**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY**

# ANTIOXIDANT CAPACITY AND FLAVONOID DIVERSITY OF *PROSOPIS FARCTA*

**M.Sc. THESIS**

**İlkin AYDIN**

**Department of Chemistry**

**Chemistry Programme**

**SEPTEMBER 2015**

**ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY**

# ANTIOXIDANT CAPACITY AND FLAVONOID DIVERSITY OF *PROSOPIS FARCTA*

**M.Sc. THESIS**

**İlkin AYDIN**
**(509121069)**

**Department of Chemistry**

**Chemistry Programme**

**Thesis Advisor: Prof. Dr. Birsen DEMİRATA ÖZTÜRK**

**Anabilim Dalı : Herhangi Mühendislik, Bilim**

**Programı : Herhangi Program**

**SEPTEMBER 2015**

**İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ**

# *PROSOPİS FARCTA*( ÇETİ) BİTKİSİNİN MEYVE KISMININ ANTİOKSİDAN KAPASİTESİ VE FLAVONOİD İÇERİĞİ

**YÜKSEK LİSANS TEZİ**

**İlkin AYDIN**
**(509121069)**

**Kimya Anabilim Dalı**

**Kimya Programı**

**Tez Danışmanı: Prof. Dr. Birsen DEMİRATA ÖZTÜRK**
**Anabilim Dalı : Herhangi Mühendislik, Bilim**
**Programı : Herhangi Program**

**EYLÜL 2015**

İlkin AYDIN, a M.Sc. student of ITU Graduate School of Science engineering and technology student ID 509121069, successfully defended the thesis entitled "ANTIOXIDANT CAPACITY AND FLAVONOID DIVERSIFICATION IN BEANS OF PROSOPIS FARCTA " which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

**Thesis Advisor :** **Prof. Dr. Birsen DEMİRATA ÖZTÜRK ….....**
İstanbul Technical University

**Jury Members :** **Prof. Dr. İkbal KOYUNCU …….**
Yıldız Technical University

**Doç. Dr. Gülçin Yılmaz ……**
Istanbul Technical University

**Date of Submission : 15 September 2015**
**Date of Defense : 14 June 2015**

***To my precious grandmother and grandfather…***

## FOREWORD

I woud like to express my sincere gratitude to my thesis supervisor Prof. Dr. Birsen DEMİRATA ÖZTÜRK for her endless supervision, guidance, encouragement, support, optimism and giving me the chance to work this study.

I express my special thanks to Dr. Dilek ÖZYURT for not also unending academic support and advices but also for her friendship.

I would also like my half-mother Nurten DEMİR and my brother Ataberk AYDIN for making my life easier with giving moral support and their endless love.

I express my special thanks to Yusuf Can GERÇEK for his endless support in my academic career and expanding my scientific vision.

Lastly and most importantly, I would like to express my special thanks to my parents Aysel AYDIN and Yüksel AYDIN to whom I dedicate all my success for their never ending love, support and faith in my entire and future life.

September 2015 İlkin AYDIN

**TABLE OF CONTENTS**

**Page**

**ABBREVIATIONS**

**BHA** **:** Butylated Hydroxyanisole
**BHT** : Butylated hydroxytoluene
**TBHQ** **:** *tert*-Butylhydroquinon
**ROS** **:** Reactive oxygen Species
**RNS** **:** Reactive Nitrogen Species
**PG** **:** Propyl Gallate
**OG** **:** Octyl Gallate
**DG** **:** Dodecyl Gallate
**HAT** **:** Hydrogen Atom Transfer
**ET** **:** Electreon Transfer
**CERAC** **:** Cerium Reducing Antioxidant Capacity
**CUPRAC** **:** Cupric Reducing Antioxidant Capacity
**HPLC** **:** High Pressure Liqid Chromatography

## LIST OF TABLES

**Page**

**LIST OF FIGURES**

**Page**

## ANTIOXIDANT CAPACITY AND FLAVONOID DIVERSITY IN BEANS OF *PROSOPIS FARCTA*


### SUMMARY

Free radical is an atom, molecule or ion with an unpaired electron. Although having important role in energy circumtances for cells, oxygen association with free radicals cause oxidative stress.Oxidative stress can be defined as unbalancaed reduction and oxidation situation which damages cell structure and cause many diseases. For instance; heart and cardiovascular diseases, cancer, immune system related diseases and aging are occured by oxidative stress. Not only effective in human cells but also oxidative stress cause degradation of vegetables and fruits.

Antioxidants are compounds, which react with reactive oxygen and nitrogen species and free radicals. Free radicals reaction cause many disease in human body, likewise all type cancer or heart disease. On the other hand, free radical reaction cause deterariton on foods, cosmetic products etc. Considering harmfull effect of free radicals reactions, antioxidant compounds are important for healthy life, cosmetic industry and food industry. In wiev of the fact that with globalization and increasing interest in green chemistry, natural antioxidants source became more important. Considering synthetic antioxidant usage restricted for their toxic effects in many countries, natural antioxidants source investigation have been increased. Natural antioxidants found in plants, fruits and vegetables which are rich in antioxidant compounds. Flavonoids, caretenoids and phenolics are plant source antioxidants. Flavonoids are secondary metabolites of plants which can be classified as natural compounds. Flavonoids groups have common naturş antioxidant compound that commonly used like apigenin, myricetin etc.

*Prosopis farcta* is a small, prickly shrub, 30-80 cm tall species of prosopis and widespread in Northern Africa Asia and west to the Middle East. In Turkey, *Prosopis Farcta* only found in Şanlıurfa area. Both in Turkey and others populated *Prosopis* species have long traditional usage by local people. *Prosopis Farcta*, which collected in Şanlıurfa used for diabetic diseases as making tea. Besides using as tea, Prosopis facta mostly seen as problem by farmers because it has strong seeds. Due to this reason, *P.farcta* has forgotten usage and less assays in Turkey. On the other hand, global assays show that high degree of salt toleration, having effect on cholesterol and diabetes hurts are *P.farcta*'s important properties. In vıvo works show that *P. farcta* accelerates healing cutaneous wounds in diabetic rats. On the other hand, there are some acedemic works for *P.farcta's* effect on relaxition of rat's aorta. Besides these effects, it's antioxidant activity, antiparasitic and antimicrobial have been noted recently. There is no report to show an academic research to antioxidant effect of this plant so far. Flavonoid diversification of *Prosopis farcta* occurring two different places shows different percentage and different diversification. Having considering traditional usage, flavonoid diversity of Turkey's *Prosopis farcta* is important research issue.

In this study, antioxidant capacity of Prosopis farcta beans examine by MODIFIED CERAC, CUPRAC, MODIFIED FOLIN methods. Prosopis farcta that have been collected Şanlıurfa, storaged proper conditions in laboratory. Both infusion extraction and methanol extraction solutions are prepared by pericarps of storaged Prosopis Farcta. For antioxidant capacity analysis three different methods applied to both methanol solutions and infusion solutions. Different methods which are MODIFIED CERAC, CUPRAC and MODIFIED FOLIN applied due to having different advantages and disadvanteges against eachother. Therefore correlation in results can obtained in this study.

HPLC system used for identification of *Prosopis farcta* bean's flavonoid diversity. According to flavonoid diversity of *Prosopis farcta* have not been reported, proper parametres of HPLC system decided by several experiments. Chromatographic analysis applied to methanol solutions of prosopis farcta. First, main peaks of Prosopis Farcta solution chromatograms decided. Then,chromatograms of standards solution database formed. Flavonoid diversity of prosopis farcta are identified with standard addition methods by comparing chromatograms.

According to results of this study, differences between infusion and methanol/water extraction solution observed in antioxidant capacity assays.Samples of infusion solution has higher antioxidant capacity than methanol/water extraction samples. Due to qualitative and quantitative results, based on HPLC, flavonoids diversity show differance from Tunusia populated *Prosopis farcta* beans. Quercetin, cafeic acid and gallic acid are found in beans of *P.Farcta* populated in TURKEY. On the other hand, base peaks of *Prosopis farcta* beans couldn't identified in this study.

# *PROSOPİS FARCTA* BİTKİSİNİN MEYVE KISMININ ANTİOKSİDAN KAPASİTESİ VE FLAVONOİD İÇERİĞİ

## ÖZET

Serbest radikaller eşleşmemiş elektrona sahip atom, molekül ya da iyonlardır. Oksijen canlıların enerji döngüsünde önemli bir role sahip olmasına rağmen, serbest radikallerle etkileşerek oksidatif strese sebep olmaktadır. İndirgenme ve yükseltgenme reaksiyonları arasındaki dengesizlikten kaynaklanan oksidatif stres, hücre yapısına zarar vermekte ve bir çok hastalığa sebep olmaktadır. Kalp ve kardiovasküler rahatsızlıklar, kanser, immün sistem ile ilgili hastalıklar ve yaşlanma oksidatif stresin yol açtığı hastalıklara örnektir. Oksidatif stres insan hücrelerini etkilemesinin yanında, sebze ve meyvelerde de bozunmalara yol açmaktadır.

Antioksidan maddeler, reaktif oksijen ve nitrojen türleri ve serbest radikallerle tepkimeye giren bileşiklerdir. Reaktif oksijen türleri canlı vücudunda hücrede degrasyona sebep olduklarından bir çok hastalığa yol açabilmektedirler. Hücre degradasyonun sebep olduğu her çeşit kanser türleri, kalp hastalıkları, cilt rahatsızlıkları gibi hastalıklara sebep olmaktadır. Bunun yanısıra hazır gıda sektörü ve kozmetik sektörü gibi hızlı ulaşım gerektiren sektörlerde yol açılan mikrobiyolojik reaksiyonlar nedeniyle de reaktif oksijen türleri önemli bir sorun oluştıurmaktadır. Serbest radikallerin olumsuz etkileri göz önüne alınacak olursa, antioksidan bileşikler sağlıklı bir yaşam için oldukça önemlidirler. Sağlıklı yaşamın yanısıra bahsedilen nedenlerden dolayı globalleşen dünyanın sektörel olarak da büyük bir ihtiyacını oluştuırmaktadırlar.

Antioksidanlar çeşitli şekilde sınıflandırılabilirler. En temel sınıflandırma doğal ve sentetik antioksidan olarak yapılmaktadır. Sentetik antioksidanların kullanımı oluştırdukları toksik etkilerden dolayı ülkeden ülkeye değişen oranlarda yasaklanmıştır. BHT, TBHQ yasaklanan sentetik antioksidanlardan bazılarıdır. Bu yasaklama için doğal antioksidan kaynaklara olan ilgiyi artırmaktadır. Doğal antioksidanlar hayvan kaynaklı olmakla birlikte, çoğunluk olarak bitki kaynaklarından oluşmaktadır. Bitki, meyve ve sebzeler antioksidan içerik açısından oldukça zengindir. Bitkilerin antioksidan kaynağını ikincil metabolitler oluşturmaktadır. Doğal bileşikler olarak da adlandırılan ikincil metabolitler flavonoidler, karetenoidler ve fenolik bileşiklerden oluşmaktadır.

Flavonoidler bitkilerin ikincil metaboklitlerinden olup fenil benzopiren yapısı A,B ve C halkalarından meydana gelmektedirler. Halkalara bağlanan farklı sübstitüentlerin oluşturdukları konjügasyonlar ile farklı alt grup flavonoidler oluşmaktadır. Flavonoidler sınıfça daha fazla bileşik içermekte ve miresetin, kuarsetin gibi yaygın olarak kullanılan antioksidan bileşiklerini kapsamaktadır. Yeşil kimya çevresel kirliliği ve sentetik kullanımını minimanize ederek sentez, üretim ve proses oluşturmayı amaçlamaktadır. Sekonder metabolitlerin aydınlatılması bu açıdan önem oluşturmaktadır. Bunun yanı sırıa antioksidan içeriği analizi ile korelasyonun

sağlanması için bu çalışmada Prosopis Farcta bitkisinin flavonoid içeriği araştırılmıştır.

