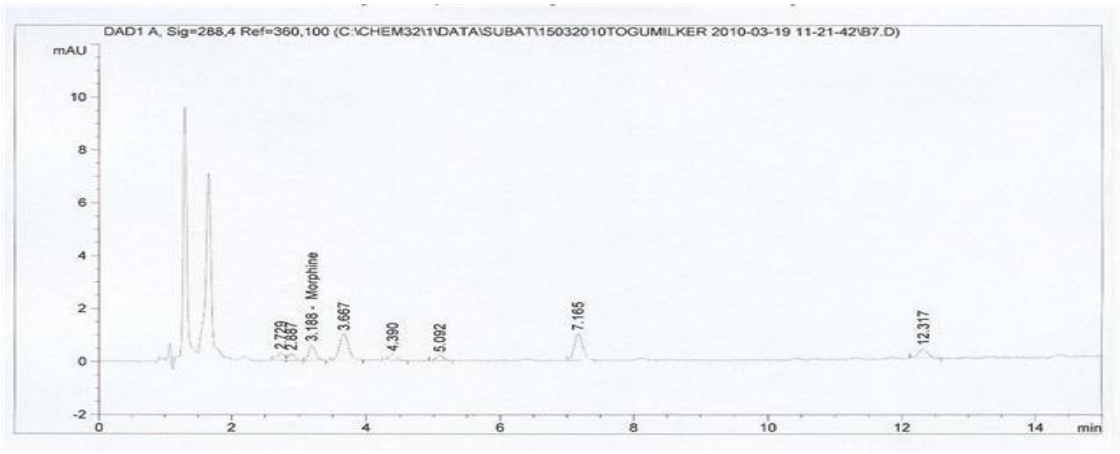

Figure 3.3 HPLC chromatogram of morphine in poppy seed sample (1.4 µg/ml)

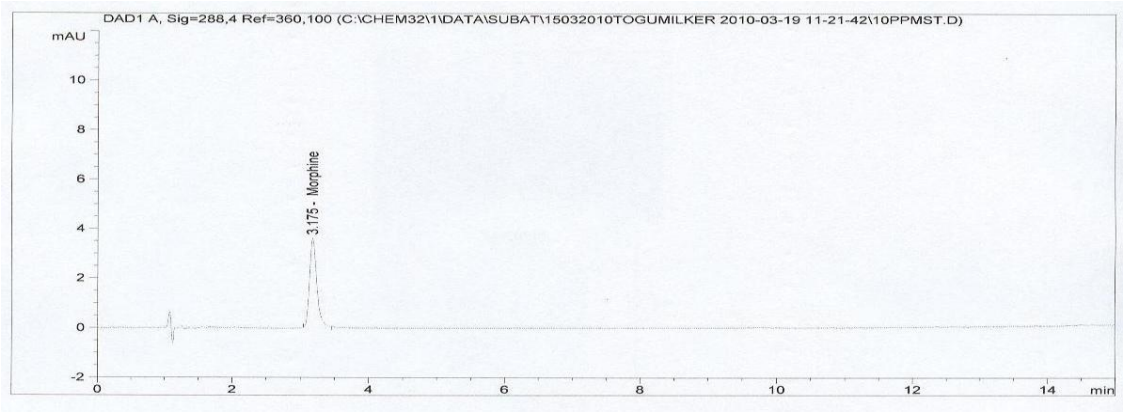

Figure 3.4 HPLC chromatogram of standard morphine solution (10µg/ml)

parallel samples were prepared from each poppy seeds samples for the determination of morphine content and they were analyzed by described HPLC method (2.2.1). The morphine content of 39 samples which were collected from 13 provinces and 35 different fields were ranged from concentrations 9.73 µg/g to 37.46 µg/g.

The average morphine content was calculated as 23.67 µg/g as shown in Table 3.1.

As an example, HPLC chromatogram taken during these studies was given in Figures 3.2 and Figure 3.3. The results were classified due to poppy seed colours varieties in Table 3.3. The morphine contents in blue, yellow and white colour poppy seeds were determined as average 21.79, 23.01 and 27.6 µg/g respectively.