*Prosopis*'in bir alt türü olan *Prosopis farcta* 30-80 cm uzunluğunda küçük dikensi ağaçsılar olup, genellikle Kuzey Afrika ve Orta Doğu'nun doğusuna yayılmıştır.Türkiye'de sadece Şanlıurfa'da yetişmektedir. *Prosopis farcta'* nın yerel halk tarafından geleneksel kullanımı diyabetik rahatsızlıkları gidermesi amacıyla çayının yapıması şeklindedir. Çay içiminin yanında yaraları iyileştirmesi için merhemi hazırlanarak direk tedavisi olduğu da kaynaklarda yer almıştır. Bunun yanısıra Türkiye'de kullanımı günümüzde yaygın olarak görülmektedir. Aksine tarım sektöründe çiftçiler için sahip olduğu kalın ve sert köklerinden dolayı sorun oluşturmaktadır. Yapılan tek çalışma da tarım için biyolojik mücadelesi yönündedir. Türkiye'de içerik analizi yapılmamasın ragmen yapılan global araştırmalarda yüksek tuz toleransı, kolestrol ve diyabetik yaralar üzerine etkisi bitkinin önemli özelliklerinden birkaçı olarak yer almıştır. Yapılan in-vivo çalışmalar, *P. farcta*'nın diyabetik farelerin derilerinde oluşan yaraların iyileşmesindeki etkiyi göstermiştir. Öte yandan bazı akademik çalışmalar, aort damarının genişlemesinde *P.farcta'nın* etkisini incelemiştir. Bu etkilerin yanı sıra, *P.farcta'nın* antioksidan, antiparasetik ve antimikrobiyal etkilerinin çalışıldığı bir yayın yapılmamıştır. Yapılan bir araştırmaya göre *P. farcta'nın* flavonoid içeriği farklı iki bölgede yetişenleri arasında farklılık göstermiştir. Geleneksel kullanımı da göz önüne alınacak olursa, Türkiye' de yetişen *Prosopis farcta'*nın flavonoid içeriğinin aydınlatılması önem ihtiva etmektedir.

Bu çalışmada *P. farcta'nın* antioksidan kapasitesi MODIFIED CERAC, CUPRAC, MODIFIED FOLIN yöntemleri ile incelenmiştir. Üç farklı yöntemin uygulanmasının amacı uygulanan yöntemlerin birbirinden farklı olarak içerdikleri üstünlüklerden ve sonuç olarak raporlama yapılırken aralarındaki korelasyonun incelenmesi amacıyladır. MODIFIED CERAC yöntemi cerium iyonun antioksidan bileşik ile redox reaksiyonunda dayanırken, CUPRAC yöntemi bakır(II) iyonunun antioksidan bileşik ile redox tepkimesine dayanmaktadır. MODIFIRED FOLIN yöntemi folin reaktifinin reaksiyonunu içeren, hazırlanan çözeltirler açısından daha hassas analiz edilmesi gerekn bir yöntemdir. Antioksidan kapasite tayini için Şanlıurfa'dan toplanan ve laborauarda uygun koşullarda saklanan bitkinin meyve kısmının toz örnekleri hazırlanıştır.Toz örnekler her seferinde analiz edilmeden önce robotla hazırlanmıştır. Toz örneklerden metanol ekstraksiyonu ve infüzyon ekstraksiyonu çözeltileri hazırlanmıştır. Metanol ekstraksiyon çözeltisi hazırlanmadan önce uygun oranı belirlemek için farklı oranlarda metanol-su çözeltirleri hazırlanarak, analiz edilmiştir. Bu sonucunda 80% metanol-su çözeltisi uygun görülmüştür. Hazırlanan infüzyon ve metanol bitki ekstratların analizi ile MODIFIED CERAC, CUPRAC, MODIFIED FOLIN yöntemleriyle gerçekleştirilmiştir. Elde edilen spektroskopik sonuçlar kaydedilmiştir.

Flavonoid içeriğinin aydınlatılması HPLC analizi gerçekleştirilmiştir. HPLC analizine başlamadan önce öncelikle program sisteminin ve uygun mobil fazlarının belirlenmesi için çalışmalar gerçekleştirilmiştir. Program sistemi oluşturuldaktan sonra bitkinin meyve kısmını içeren metanol çözeltisinin kromatogramı alınmış, ana pikleri belirlenmiştir. Aynı programda standart çözeltiler de analiz edilerek, kromatogramlarının data bankası oluşturulmuştur. Standartlar ve Prosopis Farcta'nın kromotagramları kıyaslanarak uyuşan standartlar, standart ekleme yöntemi için seçilmiştir. Standart ekleme yöntemiyle, belirlenen üç standart için Prosopis farcta bitkisinin flavonoid içeriğinin kantitatif analiz sonuçları oluşturulmuştur. Oluşturulan

sonuçlar, yurtdışında yapılan sonuçlar ile kıyaslanarak Türkiye'deki Prosopis Farcta bitkisinin içeriği kıyaslanmış ve aydınlatılmıştır.

Sonuç olarak, infüzyon ve metanol ekstratlarının antioksidan kapasiteleri kıyaslanmış ve infüzyon çözeltisinin daha yüksek antioksidan kapasitesine sahip olduğu gözlenmiştir. Bu sonuçç geleneksel kullanımı destekler bir sonuç olup, sadece tarımsal mücadele için çalışmalar yapılan Prosopis Farcta bitkisinin başka çalışmalarının yapılması gerektiği ve sağlık açısından kullanımı için çalışmalarının artırabileceğini göstermektedir. HPLC ile gerçekleştirilen analizler doğrultusunda Tunus'ta yetişen *P. Farcta* ile flavonoid içeriğinin farklılık gösterdiği ispat edilmiştir. Kuarsetin, kafeik asit ve gallik asit Türkiye'de yetişen Prosopis farct bitkisinde bulunan flavonoidlerdir. Bunun yanısıra ana piklerin aydınlatılması bu çalışmada gerçekleştirilememiştir.

## 1. INTRODUCTION

In last decades, there is an increasing interest in natural antioxidant assays especially plant origin antioxidants. Antioxidants are compounds that can react with free radicals and terminate the chain reaction before cells structue are damaged. Lipid oxidation, which is a chemical reaction that occurs in foods, cause rancid flavour and rancid aroma that makes food useless. Restricted usage of synthetic antioxidants in food industry, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ), plant source antioxidants become more important for food preservation [1]. Natural antioxidants are not only used in food industry, but also used in cosmetic and pharmaceutıcal industry as ingredients and stabilizier.

Plant source antioxidants are phenolics, flavonoids and caretenoids compounds that have an important role not only for plants but also human health. Flavonoids are plants secondary metabolites which basically occurred by two phenolic and heterecylic rings. Flavonoids, that can be divided many sub groups, are the most common group in the human diet. They inhibite the some enzym activity and cause healing cardiovascular disease [2]. Also flavonoids are effective on decelerating aging.

Several uses and effects have been reported of prosopis species. Phenolics, flavonoids, anthocyanins, and alkaloids profile of *Prosopis alba* and *Prosopis nigra* are studied and main compounds were found asquercetin O-glycosides and apigenin-based C-glycosides [3]. Quercetin is mostly founded and effective flavonoids in nature. On the other hand, Harzallah-Skhiri stated that *Prosopis farcta* beans major flavonoid compounds are cafeic acid and it's derivaties. Flavonoids diversity shows difference according to where plants habitat is [4]. Turkey's species of *Prosopis farcta*, whom antioxidant capacity has not been studied, has been used for many years for curing dierhea and epidemis disease.

The main objectives of this study are; (1) to investigate the antioxidant capacity of *Prosopis Farcta* populated in Turkey (2) research flavonoid diversity in beans organ of *Prosopis Farcta* (3) explain the correlation between traditional usage of prosopis farcta and its antioxidant capacity (4) create new employment opportunities

## 2. LITERATURE REVIEW

### 2.1 Free Radicals

Free radical can be defined as an atom, molecule or ion with an unpaired electron which may be charged or uncharged. Free radical can occurred by two sources; endegoneus source and exogoneus source. Endegoneus free radicals are produced by biochemical process in cells. For example, autooxidation of biological molecules like hemoglobin can produce free radicals. Endegoneus product free radicals mostly consist reactive oxygen species. Exogoneus sourced free radical production triggered by different environmental situations. For instances, air pollution by automobile exhaust include polycylic aromatic carbon which cause generation of free radicals [5].Common free radical examples both endegoneus and exogoneus sourced shown in table 2.1.

Oxygen plays an important role in energy circumstances of cells to survive. On the other hand, oxygen can be harmfull at some situation and may damage to cells. Disadvantage of oxygen may seen associatiotion with free radicals. These disadvantage occurred by reactive oxygen species. Besides source classification, free radicals can be divided in two groups according to chemotaxonomy ;reactive oxygen species( ROS) and reactive nitrogen species(RNS). Reactive oxygen species are chemically reactive molecules containing oxygen. Superoxıde, hydroxyl, peroxyl and hydrogen peroxide are important examples of ROS [6]. ROS are occured naturally in cells, but enviromental situtaion can increase the level of ROS. Reactive nitrogen species are nitric oxide-derived compounds. Nitroxyl anion, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols,and dinitrosyl iron are examples of RNS [7].

Oxidative stress is defined for excrescence reactive oxygen species occurred by unenabled balance between reduction and oxidation. Oxidative stress may cause many harmfull effect not only in human cells but also cause damage for food, drugs etc [8]. On the other hand RNS cause nitrosative stress which has same effect as oxidative stres

**Table 2. 1:** List of ROS, RNS and free radicals.

| **COMMON FREE RADICALS, ROS and RNS** | |
|---|---|
| ▪ Superoxıde anion radical | ▪ Lipid peroxyl radical |
| ▪ Hydroxyl radical | ▪ Lipid alkoxyl radical |
| ▪ Peroxyl radical | ▪ Nitric oxide |
| ▪ Hydroperoxyl radical | ▪ Nitroxyl cation |
| ▪ Lipid radical | ▪ Nitrous acid |
| ▪ Hydrogen peroxide | ▪ Nitrous oxide |

Besides physiological roles of ROS, oxidative stress damage biomolecules like lipids, proteins, carbonhydrates and nucleic acids. Lipids are fundamantel organic compounds for organism. ROS makes peroxidation reaction with lipids which cause deformation of cells and fibers. Peroxidation reaction is a chain reaction that involves three steps: initation, propagation and termination.

$$LH + R^{\cdot} \longrightarrow L^{\cdot} + RH \qquad (2.1)$$

In reaction 2.1 initation step of peroxidation shown.Whereas, LH is the unsaturated lipid interacting with $R^{\cdot}$ as initiating oxidizing radical. This interaction forms a highly reactive allyl radical (denoted as $L^{\cdot}$) that has a tendency to react with oxygen to form lipid peroxyl radical ($LOO^{\cdot}$) as the starter of propagation step. Propagation step with further oxidizatin of the lipid, lipid hydroperoxydes are produced. If the free radicals meet and form non-radical compounds, the process is called as termination.

ROS are less effective on protein than lipids. Sulphure and unsaturrated band containing aminoacids react with ROS and product sulphure radical species [8]. Sulphure radicals brokes the configüration of proteins which ends losing metabolies activity.

Considering reaction ROS with lipids, proteins, carbonhydrates, ROS may effect DNA structure. unpredictable effect ofoxidation in DNA can lead to cancers. The other free radicals oxidation reaction can lead many disease [5];

• Heart and cardiovascular diseases

• Lung diseases

• Alcohol-related diseases

• All types of cancers

• Aging

• Skin diseases

• Eye disorders

• Immune system related diseases

• Central nervous system diseases

• Radiation injury

• Kidney diseases

• Gastrointestinal diseases

## 2.2 Antioxidants

Antioxidants may be defined as against compounds to free radicals. They defend the cell with inhibiting the oxidation reaction [9]. As considering oxidation reaction's effect to cells and disease that can caused by oxidative stress, antioxidants play an important role both in humans health and all living organism. Besides this important role, antioxidants are one of the main key of private sector. All area of industry likewise food, drug and cosmetic industry etc. needs exegeneous source antioxidants to protect their products from degredation.

Like free radicals, antioxidants may be classiffied as endegeneous source and exogeneous source. Besides source come classification, antioxidants are divided in two groups; natural antioxidants and synthetic antioxidants [1].

Synthehic antioxidants have been used since 1950s for food protection. Common antioxidants are tertiary-butylhydroquinone (TBHQ), butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT), propyl gallate (PG), octyl gallate (OG), and dodecyl gallate (DG)[10].All these antioxidants are phenolic compounds, but they have different physical and chemical properties. For example, BHA is particularly suitable in baked and fried foods but TBHQ is more suitable preventin poly unsaturatted oils.

Besides providing food protection, using synthetic antioxidants in food industry has been quastionable for years. Some synthetic antioxidants usage is stricted many

countries or allowed only little dozage. TBHQ is permitted to be used in foods up to a maximum of 200 mg/kg in countries such as European Union, China, the United States, Australia, Brazil, New Zealand, and the Philippines. Many countries including Japan don't allowed usage BHA in food industry due to it's high carcinogenic effect. Therefore searching natural antioxidants has become more important years by years. On the other hand, manufacturing product by green chemistry and natural products has become more important issues both consumer and sector's leader of all industry area. This attention increase the importance of natural antioxidants which can be used in product safely.

Natural antioxidants commanly may classified in two groups; plants sourced and animal derived sourced antioxidants. Natural antioxidants in animal food are aminoacids, peptides and proteins compounds. Plants sourced antioxidants come from different parts of plants; seed, fruit, leaves etc. Plants are main source of exegenous sourced antioxidants. Seconder metabolites in plants are main source of natural antioxidans.Comparing plant source and animal source antioxidants, plant sourced antioxidants assays are more valuable due to manageblity in industry. On the other hand antioxidants can be classified as water soluble antioxidants and oil soluble antioxidants.

### 2.2.1 Plant origin natural antioxidants

Plants occurred by different parts which both of them responsible for different biological activities.Basically plants occurred by root, leaf, steam, flower and fruit. For example, roots are responsible for absorbing water and mineral from soil, while leaves are responsible in absorbing sunligthing to use in photosynthesisprocess [11]. All these parts of plants have different antioxidants constituents that have different pharmaceutics effect. For example; carrot's, whom antioxidants constituents are carotenoids, roots used for bronchitis and chest troubles [12]. On the other hand, turmeric leaves used for cleaning blood and cough and it has curcumin, eugenol, pinene as antioxidant compounds. Besides this specific example, generally most common plants sourced antioxidants can listed as ascorbic acid, Vitamin-E and carotenoids.

Ascorbic acid, which known as vitamin C, cannot be produced by human system. It exists in many fruits and plants like tomato, strawberry, orange etc. Ascorbic acid,

which chemical structure shown in Figure 2.1, inhibits the lipid peroxidation reaction and reacts single oxygen with fast rate [13].

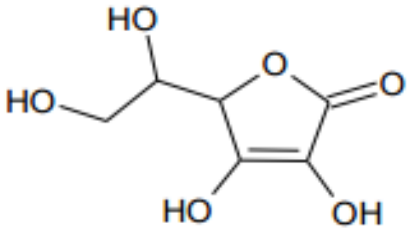
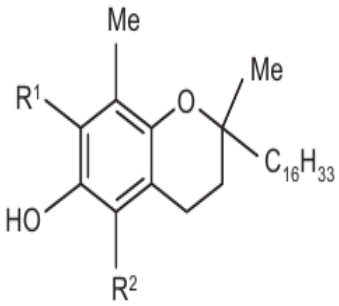



**Figure 2. 1:** Chemical structure of ascorbic acid.

Vitamin E, which also named as tocopherols, exists mostly in plant source. Vitamin-E protects the cell membrane with inactivation of lipid peroxidase. Most active and important four tocopherols are α- tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol, which shown in Figure 2.2 [14]. For example, Egg, grain and products, peas and vegetables are main source of vitamin-E.




$R^1$ $R^2$ =Me, α- tocopherol

$R^1$= H, $R^2$= Me, β-tocopherol

$R^1$ $R^2$=H, δ-tocopherol

$R^1$ =Me $R^2$ = H, γ-tocopherol

**Figure 2. 2:** Chemical structure of Vitamin-E and derivations.

Carotenoids are plants pigments that shown in Figure 2.3. They are responsible for color in plants, fruits and vegetables. There are several form of carotenoids, but β-

carotene, lycopene and lutein are most important carotenoids. B-carotene, which is occurred isoprene units, precursor of vitamin A.

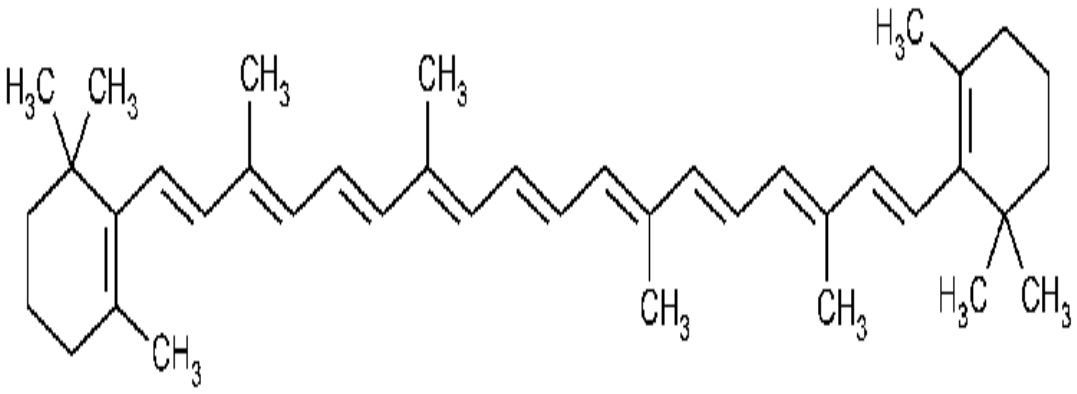



**Figure 2. 3:** Chemical structure of carotene.

### 2.3 Secondary Metabolites

Secondary metabolites, which are also known as natural products, are organic compounds produced by plant.They do not effective in growth, development, or reproduction of any organism in plants. Primary metabolites, which are phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, have metabolitic roles contary to natural products. On the other hand, natural products are important due to usage by humans as in drugs, medicine or flavouring agent.

Secondary metabolites classification can be done on the basis of the chemical structure composition, their solubility in various solvents, or the pathway by which they are synthesized. Briefly, they can be divided in four groups; terpenoids, phenolics, alkoloids and flavonoids [15].

Terpenoids, which also called isoprenoids, main skelation derived from five carbon units.This five carbon units are called isoprene unit. Terpens combination occured by different connecting of isoprene units which is known as isoprene rule. These connection can be head to head, head to tail and head to middle. Terpenoids classification based on number of connectted isoprene units( Table2.2)

**Table 2. 2:** Classification of terpenoids.

| Classification Name | No Isoprene Unıts |
|---|---|
| Hemiterpenoids | 1 |
| Monoterpenoids | 2 |
| Sesquiterpenoids | 3 |
| Diterpenoids | 4 |
| Sesterterpenoids | 5 |
| Triterpenoids | 6 |
| Tetraterpenoids | 8 |

Terpenoids have been used for their pharmacological activities for years. Likewise digitoxigenin have been used in treatment of congestive heart disease [16]. Taxol, which is cylic type terpenoids, have been used as anticancer drug. On the other hand, terpenoids can be used as fragrance ingredient in cosmetic industry. For example; carvone used for spearmint odour, linalool for floral odour, menthol for mint odour etc..

Alkoloids were defined as nitrogencontaining basic compounds of plant origin at first time. After research for years, time showed that this definition is not enough due to researcher proved thatexistence of alkoloids biosynthesized by animals. Animals have been used alkoloids compounds as defence agent, sexual signaling etc.. For exampe the frog Bufo marinus accumulates a considerable amount of morphine in its skin [15].

Plants alkoloids have been used for their pharmaceutical effect by people in medicine since ancient times. For instance morphine, which is extracted from Papaver somniferum, has been used as pain relivier especially at war times. More detailed examples of alkoloids usage shown in Table 2.3. Morphine was the first isolated

alkoloids from plant by German chemist Friedrich Sertürner in 1804. More than 12,000 alkaloids have been isolated since the discovery of morphine.

**Table 2. 3:** Usage of some alkoloid examples.

| Alkoloids | Plant Source | Usage |
|---|---|---|
| Codeine | *Papaver somniferum* | Analgesic and antitussive |
| Caffeine | *Coffea arabica* | Central nervous system stimulant |
| Berberine | *Rhizoma Coptidis* | Anticancer drug |
| Quinine | Cinchona officinalis | Antimalarial |

Phenolic compounds are secondary metabolites which comprised simply aromatic ring and substituents. Phenolic compounds classification are made via number of aromatic ring and substituents that compounds have. Basic phenolic compounds include six carbon ring unit. Substitent attached to phenolic rings makes classification and difference between compounds. Classification and examples of groups written in Table 2.4. They have wide range of physiological effects, like anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects.Mostly used antioxidants like gallic acid are phenolic type compounds whom examples seen in Table 2.4. Phenolic compounds widely arange $C_6$ to $(C_6\text{-}C_3\text{-}C_6)_n$ strcture. Gallotannins are examples of $(C_6\text{-}C_3\text{-}C_6)_n$ structure phenolic compounds which are importsnt in cosmetic industry and can be used in cleansing products. Also they can act as antioxidant compounds like others phenolic compounds.

**Table 2. 4:** Examples of phenolic compounds.

| **Classification Name** | **Structure** | **Example** |
|---|---|---|
| Simple phenolics | $C_6$ | Catechol |
| Hydroxybenzoic acids | $C_6$-$C_1$ | Gallic acid |
| Acethophenones,phenylacetic acids | $C_6$-$C_2$ | 3,4-dihydroxyphenylethanol |
| Hydroxycinnamicacid, phenylpropanoid | $C_6$-$C_3$ | Cafeic acid |
| Napthoquinones | $C_6$-$C_4$ | Juqlone |
| Xanthones | $C_6$-$C_1$-$C_6$ | Xanthene |
| Stilbenes, anthraquinones | $C_6$-$C_2$-$C_6$ | Resveratrol |
| Flavonoids, isoflavonoids | $C_6$-$C_3$-$C_6$ | Quercetin |
| Lignans, neolignans | $(C_6$-$C_3)_2$ | Pinoresinol |
| Biflavonoids | $(C_6$-$C_3$-$C_6)_2$ | Sulcatone |
| Lignins | $(C_6$-$C_3)_n$ | Lignin |
| Condensedtannins | $(C_6$-$C_3$-$C_6)_n$ | Gallotannin |

### 2.3.1 Flavonoids

Flavonoids are actually sub-groups of phenolic compounds. They are the largest and most studied classes of seconder matabolites [17]. Their basic skeleton consists of two phenolic rings and one heterocylic ring. Shown in Figure 2.4 that phenolic rings are named A and B ring, heterocylic ring is named as C ring. Basic skeleton is known as flavone backbone.

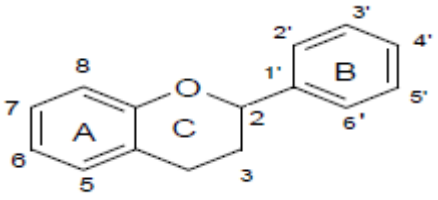
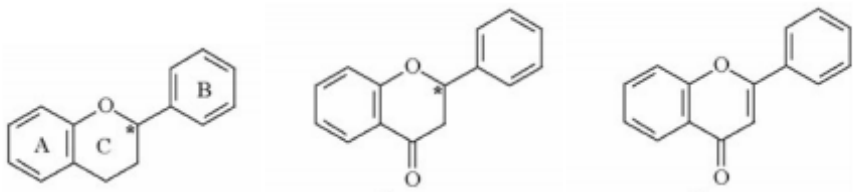
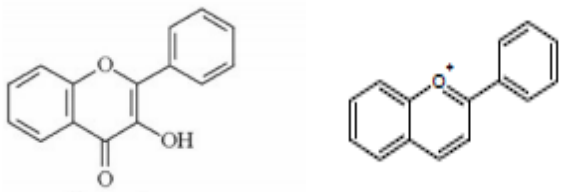



**Figure 2.4 :** Flavone backbone structure.

Classification of flavonoids are made via derivation of this flavone backbones.These classsification differ to oxidation and saturation of C ring and attachment B ring to 2 or 3 carbon of C ring. According to substituent attachment; flavon, flavonone and flavonol groups are occurred. Difference between subgroups shown in Figure 2.5 according to substituent attachment.




flavan flavanone flavone

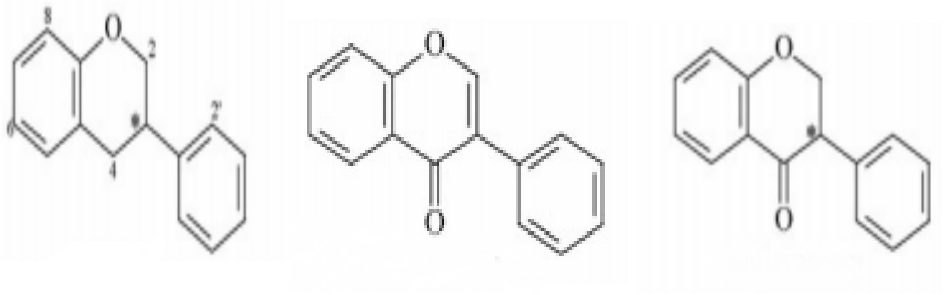



flavanol anthociyanidin

**Figure 2. 5:** Chemical structureof flavonoids subgroups.

Besides, subgroups that mention in figure 2. 5, isoflavonoids are different subgroups of the flavonoid (Figure 2.6). They have 3-phenylchromen-4-one backbone, while flavonoids have 2-phenylchromen-4-one backbone.