Table 3.1 Morphine content in samples collected from 13 provinces

Province	**Sample Number**	**Harvested field**	**Seeds colour**	**Morphine content (µg/g)**
Afyonkarahisar	1	Şuhut	yellow	15.66 ± 0.12
"	"	Çobanlar	blue	16.06 ± 0.20
"	"	İhsaniye	blue	9.73 ± 0.17
"	"	Sinanpaşa	yellow	22.96 ± 0.45
"	3	Sinanpaşa	blue	21.06 ± 0.20
"	1	Bolvadin	yellow	35.86 ± 0.17
"	"	Sincan	yellow	20.09 ± 0.29
"	"	Saraydüzü	white	20.53 ± 0.42
"	"	Sandıklı	blue	34.20 ± 0.45
"	"	Çobanlar	white	23.90 ± 0.58
Amasya	"	Taşova	blue	30.66 ± 0.65
"	"	Merzifon	yellow	20.00 ± 0.29
Balıkesir	"	Bigadiç	blue	30.16 ± 0.97
"	"	Bigadiç	white	30.13 ± 0.28
Burdur	"	Tefenni	yellow	34.10 ± 0.24
"	"	Tefenni	white	34.26 ± 0.25
Çorum	"	Laçin	white	35.86 ± 0.17

Table 3.1 Morphine content in poppy seed due to 13 provinces (continue)

Province	**Sample Number**	**Harvested field**	**seed colour**	**Morphine content (µg/g)**
Denizli	1	Çivril	blue	22.26 ± 0.77
“	“	“	yellow	20.83 ± 0.53
“	“	Acıpeyam	white	21.00 ± 0.37
Eskişehir	“	Sivrihisar	blue	11.93 ± 0.33
Isparta	2	Gönen	white	31.73 ± 0.53
Konya	1	Akşehir	white	35.96 ± 0.40
“	“	Akşehir	yellow	22.16 ± 0.89
Kütahya	“	Central	yellow	12.86 ± 0.40
“	“	Central	white	15.10 ± 0.61
“	“	Central	blue	14.83 ± 0.13
Manisa	“	Gördes	blue	37.46 ± 0.99
“	“	Selendi	blue	13.03 ± 0.65
“	“	Selendi	yellow	26.86± 0.54
Tokat	“	Erbaa	blue	18.06 ± 0.13
“	“	Erbaa	yellow	21.80 ± 0.24
Uşak	“	Banaz	blue	17.63 ± 0.33
“	“	Central	blue	20.06 ± 0.33
“	“	Ulubey	blue	29.73 ± 0.60
Total	**39**		**Average**	**23.67 ± 7.91**

Table 3.2 Morphine content in different colors of poppy seed samples

Sample number	Yellow poppy (μg/g)	Blue poppy seed (μg/g)	White poppy seed (μg/g)
1	15.66 ± 0.12	16.06 ± 0.20	20.53 ± 0.42
2	22.96 ± 0.45	9.73 ± 0.17	23.90 ± 0.58
3	35.86 ± 0.17	21.06 ± 0.20	30.13 ± 0.28
4	20.09 ± 0.29	34.20 ± 0.45	34.26 ± 0.25
5	20.00 ± 0.29	30.66 ± 0.65	35.86 ± 0.17
6	34.10 ± 0.24	30.16 ± 0.97	21.00 ± 0.37
7	20.83 ± 0.53	22.26 ± 0.77	31.73 ± 0.53
8	22.16 ± 0.89	11.93 ± 0.33	35.96 ± 0.40
9	12.86 ± 0.40	14.83 ± 0.13	15.10 ± 0.61
10	26.86 ± 0.54	37.46 ± 0.99	
11	21.80 ± 0.24	13.03 ± 0.65	
12		18.06 ± 0.13	
13		17.63 ± 0.33	
14		20.06 ± 0.33	
15		29.73 ± 0.60	
Average	**23.01 ± 6.65**	**21.79 ± 8.36**	**27.60 ± 7.22**

3.2 Determination of total phenolic in the poppy seeds

The total phenolic content of poppy seeds samples were determined by using the Folin-Ciocalteu reagent and colorimeric method according to Singelton et al., (1999) using gallic acid as standard and the results were calculated as gallic acid equivalent.

3.2.1 Preparation of standard solutions for gallic acid

1.0 mg/L stock solution was prepared by 50 mg gallic acid was dissolved in 50 ml demineralized water in volumetric flask . 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL gallic acid standard solutions were prepared through 0.5, 1.0, 2.0, 3.0, 4.0 and 5 ml were taken from stock gallic acid solutions into 10 ml volumetric flask each and complated by deionized water until mark.