Flavonoids and isoflavonols has many pharmaceutical effect. Some examples and gropus listed in table 2.5. Antioxidant capacity is one of the most important properties that they show. For example quercetin, which shows also anti inflammatory and anti-allergic effect, has greater antioxidant effect than vitamin C.Alsoresveratrol, that exists in red grape, has anti-cancer action. Besides these examples epicatechin, which is found in high concentration in many fruits and vegetables especially in green tea, has a variety of beneficial effects to human health. One effect is improving glucose tolerance and reducing glycosylated hemoglobin levels in non-obese diabetic mice [18]. Also, it acts as antioxidants by inhibiting lipid peroxidation and oxygen radical scavenging.




isoflavan isoflavone isoflavanone

isoflavanol

**Figure 2.6:** Classifaction of isoflavonoids.

**Table 2. 5:** Examples of some flavonoids effect[19].

| Flavonoid Group | Examples | Source | Effect |
|---|---|---|---|
| Anthociyanin | Cyanidin | Bilbery, Blackberry,blueberry | Antioxidant, reduce obesity |
| Flavanone | Naringenin | Orange, Grapefruit, Lemon | Antioxidant, İmmun system modulator |
| Flavon | Apigenin | Chamomile tea | Nneuroprotective effect |
| Flavanol | Myricetin | Red wine, nuts, berry | Antioxidant, anticarcinogen |
| Isoflavones | Genistein,Glycitein | Soy beans, Soy milk | Anthelmintic |

## 2.4 *Prosopis Farcta*

*Prosopis farcta* is a small, prickly shrub, 30-80 cm tall species of prosopis and widespread in Northern Africa Asiaand west to the Middle East. *Prosopis farcta*, which is belong to leguminosae family and mimosoideae subfamily, found in only Şanlıurfa area in Turkey*(*Figure 2.6). *P. Farcta* has long traditional usage by local people. The beans, that collected end of the summer(Figure 2.7) used for treatment of enteric disease. According to them, after boiling beans, regularly usage of tea effects the diearhea. On the other hand, it's leaves used for treatment of epidermic disease [20].

Besides it's advantage in treatment of epidemic and endemic disease, Akkuzu says this plant may cause a problem for farmers. *Prosopis farcta* has deeper strong seed which cause a damage on farm machines and devices. In consequences of damage, farmers have to deal with economical loss and waste of time. Akkuzu pointed that glyphosate isopropylamine salt (750 cc/da), glyphosate diamonium salt (1000 cc/da), glyphosate potassium salt (1000 cc/da) and 2,4-D (200 cc/da) were found as more effective for chemical managament with *prosopis farcta* [21].

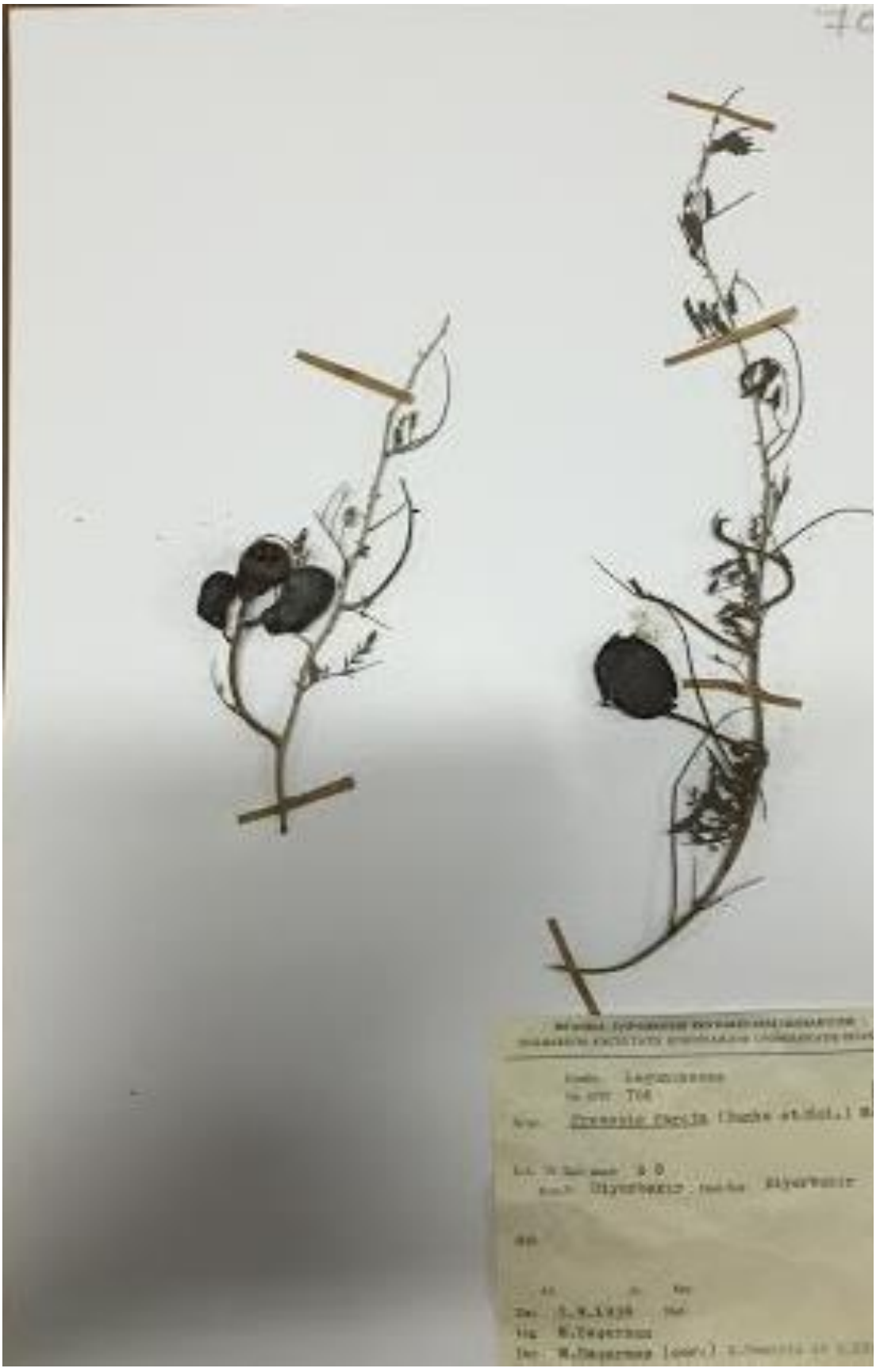


**Figure 2. 6:** *Prosopis Farcta* from Flora databank.

Different cultures have been used their local plants for medicanal purpose since many years. Asoldohi and Abassi pointed the historical usage of prosopis farcta for reducing cardiac or chest pain in İran. According to their in vivo study, prosopis farcta extract showed a relaxition effect on aort and smooth muscle. Through their study, they suggested the investigation of prosopis farcta extract effect on hypertension [22]. There haven't been any published paper about hypertension effect of *P.Farcta* but Omodi and Ansari had a research on *P. Farcta* beans effect on hdl and ldl cholesterol. According to their study, *P. farcta* beans increased hdl cholesterol and increase ldl cholesterol in ostriches [23]

**Figure 2. 7:** *Prosopis Farcta* from Şanlıurfa.

*Prosopis farcta* not only used in alternative medicine but also have many effect which is proven by academic studies. Besides invivo studies, there haven't been published work about antioxidant capacity of *prosopis farcta*. Shkiri and Jannet stated that two prosopis farcta that located in North and South Tunisia have different major flavonoid component [24].These major and minor components listed in Table 2.6.Considering this study and importance of usage in traditional medicine, properties of prosopis facta in Turkey sould be identified.

**Table 2. 6:** Flavonoid diversity of *P.farcta* from Tunusia.

| Compound | Branches | Roots | Leaves | Flowers | Pericaps |
|---|---|---|---|---|---|
| Myricetin 3-O-glucoside | M* | a | M | major | M++ |
| Isovitexin | M | a | Major | M++ | M |
| Rutin =Quercetin glycoside I | major | a | M | M++ | M |
| Isorhamentin 3-O-rutinoside | m+ | a | M | m | major |
| 5-deoxyluteolin | m+ | major | A | a | M++ |
| Cafeic acid and derivative | M | m+ | A | a | major |

*: m: minor; m+: c : appreciate ; m++:abundant; a: absent

### 2.5 Literature Review of Antioxidant Capacity Methods

Antioxidant capacity methods can be classified as hydrogen transfer reaction (HAT) based methods and electron transfer (ET) reaction based methods. These HAT based and ET based methods listed in Table 2.7. HAT-based methods measure the classical ability of an antioxidant to scavenge free radicals by hydrogen donation to form stable compounds. HAT-based methods generally occured synthetic free radical generator, oxidizable radical probe and antioxidant compound [25]. While synthetic free radical generator produce peroxyl radicals, oxidizable radical probe used for create compotitive kinetic between antioxidant and peroxyl radical. Whereas HAT-based methods pH dependent and are generally quite rapid, ET-based methods can be relatively slow and need long times to reach completion. Et- based methods involve antioxidant and oxidant compound. As well as antioxidant compund gives an electron

to oxidant compound, color change can be seen in oxidant compound [26]. The degree of the color change is proportional to the antioxidant concentration. Parameters explained individually.

**Table 2. 7:** Antioxidant capacity methods classification.

| HAT BASED METHODS | ET BASED METHODS |
|---|---|
| OXYGEN RADICAL ABSORBANCE CAPACITY ASSAY | TROLOX EQUIVALENCE ANTIOXIDANT CAPACITY ASSAT |
| TOTAL PEROXYL RADICAL-TRAPPING POTENTIAL ASSAY | FERRİC İON REDUCİNG ANTİOXİDANT POWER |
| CROCIN BLENCHING ASSAY | DPPH RADICAL SCAVENGING CAPACITY ASSAY |
| | FOLIN-CIOCALTEU REAGENT ASSAY |
| | COPPER ION REDUCTION ASSAY |
| | CERIUM ION REDUCTION ASSAY |

### 2.5.1 Cerium Reducing Antioxidant Capacity Assay

Cerium ion reduction antioxidant capacity assay (CERAC) based on the reaction between Ce(IV) ion and antioxidant compounds. Ce(IV) ions absorbs the light maximum at 320 nm, after reaction with antioxidant components maximum absorbance value decrease. Through this decrease, total antioxidant capacity n example can be calculated [27]. Only problem in CERAC assay that Cerium ( IV) not only react with antioxidant compounds but also oxidize citric acid and simple sugar. Reaction with citric acid cause an error for total antioxidant capacity analysis.

Modified cerac method was developed for selectively oxidization of antioxidant compounds, there for minimize the error in total antioxidant capacity measurement. Selective oxidization is possible with adjusting the $H_2SO_4$–$Na_2SO_4$ composition in cerium solution [28].

### 2.5.2 Cupric Reducing Antioxidant Capacity Assay

Cupric Reducing Antioxidant Capacity (CUPRAC) assay based on the reaction between Cu(II)-Nc (cupric(II)-neocuproine (2,9-dimetil-1,10-phenanantrolin) reactive and antioxidant components. After reaction with antioxidant compounds, Cu(I)-Nc (cupric(I)-neocuproine) chelate (Figure 2.8) occured that absorbance masurement at 450 nm lead to calculation of antioxidant capacity [29].

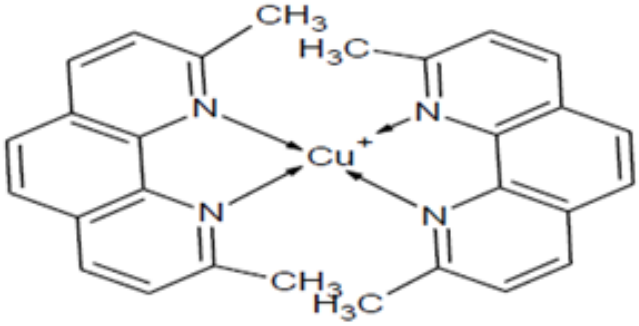



**Figure 2. 8:** Chemical structure of Cu(II)-Nc.

These method has many advantages than other methods. First of all, due to the lower redox potential of the CUPRAC reagent, reducing sugars and citric acid are not oxidized with the CUPRAC reagent [30]. Also the CUPRAC reagent, which is more stable than other reagent, is fast enough to oxidize thiol-type antioxidants. Furthermore, the method can currently measure hydrophilic and lipophilic antioxidant capacity. On the other hand, the only disadvantage of CUPRAC method is reaction time for complex antioxidant analysis.

### 2.5.3 Modified Folin-Ciocalteu Assay

Folin-Ciocalteu assay based on the measurement of molibden(V) ion absorbance at 765 nm wavelength. Folin-Ciocalteu reagent (FCR) is mixture of phospomolybdate and phospotungstate ($3H_2O.P_2O_5.13WO_3$. $5MoO_3.10\ H_2O$). Folin-Ciocalteu assay studied at pH 10 and depends on the reduction, shown in equation 2.2, Mo(VI) ion to Mo(V) ion [31].

$$Mo(VI)(yellow)+e \rightarrow Mo(V)(blue) \qquad (2.2)$$

Folin-Ciocalteu methods was insufficient to measure lipophilic antioxidant, thus Modified Folin method was developed and applied successfully to antioxidant capacity assay like quercetin, caffeine, catechin etc.. Modified methods occured by these conditions;

1. Dilution ratio of aqueous FC reagent with iso-BuOH (1:2, v/v)
2. Final NaOH concentration of $3.5 \times 10^{-2}$ M,
3. After reaction time of 20 min and maximum absorption wavelength of 665 nm.

# 3. EXPERIMENTAL

## 3.1 List of Chemicals Used

Cerium sulfate tetra hydrate (IV) (Ce(SO4)2.4H2O), sulphuric acid (H2SO4), sodium sulphate (Na2SO4), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one), ammonium acetate ($CH_3COONH_4$), neocuproin (2,9-dimethyl-1,10-phenanthroline), copper chloride di hydrate ($CuCl_2.2H_2O$), Folin-Ciocalteu reagent, sodium hyrodxyate (NaOH), rutin ($C_{27}H_{30}O_{16}$), caffeic acid ($C_9H_8O_4$), gallic acid ($C_7H_605$), tannic acid ($C_{76}H_{52}O_{46}$), methanol ($CH_3OH$), ethanol ($C_2H_5OH$), hydrochloric acid (HCl), luteolin ($C_{15}H_{10}O_6$), apigenin($C_{15}H_{10}O_5$), myricetin($C_{15}H_{10}O_8$).All chemicals were HPLC grade.

## 3.2 List of Devices Used

For these assay UV-vis spectra recording and absorbance measurements were made with Varien Cary 100 model UV/vis spectrophotometer (Mulgrave, Victoria, Austrilia) equipped with a pair of matched quartz cuvettes of 1 cm light path. Flavonoid diversity experiments were made by Elite Lachrom HPLC with C18 coloumn, UV dedector and D&W2 light source. Solutions for antioxidant assays are measured on Gec Avery assay balance. For preperation of *Prosopis Farcta* beans extract Edmund Buhler Tubingen shaker and Magic Bullet electrical mill was used. Liquid sampling made with ependorf micropipettes with different volume.

## 3.3 Preparation of Solutions Used

### 3.3.1 Solutions of Modified-CERAC assay

Ce(IV) sulphate solution at $2x10^{-3}$M concentration was prepared by dissolving 0.04g $Ce(SO_4)_2.4H_2O$ in water, transferring to a 50 mL volumetric flask, adding 8.41 mL of concentrated $H_2SO_4$ to prevent ceric ion hydrolysis, and diluting to the mark with double-distilled water. For 1,0 M $H_2SO_4$ solution 35.51 g sodium sulphate dissolved with deionized water in 250 mL volumetric flask.

### 3.3.2 Solutions of CUPRAC assay

0.134 g $CuCl_2.2H_2O$ was measured and dissolved in 100 mL water for preparing $1\times10^{-2}$ M copper chloride solution. For $3\times10^{-3}$ M neocuproin solution 0,156 g neocuproin was measured and dissolved with ethanol in 100 volumetric flask. 1, 0 M ammonium acetate was prepared via measuring 19, 27 g and diluting with water to the mark in 250 mL volumetric flask.

### 3.3.3 Solutions of MODIFIED- FOLIN assay

0.1 M sodium hydorxylate solution prepared by measuring 0.4 g NaOH and dissolved in 100 mL distilled water. Folin reagent mixed with butanol in 1:2 rations, after shaked two components, solution was extracted and yellow phase used for assay.

## 3.4 Preperation of *Prosopis farcta* extract

### 3.4.1 Infusion solutions

First *Prosopis Farcta* beans powdered with electrical mill. From powder of herbs, 1 g was measured, shaked into 250 mL hot water. Then wait in hot water until 3 minutes. After 5 minutes infusion extraction, solutions were filtered and transferred to the 250 mL volumetric flask. Solutions diluted to the mark with deionized water.

### 3.4.2 Methanol extraction solutions

Methanol extract solutions of *Prosopis Farcta* was prepared through the process that is written in the below paragraph.

First, 20 ml 80% (v/v) methanol solution added to 1 g powdered beans and shaked for one hour with 350 rpm. End of the one hour, solution filtered. Secondly, 20 ml 80% (v/v) methanol was added to residue and shaked for 45 minutes with 350 rpm. Then the same filtration process applied. Finally, after added 10 ml 80% (v/v) methanol solution to second residue, extract was shaking for 15 minutes with 350 rpm. End of these three steps, all filtrate taken in 50 mL volumetric flask and diluted to the mark with 80% (v/v) methanol solution.

## 3.5 Solutions of HPLC assay

### 3.5.1 Mobile phase solutions

For HPLC assay of *Prosopis farcta*, mobile phase solutions were prepared. First 2% $H_3PO_4$ solution was prepared via diluting 0.69 ml $H_3PO_4$ TO 500 ml. Then, 80%

methanol solution was prepared via mixing 8:2 ratio of methanol –water. Methanol solution degased in ultrasonic bath before used as mobile phase.

### 3.5.2 Standard solutions of HPLC assay

For standard addition part of HPLC assay $1\times10^{-3}$ M quarcetin, gallic acid and cafeic acid stock solutions were formulated. 0.008 g quarcetin, 0.002 g cafeic acid and 0.004 g gallic acid were measured and diluted with %80 (v/v) methanol in 25 ml volumetric flask. Other solutions with different molarities formulated from stock solutions.

### 3.5.3 *Prosopis Farcta* solutions

5 g powdered prosopis farcta beans 80% methanol extract was prepared. For hydrolyzing, 5 mL HCl and 1 mL methanol added to 30 mL methanol extract and completed to 50 mL via distilled water. This solution refluxed for two hours before used in HPLC.

## 3.6 List of methods used

In this study for antioxidant capacity experiments MODIFIED-CERAC, CUPRAC and MODIFIED FOLIN methods used. On the other hand, for flavonoids diversity HPLC analysis applied.

### 3.6.1 Modified- CERAC method

Modified- Cerac method calculate the antioxidant capacity of samples through the change of absorbance value Ce (IV) ion. Difference Modified-CERAC method from classical CERAC method based on selectively oxidation of antioxidant compounds.

1,0 mL $2\times10^{-3}$ M Ce(SO4)2 solution + 7,0 mL 1,0 M $Na_2SO4$ solution + x mL antioxidant compound was put in test tube and diluted to 10 ml with deionized water. After shaked via vortex , samples are waited for 30 minutes at room temperature. Than all samples absorbance was measured at 320 nm against to blank solution. In this study, as antioxidant compounds quercetin was used. Both ınfusion and methanol extract of *prosopis farcta* absorbance was measured.

### 3.6.2 CUPRAC method

CUPRAC method worked on reduction of Cu (II)-Nc blue complex to Cu (I)-NC yellow complex via interaction with antioxidants and complex. According to this visible change, total antioxidant capacity can be measured.

Solutions are formulated according to below mix formulation. After waited 30 minutes, all samples absorbance value was measured at 450 nm against to blank solution.

1,0 mL 1,0 × $10^{-2}$ M $CuCl_2$ solution + 1,0 mL 7,5 × $10^{-3}$ M neocuproin (Nc) solution + 1,0 mL 1,0 M $NH_4Ac$ solution + x mL antioxidant solution + (1,1-x) mL $H_2O$

This ratio both applied to quercetin standard solution, infusion solution and methanol extraction of *prosopis farcta.*

### 3.6.3 Modified Folin-Ciocalteu method

Solutions for modified Folin-Ciocalteu method were formulated as written below.

0,3 mL Folin reagent + 3.5 ml NaOH + x mL antioxidant + (6,2- x) mL $H_2O$

After solutions were waited for 20 minutes, absorbance value was measured at 665 nm against to blank solution. This formulation applied to standar quercetin solutions. Also infusion and methanol extract absorbance value was measured at 665 nm.

## 3.7 HPLC method

| | |
|---|---|
| **Coloumn** | C-18 kolon (10 μm) [250x4.6 mm(ID)] |
| **Oven temperature** | 30°C |
| **Dedector** | UV dedector |
| **$\Lambda_{max}$** | 280 nm |
| **Mobile phase** | A: %100 MeOH B: %2 o-$H_3PO_4$ |
| **Flow rate of mobile phase** | 1 mL/m |
| **Pressure** | 300 bar |

Phase program of HPLC methods shown in table 3.1, based on percentage taken programme applied both standars and samples.

**Table 3. 1:** Phase programe of HPLC.

| TIME(min) | % A PHASE | %B PHASE |
|---|---|---|

| | | |
|---|---|---|
| 0 | 10 | 90 |
| 5 | 20 | 80 |
| 8 | 30 | 70 |
| 10 | 40 | 60 |
| 15 | 50 | 50 |
| 23 | 55 | 45 |
| 25 | 60 | 40 |
| 28 | 70 | 30 |
| 30 | 80 | 20 |

### 3.8 Calibration graphipis of antioxidant methods

#### 3.8.1 Quercetin calibration graphic via Modified-CERAC method

Standard solutions that having concentration between $1\times10^{-5}$ and $1\times10^{-6}$ were preperead by dilution of stock quarcetin solution. Each measurement was carried out in dublicate. Through avarage results, concentration and absorption graphic was drawn. According to calibration curve equation, written in Figure 3.1, molar absorptivity (L $mol^{-1}$ $cm^{-1}$) was calculated. Table 3.2 shows the avarage value of molar absortivity for Modified-Cerac method

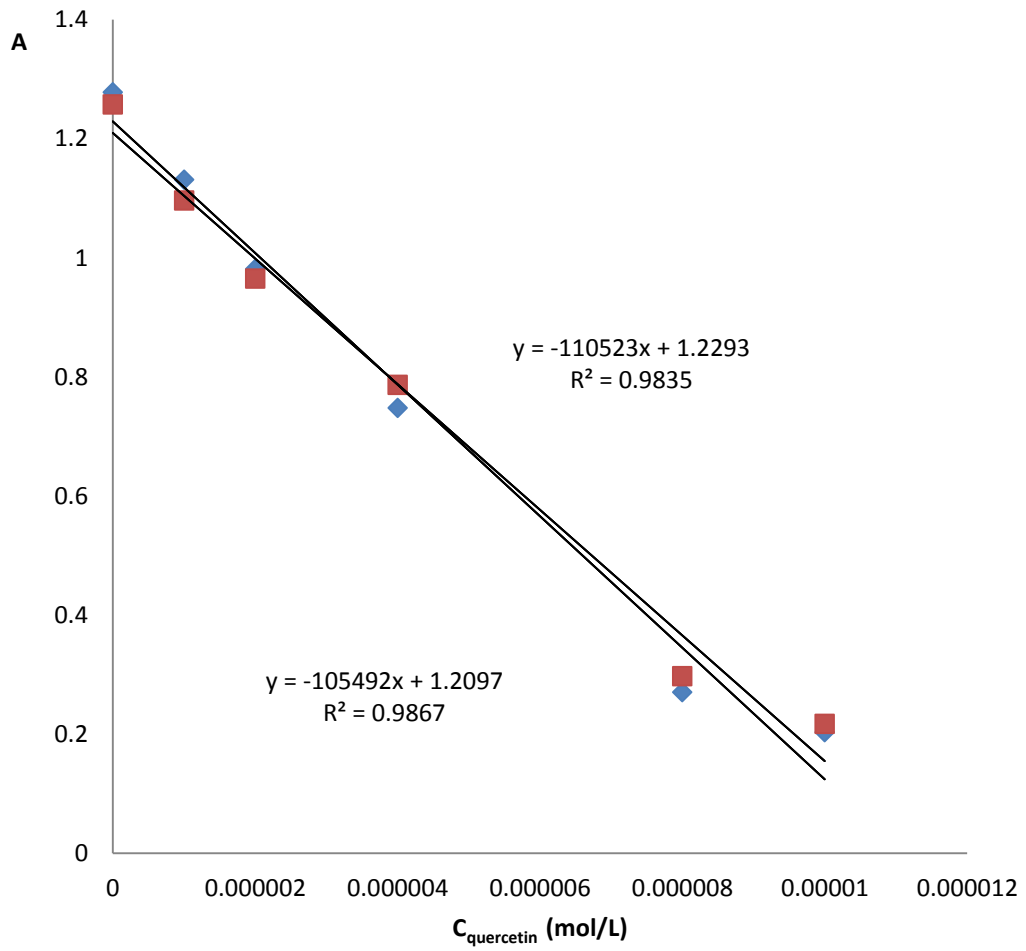



**Figure 3. 1:** Calibration graphic of quercetin via Modified-CERAC method.

**Table 3. 2:** Molar absortivity of quercetin via Modified-CERAC method.

| | **Molar absorsivitiy(Lmol$^{-1}$cm$^{-1}$)** |
|---|---|
| 1 | 11052 |
| 2 | 10549 |
| **Avarage value** | 10800 |

### 3.8.2 Quercetin calibration graphic via CUPRAC method

Quarcetin solutions that having different concentration were preperead from stock solution. Each measurement was carried out in dublicate. Through avarage results, concentration and absorption graphic was drawn. According to calibration curve equation, written in Figure 3.2, molar absorptivity for quercetin was calculated. Table 3.3 shows the avarage value of molar absortvity for Cuprac method.