3.2.2 Method optimization

To optimize the amount of total phenol content of the extraction method from poppy seeds, percolation time (Table 3.4) and temperature (Table 3.5) were examined. The solvent (Table 3.3) and ultrasonification effect was also studied (Table 3.6). According to resulst from Table 3.6 there was not a positive effect gained by sonification step. As a result, after optimization steps, maximum total phenol amounts were obtained in 4 hours and at 25ºC without ultrasonification, and water was chosen as solvent. All determinations were performed in triplicate and the results were indicated as (Mean ± S.D)

3.2.2.1 Optimization for solvent

Optimization for solvent was studied with methanol and water to prefer an effective solvent to extract polyphenolic substances. The results were significantly different as about ten fold as seen in table. Therefore water was chosen as an effective solvent.

Table 3.3 Optimization for solvent

Solvent	Total polyphenols (mg/g)
Methanol	0.49 ± 0.07
Water	3.28 ± 0.03

3.2.2.2 Optimization for time

Optimization studies were tried to determine effective time condition for extraction of maximum amount of TP from poppy seeds. During study, temperature was keep at 25ºC for sellected incubation period (1 – 24 hrs) and water was used as solvent. Because of the results were not significantly different, hence incubation period was chosen as 4 hours.

Table 3.4 Optimization for time

Incubation periods (Hours)	Total polyphenols (mg/g)
0	1.45 ± 0.09
1	2.37 ± 0.14
2	3.04 ± 0.12
4	3.47 ± 0.20
6	3.45 ± 0.06
24	3.33 ± 0.12

3.2.2.3 Optimization for temperature

Optimizations for extraction temperature were examined to determine effective temperature for the duration of incubation. Temperatures of 25, 40 and 60 ºC were chosen for extraction of TP in poppy seeds. The extraction period was chosen as 4 hr in the previous optimization study. As the results were displayed in Table 3.5 most effective extraction temperature was selected as 25 ºC, although there were no significant differences among the temperatures examined.

Table: 3.5 Optimization for temperatures

Temperature (ºC)	Total polyphenols (mg/g)
25	3.42 ± 0.06
40	2.99 ± 0.12
60	2.34 ± 0.08

3.2.2.4 Optimization for ultrasonification

Optimization studies were also extended to examine the effect of ultrasonification. Ultrasonification was applied at 25ºC and for 30 minutes before the incubation period of extraction. Results were tabulated in Table 3.6, and there was no observed statistical differece between the total phenol amount of popy seeds with or without application of utrasonification step. Therefore utrasonification was not applied befor incubation period**.**

Table 3.6 Optimization for ultrasonification

Ultrasonification	Total polyphenols (mg/g)
With	3.02 ± 0.38
Without	3.12 ± 0.32

3.2.3 Calculation of Total Phenolic Content in Poppy Seeds Samples

The calibration curve (Figure 3.5) was created with prepared standart solutions (3.2.1) as absorbance vs. concentration to determine the TP levels in the samples. Regression coefficient had been calculated by the instrument and it was found as 0.9968. The concentration of samples were obtaind as mg/mL by using absorbance versus concentration graph. Then, (mg) of TP content in (g) of poppy seed samples was calculated from the following equation:

TP content (mg/g) = (Cse x Vse) / Ws

Cse = Concentration of TP in sample extract (mg/ml)
Vse =Total volume of sample extract (ml)
Ws = Weight of sample (g)

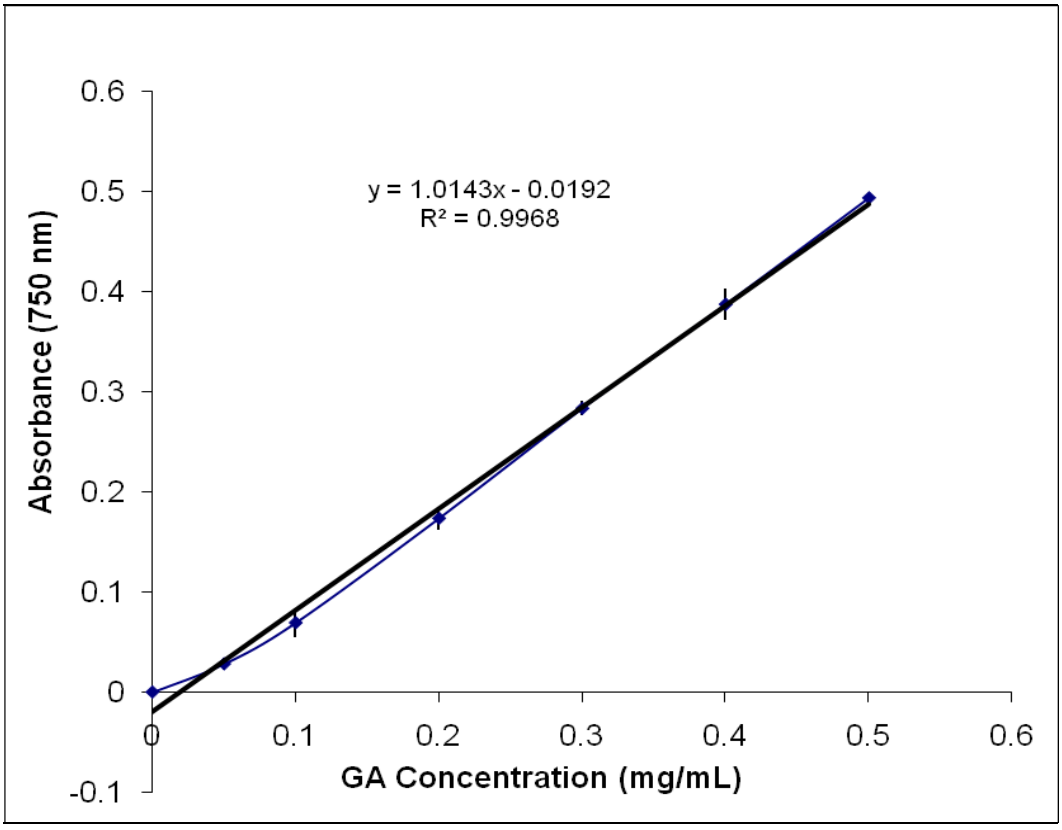

Figure 3.5 Calibration curve of gallic acid standard concentration

Total phenolic determination studies were carried out for extraction parameters as solvent, time, temperature and ultrasonification need. Therefore the optimum conditions for extraction of polyphenolic compounds were obtained in 4 hours, at 25^{O}C, without ultrasonification in the presence with water. As a result, the total phenolic content in white, yellow and blue colour seed were found as 4.44, 3.05 and 3.67 mg/g respectively. The results were shown that the white colour poppy seeds have more TP than yellow and blue colours. Nonetheless, statistically there was a significant difference only between yellow and white poppy seed when analysed in 95% confidence interval (Table 3.7).

Table 3.7 The concentration of total phenolic of poppy seeds

Poppy seeds	Total phenolic (mg/g)
White	4.44 ± 0.12
Yellow	3.05 ± 0.05
Blue	3.67 ± 0.11

CHAPTER IV

CONCLUSIONS

In this study Turkish origin poppy seeds which were collected from 13 provinces and 35 harvesting fields as different coloured varieties were contained (9.73 – 37.46) µg/g morphine and average value was calculated as 23.67 µg/g. The HPLC system was used to determine morphine content. This results were obtained from original material that only sieved through a 500 micron sieve to separate poppy capsule particles. Some studies need to determine morphine content in seeds which were higher purity and soaked in water to reduce values.

We chose a simple and single step extraction method by elution through prepared activated aluminum oxide column with water. The results were shown at an elution profile (Figure 3.1) to determine the required time for extraction and extraction yield. Other alternative extraction methods were multiple stage (solid-liquid, liquid-liquid extractions, evaporation, redissove, etc). The multiple stages can have negative effect on the overall extraction yield.

The total phenolic content of poppy seeds samples were determined by using the Folin-Ciocalteu reagent and colorimeric method. Gallic acid was chosen as Standard which is based on the availability of a stable and pure substance and the results were calculated as GAE. Optimization studies were done to

determine the conditions for extraction of polyphenolic compounds. Therefore, effective extraction condition was obtained in 4 hours, at 25 ºC, without ultrasonification and with water. Results have revealed that TP contents of poppy seeds had very low solubility in methanol. As a result, the total phenolic content of white, yellow and blue coloured popy seeds, were obtained as 4.44, 3.05 and 3.67 mg/g respectively by water extraction.