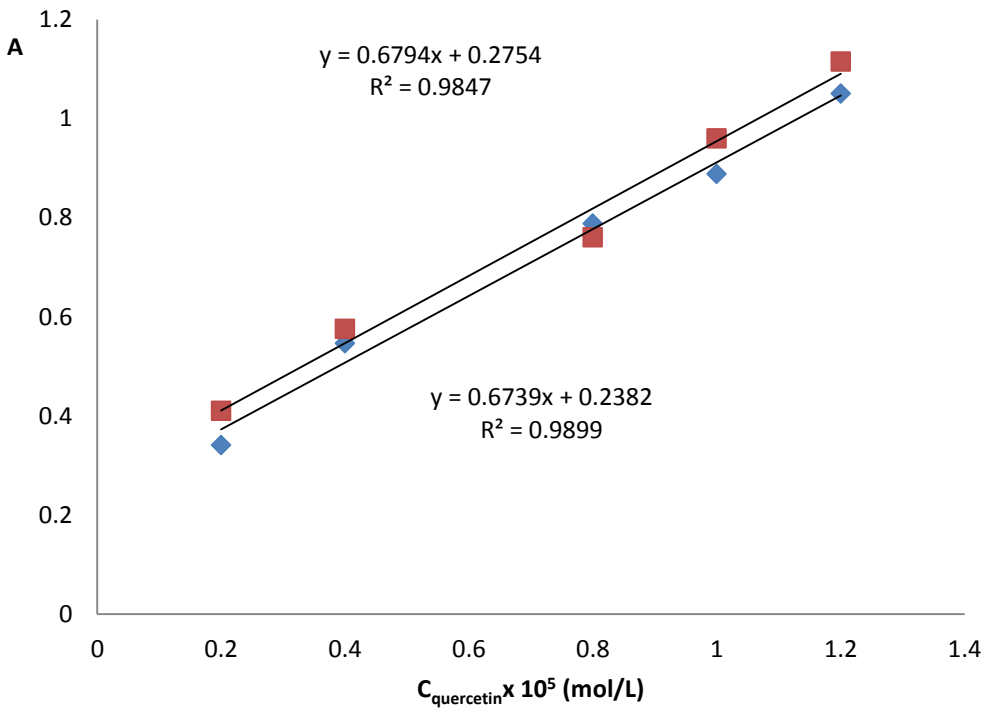



**Figure 3. 2:** Calibration graphic of quercetin via CUPRAC method.

**Table 3. 3:** Molar absorbtivitiy of quercetin via CUPRAC method.

| | **Molar absorsivitiy($Lmol^{-1}cm^{-1}$)** |
|---|---|
| 1 | 67900 |
| 2 | 67300 |
| **Avarage value** | 67600 |

### 3.9 Determination the total antioxidant capacity of *prosopis facta* beans extracts

#### 3.9.1 Application of Modified- CERAC method to extracts

Formulation of Modified-CERAC assay was applied to plant extracts. For experiment extracts put in to test tubes which include 1 mL $2\times10^{-3}$ Ce(IV) solution and 7 mL $Na_2SO_4$. Volume of extracts were between 0,2 mL to 1 mL. After waited for 30 minutes, all solutions absorbance was measured and graphic of absorbance was drawn both for methanol solution and infusion solution Figure 3.3 and Figure 3.4.Antioxidant capacity, that written in, was calculated. All measurement, that applied both infusion extract and methanol extract, was triplicated. Values that shown are avarage values(Table 3.4).

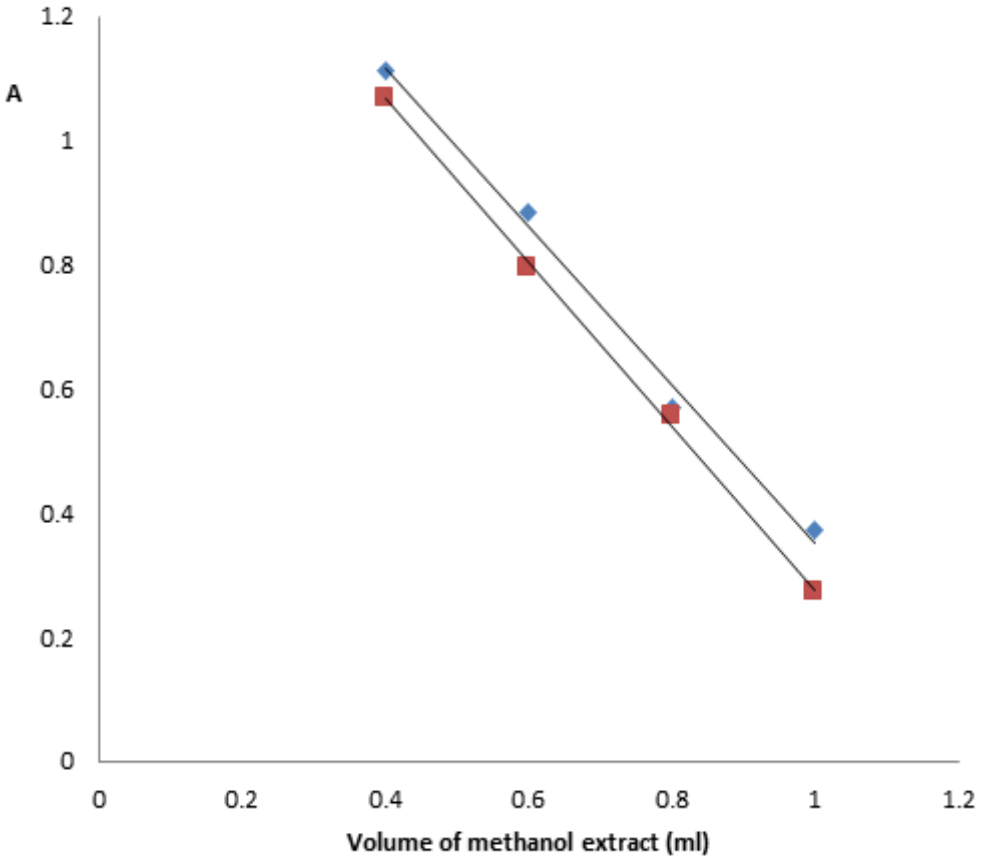



**Figure 3. 3:** Linear absorbance graphic of *P.farcta* methanol extract solution.

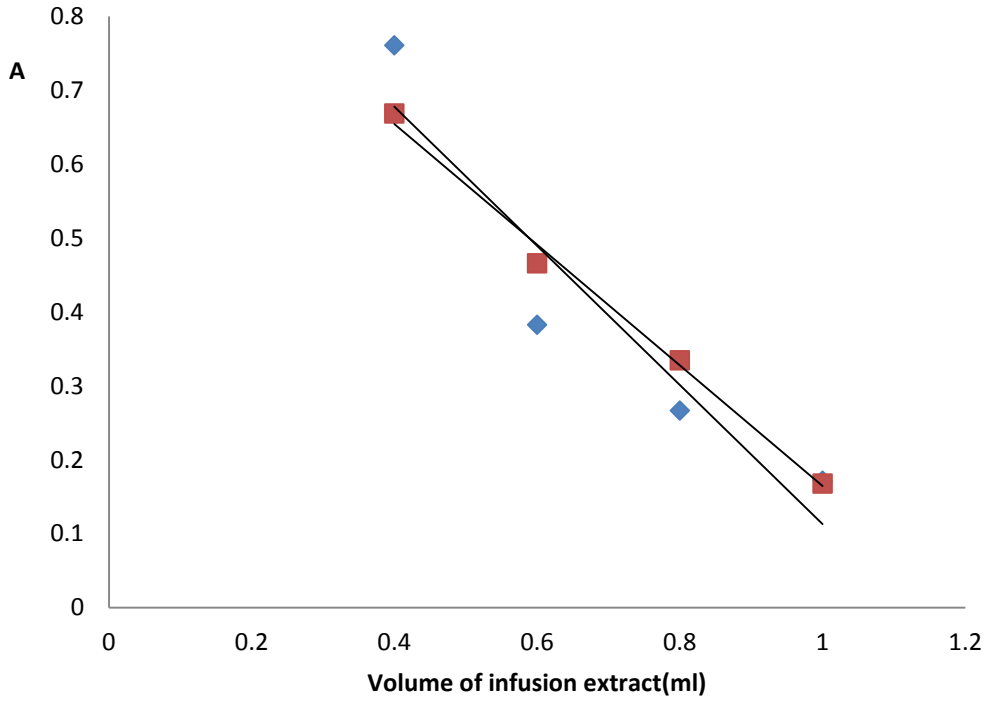



**Figure 3. 4:** Linear absorbance of graphic of infusion extract solution.

Both graphics, Figure 3.3 and Figure 3.4, improve that absorbance of Ce(IV) ion decrease while amount of *prosopis farcta* extract solution increase. This situtaion occurs due to reaction between Ce(IV) ion and antioxidant components that *prosopis farcta* has.

**Table 3. 4:** Amount of quercetin both in infusion solution and methanol extraction solution.

| SAMPLE | mmol quercetin/ g extract |
|---|---|
| 80 % Methanol extraction solution | $3{,}8\times10^{-4}$ |
| Infusion solution | $3{,}4\times10^{-1}$ |

### 3.9.2 Application of CUPRAC method to extracts

For CUPRAC assay, different volume of extract solutions that exist between 0,1 ml to 1 ml, put into test tubes. Each test tubes include 1 ml $1{,}0 \times 10^{-2}$ M $CuCl_2$ solution, 1,0 mL $7{,}5 \times 10^{-3}$ M neocuproin (Nc) solution and 1,0 mL 1,0 M $NH_4Ac$ solution. Final volume of tubes was 4,1 mL. After 30 minutes, methanol extract solutions and infusion solutions absorbance values were measured at 450 nm against to blanck solution. Total antioxidant capacity was calculated through equation 3.1 These equation applied to all antioxidant capacity assay that used in this study. All measurements repeated twice as others assays. Values, shown in Table 3.5, were calculated from the aritmetic mean. Detailed of equations shown in 3.1 that written in below.

**Antioxidant capacity=[A/εq] ×Vf/Vi×DF×Ve/m** (3.1)

A: Absorbance of example

DF=Dilution factor

Vf: Final volume of sample

Vİ:initisl volume of sample

Ve=volume of extraction solution

m = Measurement of example(g)

εq= Quercetin molar absortion coefficient

**Table 3. 5** Amount of quercetin both in infusion and methanol extact solution via CUPRAC method.

| **SAMPLE** | **mmol quercetin/ g extract** |
|---|---|
| 80 % Methanol extraction solution | $0{,}10\times10^{-4}$ |
| Infusion solution | $1{,}0\times10^{-1}$ |

### 3.9.3 Application of MODIFIED- FOLIN method to extracts

Modified-Folin methods procedure was applied to both infusion and methanol extraction solutions. Each sets of experiments were done twice. According to avarage value antioxidant capacity was calculated. Difference value of results shown in table 3.6.

**Table 3. 6:** Amount of quercetin both in infusion and methanol extact solution via Modified-FOLIN method.

| SAMPLE | mmol quercetin/ g extract |
|---|---|
| 80 % Methanol extraction solution | $1{,}56\times10^{-3}$ |
| Infusion solution | $7{,}2\times10^{-2}$ |

## 3.10 Determination of the *P.farcta* flavonoid diversity by HPLC

### 3.10.1 Calibration graphics of flavonoid compounds

In this study for identify the flavonoid diversity of *P.farcta'*s high performance liquid chromatoraphy was used. Mobile phase and gradient system explained in Table 3.1. For identification standard addition methods was choosen. First, HPLC gradient system programe applied to flavonoids standards : quercetin,cafeic acid, gallic acid, luteolin, apigenin, myricetin,rutin and vicenine. All standard solutions made by diluting stock solutions. Secondly, same programe applied to *P.Farcta* samples which hydrolised before. All standards chromatograms compared with *P.Farcta* chromatogram for identification. Linear correlation of increasing concentration increasing peak area seen clearly as shown in figure 3.5.