The results were shown that the white colour poppy seeds have higher total phenolic than yellow and blue coloured. Nevertheless, statistically there was a significant difference only between yellow and white poppy seed phenolic contents when analysed in 95% confidence interval.

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APPENDICES

A. Resolution 51/15

Control of international movement of poppy seeds obtained from illicitly grown opium poppy plants

The Commission on Narcotic Drugs,

Reaffirming Economic and Social Council resolution 1999/32 of 28 July 1999, Considering article 22 of the Single Convention on Narcotic Drugs of 1961,1 on theprohibition of the illicit cultivation of the opium poppy, and the Action Plan on International Cooperation on the Eradication of Illicit Drug Crops and on Alternative Development, 2 adopted by the General Assembly at its twentieth special session,

Recalling that the International Narcotics Control Board, in its report for 1995, expressed its concern about trade in seeds obtained from opium poppy plants in countries where thecultivation of opium poppy was prohibited, and urged Governments to be vigilant in order to ensure that poppy seeds traded for food purposes were not derived from illicitly cultivated opium poppy plants,[3]

Emphasizing the need to fight the illicit cultivation of opium poppy,

Noting that poppy seeds are a by-product available on a large scale from countries wherethe cultivation of opium poppy is prohibited,
Aware that, according to the provisions of the 1961 Convention, trade in poppy seeds isnot subject to international control,

Recognizing that there is a need to prohibit international trade in poppy seeds obtainedfrom illicitly grown opium poppy plants,

Recognizing also that the poppy plant used for food purposes has a low morphine contentand is therefore unsuitable for the production of opium for illicit use by drug abusers,

Concerned about the trade in seeds obtained from opium poppy plants in countries where the cultivation of opium poppy is prohibited,

Resolving to fight the international trade in poppy seeds obtained from illicitly grownopium poppy plants,

Noting with concern the substantial increase reported in the illicit cultivation of opium poppy in certain areas,

1. Urges all Member States to endeavour, consistent with their domestic laws and regulations and applicable international regulations, to import poppy seeds derived from licitly grown opium poppy crops;

2. Exhorts all Member States to be vigilant and to ensure that poppy seeds traded for food purposes are not derived from illicitly cultivated opium poppy plants;

3. Stresses the need for all Member States to strengthen their resolve to implement Economic and Social Council resolution 1999/32 of 28 July 1999;

4. Requests the International Narcotics Control Board to continue gathering information regarding the implementation of Economic and Social Council resolution 1999/32 by Member States with a view to strengthening the control of international movement of poppy seeds obtained from illicitly grown opium poppy plants and to share that information with Member States;

5. Requests the International Narcotics Control Board and the United Nations Office on Drugs and Crime to take appropriate measures to ensure the full implementation of article 22 of the Single Convention on Narcotic Drugs of 196171 by the Member States concerned.

1 United Nations, Treaty Series, vol. 520, No. 7515.

2 General Assembly resolutions S-20/4 E.

3 Report of the International Narcotics Control Board for 1995 (United Nations publication, Sales No. E.96.XI.1), para. 61.

B. Resolution 51/9

The need for a balance between demand for and supply of opiates used to meet medical and scientific needs

The Commission on Narcotic Drugs,
Recalling **Economic and Social Council resolutions** 2006/34 of 27 July 2006 and 2007/9 of 25 July 2007 and other relevant resolutions,

Recognizing that the medical use of narcotic drugs, including opiates, is indispensable for the relief of pain and suffering,

Emphasizing that the need for a balance between the global licit supply of opiates and the legitimate demand for opiates used to meet medical and scientific needs is central to the international strategy and policy of drug control,

Noting the fundamental need for international cooperation with the traditional supplier countries in drug control to ensure universal application of the provisions of the Single Convention on Narcotic Drugs of l961[1] and that Convention as amended by the 1972 Protocol[2],

Reiterating that a balance between consumption and production of opiate raw materials was achieved in the past as a result of efforts made by the two traditional supplier countries, India and Turkey, together with established supplier countries,

Noting that the stocks of opiate raw materials continue to be sufficient to cover the expected licit demand and that excessive stocks should be avoided,

Emphasizing the importance of the system of estimates, based on actual consumption and utilization of narcotic drugs, furnished to and confirmed by the International Narcotics Control Board on the extent of cultivation and production of opiate raw materials,