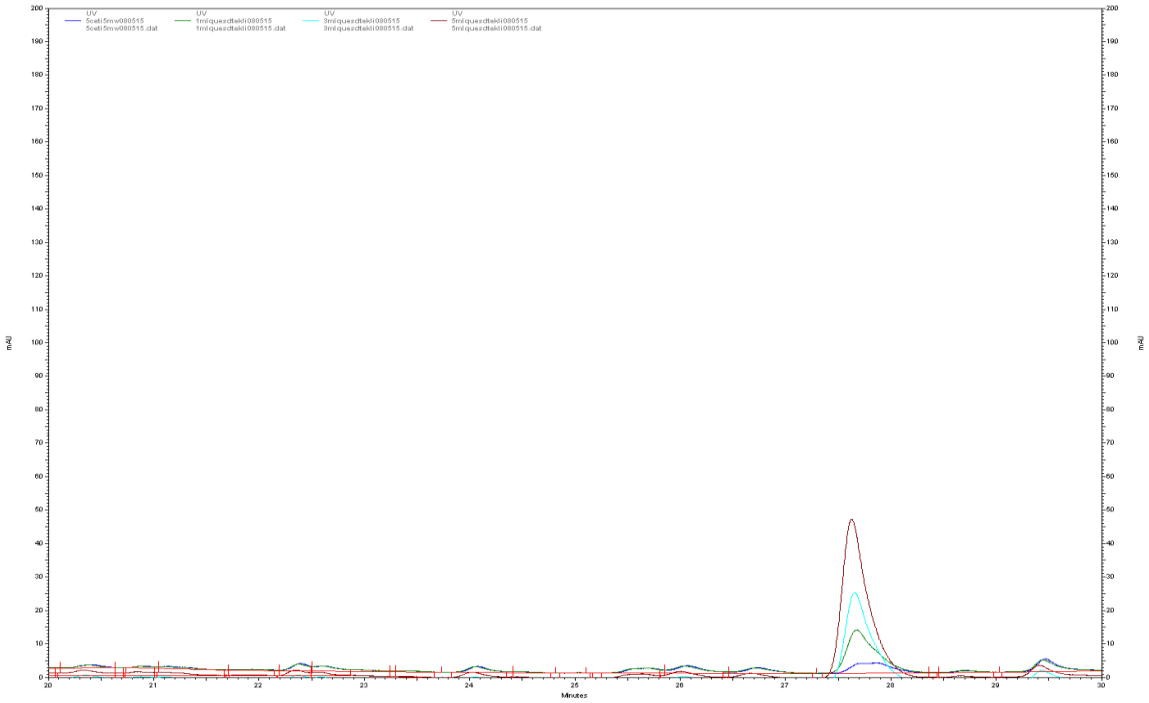


**Figure 3. 5:**Quarcetin standard addition graphic.

For quercetin standard addition method, standard solutions were prepared according to ratio: 5 mL *P.Farcta* extract + x mL quercetin + (5-x) mL% 80 methanol solution. Quercetin stock solution was $1\times10^{-4}$ M. Different concentration of quercetin used for getting equation, written in Figure 3.6, for calculation of quercetin amounts in *P.farcta* extracts.

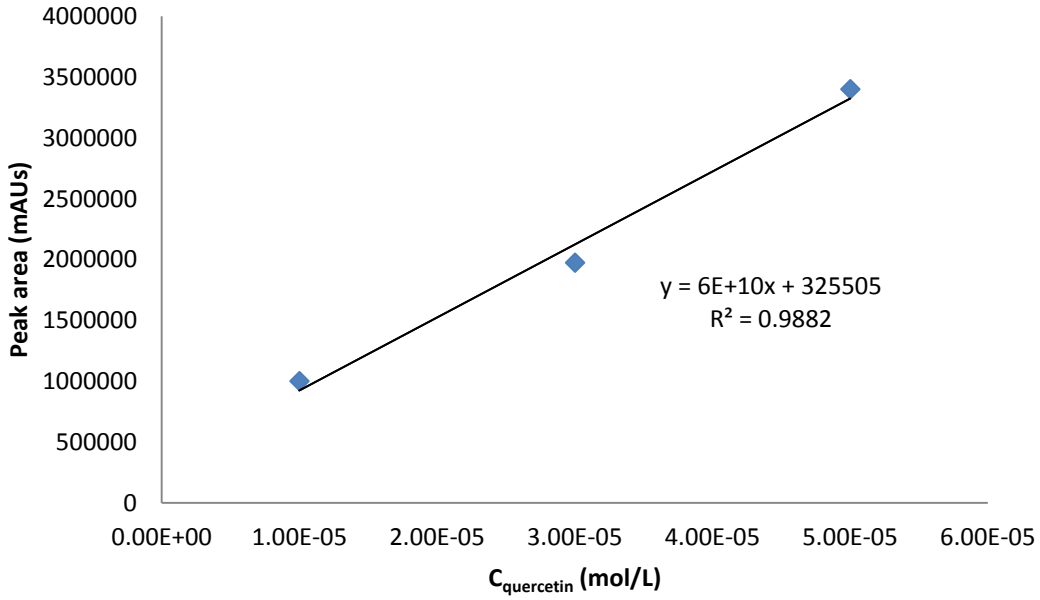



**Figure 3. 6:** Linear graphic of quarcetin peak area versus molarity.

For cafeic acid and gallic acid standard addition method, standard solutions were prepared according to ratio: 5 mL *P.Farcta* extract + x mL cafeic acid+ x mL gallic acid +(5-2x) mL 80% methanol solution. With diifferent concentration of standard solutions, linear calibration graphics draw. Help of the equation that showed in Figure 3.7 and 3.8, cafeic acid and gallic acid amounts were calculated. Cafeic acid and gallic acid stock solutions were $1\times10^{-3}$ M.

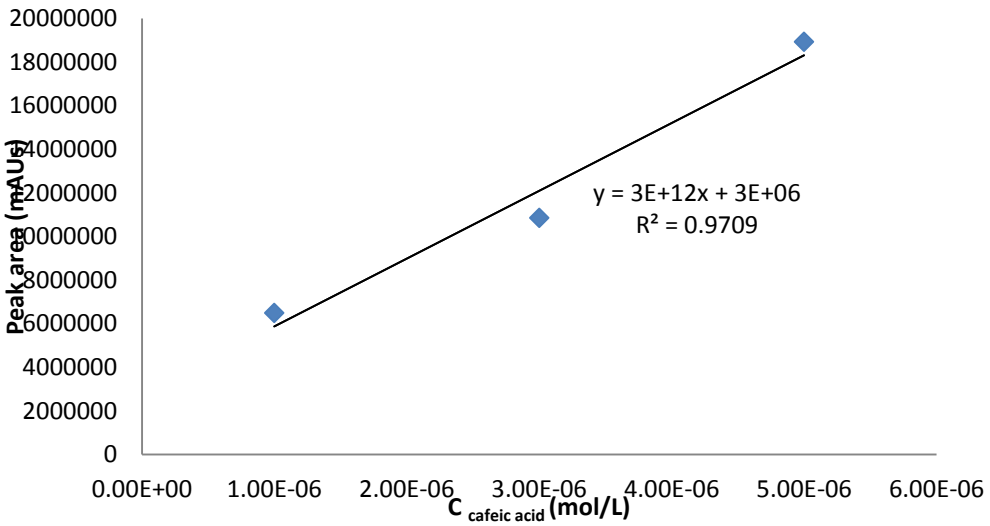



**Figure 3. 7:** Linear graphic of cafeic acid peak area versus molarity.

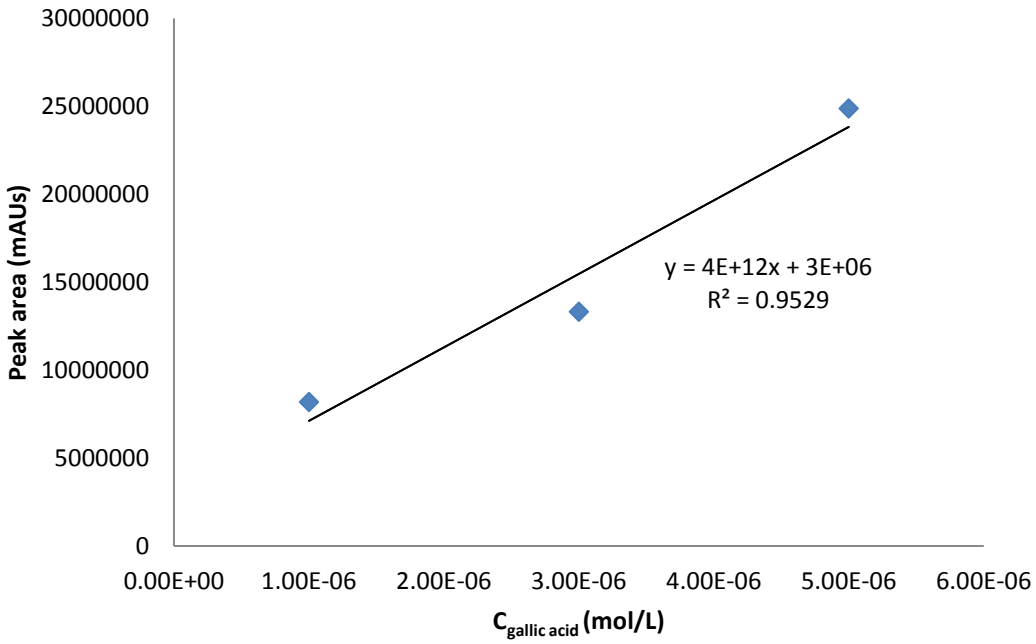



**Figure 3. 8:** Linear graphic of gallic acid peak area versus molarity.

### 3.10.2 Quantitative analyse by HPLC

According to calibration graphics of flavonoid standards, quantitatve analyse of *P.Farcta* was examined by HPLC. Amounts of quarcetin, gallic acid and cafeic acid was calculated via linear equilibrium and written in Table 3.7.

**Table 3. 7:**Amounts of quarcetin, cafeic acid and gallic acid in *P.Farcta* extract.

| **Name** | **Amount(mmol/g)** |
|---|---|
| Quercetin | $9{,}36\times10^{-05}$ |
| Cafeic acid | $2{,}12\times10^{-05}$ |
| Gallic acid | $5{,}8\times10^{-07}$ |

# 4. CONCLUSIONS AND RECOMMENDATIONS

The aim of this study is clarify the antioxidant capacity and flavonoid diversity of *Prosopis farcta* beans which shows important pharmaceutical effects and have traditional usage. The results from analyses performed on infusion and methanol extract solution of *P. farcta* beans presented and discussed in this chapter. For antioxidant capacity assays Modified-CERAC, Modified-Folin, CUPRAC methods applied on extract solutions. Flavonoid investigation identified by HPLC.

## 4.1 Total antioxidant capacity of *P.farcta*

In this study for antioxidant capacity of *P.Farcta* beans analysed by Modified-CERAC, Modified-Folin, CUPRAC methods. These three different methods applied both on %80 methanol extract and infusion solutions of *P.Farcta* beans. Beans were collected from Şanlıurfa area of Turkey. The reason for application of three different methods is different working principle between methods and comparing the calculations each other. For example; while Modified- CERAC assay oxidize the antioxidant compounds selectively, CUPRAC assay measure hydrophilic and lipophilic antioxidant capacity.

For given the results of antioxidant capacity as quercetin equivalent, molar absortiviy of different antioxidant assays were calculated through calibration graphics. Calibration graphics drawn over measurement of the quercetin standards solution absorbance value.

*P.Farcta* beans have traditional usage as tea, because of this usage assays applied on not only %80 methanol extract solution but also infusion solution. Total antioxidant capacity shows different value methods to methods. This difference can be occured by different pH conditions of assays. On the other hand, the difference of reactives which used in methods can be reason the variation of results.

According to results, showed in Table 4.1, infusion solution show have antioxidant activity than methanol solution. On the other hand, highest antioxidant capacity for infusion solution measured by CUPRAC which can explained by lower redox

potention of CUPRAC reagent and measurement both hyrophilic and lipophilic antioxidant compounds. For % 80 methanol solution the highest antioxidant capacity measured by Modified-FOLIN methods. This can be explained by pH conditions of Modified- Folin method. Modified-FOLIN methods works on pH 10 value that is higher than others methods.

**Table 4. 1:** Total antioxidant capacity of both infusion solution and methanol extracsolution of *P.Farcta.*

| SAMPLE | MODIFIED CERAC (mmol QR /g) | CUPRAC (mmol QR /g) | MODIFIED FOLIN (mmol QR /g) |
|---|---|---|---|
| **%80 Methnanol solution** | $3,8\times10^{-4}$ | $0,10\times10^{-4}$ | $1,56\times10^{-3}$ |
| **Infusion solution** | $3,4\times10^{-1}$ | $1,0\times10^{-1}$ | $7,2\times10^{-2}$ |

### 4.2 Flavonoid identification of *P.Farcta*

In this study, flavonoid diversity of *P.farcta* studied by HPLC. HPLC methods parametres showed in Table 3.1. According to parametres %80 methanol extract of *P.Farcta* analysed after hydrolisation. Table 4.2 shows retention time of componds.

**Table 4. 2**: Retention time of standard solutions.

| Name | $R_T$ (minutes) |
|---|---|
| Quarcetin | 27,53 |
| Gallic acid | 8,28 |
| Cafeic acid | 15,6 |
| Main peak | 9,1 |

According to chromatogram (Figure 4.1) four different peak found in *P.farcta* beans. For quantitative analyse, standard addition methods applied. Harzallah-Skhiri and Ben Jannet studied flavonoid diversity of *P.Farcta* populated in Tunisia. Based on their assay, standards were choosen.

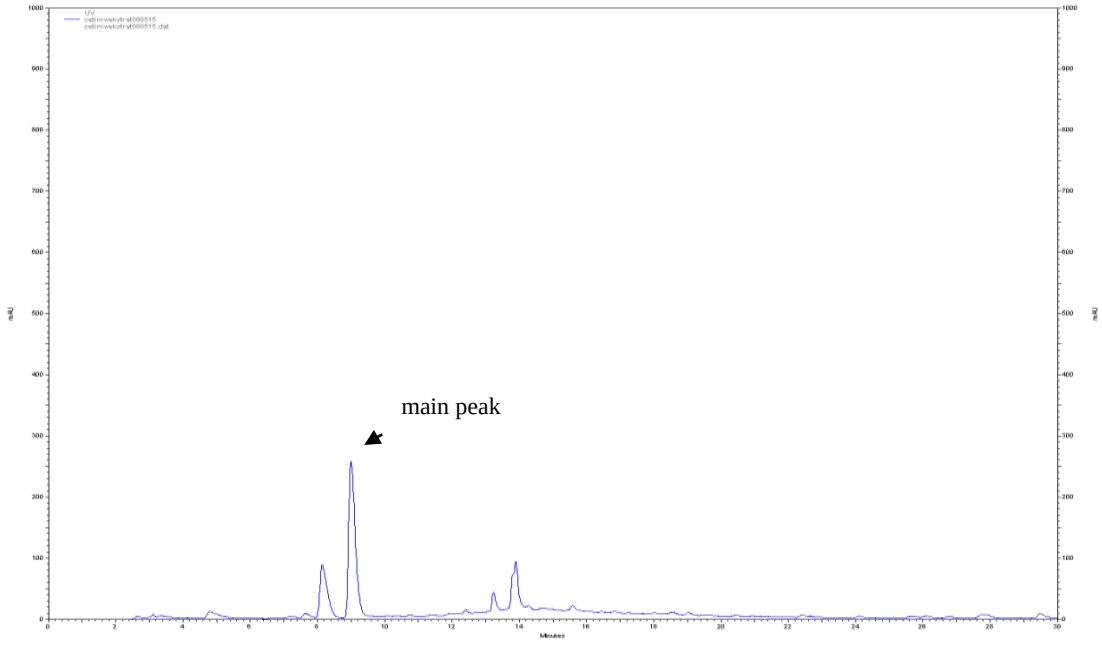



**Figure 4. 1:** HPLC chromatogram of *P.Farcta.*

Quercetin, gallic acid and cafeic acid peaks match with *P.farcta* beans. Acoording to stanndard solution chromatogram, which Figure 4.2, retention time os standards founded. Their retention time listed in Table 4.2.

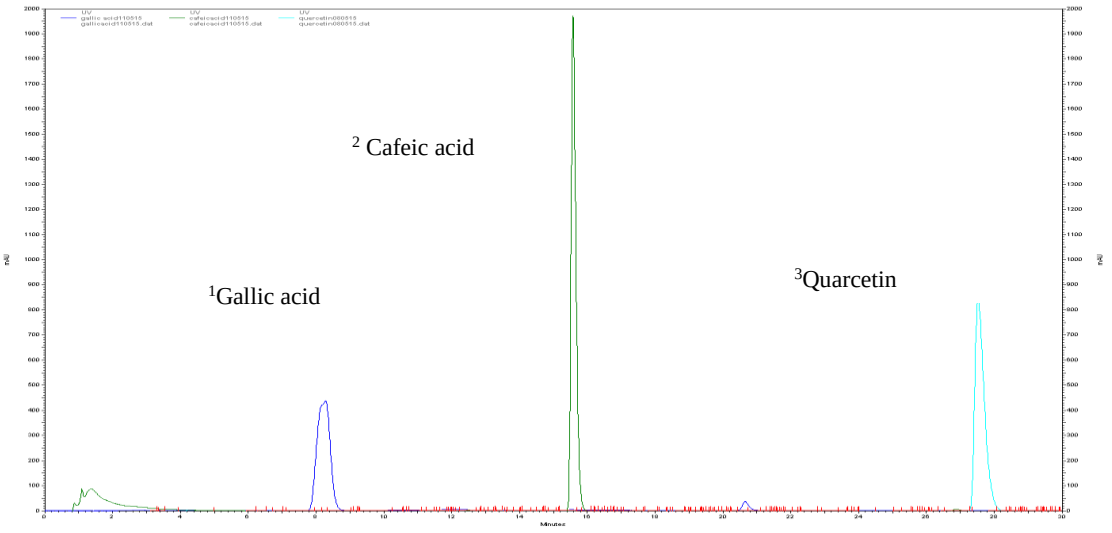



**Figure 4. 2:** HPLC chromatogram of standards.

Acorrding to calculation of flavonoids amount, written in Table 4.3, grading occured as quarcetin ›cafeic acid › gallic acid. Main peaks of *P.Farcta* beans can not identificate at this study.On the other hand, results showed that flavonoid diversity differ from Tunisia populated *P.Farcta*. Harzallah-Skhiri and Ben Jannet stated that major component of bean is cafeic acid and derivatives, in this study cafeic acid shows lower percentage than quarcetin. Considering there has no published report of *P.Farcta* grown in Turkey, main peaks must be investigate in future studies.

**Table 4.3:** Amounts of quercetin, gallic acid and cafeic acid in *P.Farcta.*

| **Name** | **Amount(mmol/g)** |
|---|---|
| Quercetin | $9{,}36\times10^{-05}$ |
| Cafeic acid | $2{,}12\times10^{-05}$ |
| Gallic acid | $5{,}8\times10^{-07}$ |

## REFERENCES


**[1] Laitonjam, W., S**.(2012). Natural antioxidants of plants acting as scavengers of free radicals, Natural Products Chemistry, 259-275.

**[2] Williams, CA., Coupland, JN.,** (1999). Lipid oxidation in food emulsions, Trends in Food Science & Technology, 83-91.

**[3] Perez, MJ.,Cuello, AS. Etc.**, (2014). Polyphenolic compounds and anthocyanin content of Prosopis nigra and Prosopis alba pods flour and their antioxidant and anti-inflammatory capacities, Food Research Internatıonal, 762-771.

**[4] Harzallah-Skhiri, F., Jannet, BH.,** (2005). Flavonoids Diversification in Organs of Two Prosopis Farcta (Banks & Sol.) Eig. (Leguminosea, Mimosoideae) Populations Occurring in the Northeast and the Southeast of Tunisia, Journal of Applied Sciences Research 1(2),130-136.

**[5] Halliwell, B.,** (2005). Free radicals and other reactive species in disease, wiley online library.

**[6] Bayır, H.,** (2005). Reactive oxygen species, Critical Care Medicine, 33,498-501.

**[7] Martínez, MC., Andriantsitohaina R., (**2009). Reactive nitrogen species: molecular mechanisms and potential significance in health and disease, Antioxid Redox Signal,3, 669-702.

**[8] Maged, Y and Marquardt, HS and Schafer, G and MaClellan, RO and Welsch, F,** (1999). Free radicals and reactive oxygen species,111-125.

**[9] Turkmen , N., Sari, F., Velioglu, S.,** (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected gren vegetables, Food Chemistry 93, 713–718.

[10] **Augustyniak A, Bartosz G, Cipak A, Duburs G, Horáková L, Luczaj W, Majekova M, Odysseos AD, Rackova L, Skrzydlewska E, Stefek M, Strosová M, Tirzitis G,Venskutonis PR, Viskupicova J, Vraka PS, Zarković N.,** (2010). Natural and synthetic antioxidants: an updated overview, Free Radical Research, 44(10),1216-62.

[11] **Macadam W.J,** (2008).Structure and Function of Plants, Wiley-Blackwell Publications.

[12] **Gupta V.K, Sharma S.K,** (2006). Plants as natural antioxidants, Natural Product Radiance 5, 326-334.

[13] **Hudson, B. J. (**1990). Food Antioksidants, USA, Elsevier Science.

[14] **Aust, O., Sies, H., Stahl, W., & Polidori, M. C.,**(2001). Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and carotenoids, Journal of Chromatography A, 936, 83-93

[15] **Croteau R., Kutchan T.M., Lewis N.G.,** (2000). Natural Products, Biochemistry & Molecular Biology of Plants, B. Buchanan, W. Gruissem, R. Jones, Eds.,1250-1316.

[16] **Belz G.G.,Breithaupt-Grögler K., Osowski U.,** (2001). Treatment of congestive heart failure – current status of use of digitoxin, European Journal of Clinical Investigation 31, Issue S2, 10–17.

[17] **Grotewold, E.,** (2006). The science of flavonoids, USA.

[18] **Kim J., Kim C.K., Moon M.K.,Kim J.S.,** (2005). Epicatechin breaks preformed glycated serum albumin and reverses the retinal accumulation of advanced glycation end products, European Journal of Pharmacology 748, 108-114.

[19] **BalasundramN., SundramK., SammanS.,** (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, Food Chemistry 99, 191–203.

[20] **Aykaç İ.,** (2014). Prosopis farcta.

[21**] Akkuzu G.,** (2014).Çeti'nin kimyasal çimlenme biyolojisiüzerine etkisi.

[22**] Asadollahi A, Sarir H,Omidi A, Torbati MB,** (2014).Hepatoprotective Potential of Prosopis farcta Beans Extracts against Acetaminophen-induced Hepatotoxicity in Wister Rats,Int J Preventine Medicine(10), 1281–1285.

[23**] Omidi A, Ansari nik H, Ghazaghi M.,** (2013). Prosopis farcta beans increase HDL cholesterol and decrease LDL cholesterol in ostriches (Struthio camelus), Tropical Animal Health and Production(45), 431-434.

[24] **Harzallah-Skhiri, F and Jannet, H Ben,** (2005). Flavonoids diversification in organs of two prosopis farcta (Banks \& Sol.) Eig.(Leguminosea, Mimosoideae) populations occurring in the Northeast and the Southeast of Tunisia, Journal of Applied Science Research (2), 130-136.

[25**] Karadag, A.,Ozcelik, B.,and Saner, S.,** (2009). Review of methods to determine antioxidant capacities,Food Analytical Methods(2), 41-60.

[26**] Huang, D., Ou, B., & Prior, R. L.** (2005).The chemistry behind antioxidant capacity assays, Journal of Agricultral and Food Chemistry, 53, 1841–1856.

[27**] Ozyurt, D., Demirata, B., & Apak, R.** (2007). Determination of total antioxidant capacity by a new spectrophotometric method based on Ce(IV) reducing capacity measurement, Talanta, 71, 1155–1165.

[28] **Ozyurt, D., Demirata, B., & Apak, R.** (2010). Modified cerium(IV)-based antioxidant capacity (CERAC) assay with selectivity over citric acid and simple sugars, Journal of Food Composition and Analysis, 23, 282–288.

[29] **Apak, R., Güçlü, K., Özyürek, M., & Çelik, S. E.,** (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity assay, Microchimica Acta, 160, 413-419.

[30]**Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S. E., Bektaşoğlu, B., Berker, K. I., Özyurt, D.,** (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay, Molecules, 12, 1496–1547.

[31] **Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R. M..** (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol, 299, 6287-6293.

[32] **Berker, K. I., Ozdemir Olgun, F. A., Ozyurt, D., Demirata, B., Apak, R.** (2013). Modified Folin−Ciocalteu antioxidant capacity assay for measuring lipophilic antioxidants, Journal of Agricultural and Food Chemistry, 61, 4783-4791.

**CURRICULUM VITAE**

**Name Surname:** İLKİN AYDIN

**Place and Date of Birth:** 16.11.1989

**E-Mail:** ilkinaydinitu@gmail.com

**EDUCATION**

**B.Sc.:** İTÜ-CHEMISTRY DEPARTMENT- 2013

**PROFESSIONAL EXPERIENCE AND REWARDS:**

**2015-** Bioarge Bitkisel Kozmetik LTD. STİ-R&D SPECIALIST

**2014-2015-**Sarıyer Kız Meslek Lisesi- PART TIME ENGLISH TEACHER

**2013-2015-**İTÜ CHEMISTRY- PART TIME LABORATORY ASSISTANT

**PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:**

**2014-** ANTIOXIDANT WORKHOP-POSTER SESSION

**2014-** ULUSAL BOTANİK KONGRESİ- POSTER SESSION