Recalling the Joint Ministerial Statement adopted during the ministerial segment of the forty-sixth session of the Commission[3], in which ministers and other Government representatives called upon States to continue to contribute to the maintenance of a balance between the licit supply of and demand for opiate raw materials used for medical and scientific purposes and to cooperate in preventing the proliferation of sources of production of opiate raw materials,

Considering that opiate raw materials and the opiates derived from them are not just ordinary commodities that can be subjected to the operation of market forces and that, therefore, market economy considerations should not determine the extent of cultivation of opium poppy,

Reiterating the importance of the medical use of opiates in pain relief therapy, as advocated by the World Health Organization,

Noting that countries differ significantly in their level of licit demand for opiates,

Noting with concern the proliferation of the cultivation of opium poppy in certain areas other than the traditional and established growing countries,

1. Urges all Governments to continue to contribute to maintaining a balance between the licit supply of and demand for opiate raw materials used for medical and scientific purposes, supporting traditional and established supplier countries, and to cooperate in preventing the proliferation of sources of production of opiate raw materials;

2. Urges Governments of all producer countries to adhere strictly to the provisions of the Single Convention on Narcotic Drugs of 1961[4] and that Convention as amended by the 1972 Protocol[5] and to take effective measures to prevent the illicit production or diversion of opiate raw materials to illicit channels, and encourages improvements in practices in the cultivation of opium poppy and the production of opiate raw materials;

3. Urges Governments of consumer countries to assess their licit requirements for opiate raw materials realistically on the basis of actual consumption and utilization of opiate raw materials and the opiates derived from them and to communicate those requirements to the International Narcotics Control Board in order to ensure effective supply, calls on Governments of countries cultivating opium poppy to limit its cultivation, taking into account the current level of global stocks, to the estimates furnished to and confirmed by the Board, in accordance with the requirements of the 1961 Convention, and urges Governments of producer countries, in providing estimates of such cultivation, to consider the actual demand requirements of importing countries;

4. Endorses the concern expressed by the International Narcotics Control Board in its report for 2005[6] regarding the advocacy by a non-governmental organization of legalization of opium poppy cultivation in Afghanistan, and urges all Governments to strongly oppose such proposals and to continue to strengthen drug control in compliance with their obligations emanating from the international drug control treaties;

5. Urges the Governments of all countries where opium poppy has not been cultivated for the licit production of opiate raw materials, in compliance with the relevant Economic and Social Council resolutions and in line with the views expressed by the International Narcotics Control Board[7] and in the spirit of collective responsibility, to refrain from engaging in the commercial cultivation of opium poppy in order to avoid the proliferation of supply sites, and calls on Governments to enact enabling legislation to prevent and

prohibit the proliferation of sites used for the production of opiate raw materials;

6. Urges the Governments of countries where opium poppy is cultivated for the extraction of alkaloids to implement and maintain adequate control mechanisms in accordance with the provisions of the 1961 Convention and that Convention as amended by the 1972 Protocol;

7. Commends the International Narcotics Control Board for its efforts in monitoring the implementation of the relevant Economic and Social Council resolutions and, in particular:

(a) In urging the Governments concerned to adjust global production of opiate raw materials to a level corresponding to actual licit requirements and to avoid creating imbalances between the licit supply of and demand for opiates caused by the exportation of products manufactured from seized and confiscated drugs;

(b) In inviting the Governments concerned to ensure that opiates imported into their countries for medical and scientific use do not originate from seized or confiscated drugs;

(c) In arranging informal meetings, during the sessions of the Commission, with the main States that import and produce opiate raw materials;

8. Requests the International Narcotics Control Board to continue its efforts to monitor the implementation of the relevant Economic and Social Council resolutions in full compliance with the 1961 Convention and that Convention as amended by the 1972 Protocol;

9. Requests the Secretary-General to transmit the text of the present resolution to all Governments for consideration and implementation.

1 United Nations, Treaty Series, vol. 520, No. 7515.

2 Ibid., vol. 976, No. 14152.

3 A/58/124, sect. II.A.

4 United Nations, Treaty Series, vol. 520, No. 7515.

5 Ibid., vol. 976, No. 14152.

6 Report of the International Narcotics Control Board for 2005 (United Natiopublication, Sales No. E.06.XI.2), para. 208

7 Report of the International Narcotics Control Board for 2007 (United Nations publication, SalesNo. E.08.XI.1).