T.R.

KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ANTIMICROBIAL, ANTIOXIDANT AND PHYTOCHEMICALS ANALYSES OF *Glycyrrhiza glabra* L. COLLECTED FROM NORTHERN OF IRAQ AND TURKEY

SORAN KAYFI NAJMALDIN

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ-TURKEY 2015

T.R.

KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ANTIMICROBIAL, ANTIOXIDANT AND PHYTOCHEMICALS ANALYSES OF *Glycyrrhiza glabra* L. COLLECTED FROM NORTHERN OF IRAQ AND TURKEY

SORAN KAYFI NAJMALDIN

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ-TURKEY 2015

M.Sc. thesis entitled "Antimicrobial, Antioxidant and Phytochemicals Analyses of *Glycyrrhiza glabra* L. Collected from Northern of Iraq and Turkey" by Soran Kaify NAJMALDIN, who is a student at the Department of Bioengineering and Sciences, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, was certified by all the majority jury members, whose signatures are given below at the date of 10.06.2015.

Prof. Dr. M. Hakkı ALMA (Supervisor) ……...…….
Department of Bioengineering and Sciences
Kahramanmaraş Sütçü İmam University

Prof. Dr. Emin ÖZKÖSE (Member) ……...…….
Department of Animal Science
Kahramanmaraş Sütçü İmam University

Assoc. Prof. Dr. Kürşat Oğuz YAYKAŞLI (Member) ……...…….
Department of Medical Biology
Kahramanmaraş Sütçü İmam University

I approve that the above signatures related to the members

Assoc.Prof. Dr. Mustafa ŞEKKELİ ……...…….
Director of Graduate School of Natural and Applied Sciences
Kahramanmaraş Sütçü İmam University

DECLARATION

I declare and guarantee that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. Based on these rules and conduct, I have fully cited and referenced all the material and results that are not orignal to this work.

Soran Kayfi NAJMALDIN

Note: Uses of the reports in this thesis, from original and other sources, tables, figures and photographs without citation, subject to the provisions of Law No. 5846 of Intellectual and Artistic Works.

This study supported by: Kahramanmaraş Sütçü İmam University.
Project Number: 2014/2-55 YLS

KUZEY IRAK AND TÜRKİYE'DEN ALINMIŞ MEYAN KÖKÜ'NÜN ANTİ-MİKROBİYAL, ANTİ-OKSİDAN ve FİTO-KİMYASAL ANALİZİ

TURKEY
(YÜKSE LİSANS TEZİ)

Soran Kayfi NAJMALDİN

ÖZET

Bu çalışmanın amacı, Irak (Erbil, Shaqlawa bölgesi, ve Süleymaniye, Dokan bölgesi,) ve Türkiye'den (Şırnak ve Ağrı) alınmış meyan kökünün (kayıngiller) serbest radikal süpürme (DPPH) and anti-mikrobiyal aktivitelerini farklı ekstraksiyon yöntemleri kullanarak bulmaktır. Ayrıca, bitkinin fito-kimyasalları LC-MS/MS yöntemiyle analiz edilmiş ve toplam ürün ekstratları ile toplam yoğunlaşmış tanenler de değerlendirilmiştir. Ekstraksiyon için hızlandırılmış çözücü ekstraksiyonu (HÇE), mikrodalga destekli ektraksiyon (MDE) ve geleneksek ekstraksiyon (GE) olmak üzere üç farklı yöntem ve üç farklı çözücü (metanol, etanol ve su) kullanılmıştır. Ekstraksiyonlarda yöntem, çözücü ve bölgelere bağlı olarak farklı verimlilik yüzdeleri elde edilmiş olup tüm bölgeler için en yüksek verimlilik yüzdesine metanolle yapılan hızlandırılmış çözücü ekstraksiyonunda ulaşılmıştır. Erbil'den alınan meyan kökü ise %22,32 ile en yüksek ürün verimlilik yüzdesine ulaşmıştır. Aynı zamanda yine bu bölgede en yüksek miktarda yoğunlaşmış tanen 20,83 mg/L görülmüştür. Anti-mikrobiyal aktivite bakımından ise disk difüzyon yöntemiyle dört Gram-pozitif, dört Gram-negatif ve üç fungus bulunmuştur. Kullanılan farklı yöntemlerde farklı derecelerde inhibisyon bölgeleri görülmüştür. Türkiye ve Irak'tan alınan meyan kökü, su ektraksiyonunda anti-bakteriyel aktivite göstermeyen Türkiye'den alınmış meyan kökü dışında, iyi derecede anti-bakteriyel aktivite göstermiştir. Meyan kökü ekstraksiyonları, *Candida albicans* mantarına karşı iyi derecede anti-fungal aktivite gösterirken *Yarrowia lipolytica* ve *Candida utilis* karşısında ise neredeyse hiç etki göstermemiştir. Meyan kökü yine tüm bölgeler için iyi derecede anti-oksidan aktivite göstermiştir. En yüksek DPPH süpürme aktivite değeri, HÇE yöntemiyle Irak'tan alınan meyan kökünde %98,57 oranında görülürken, aynı konsantrasyonla en düşük değer geleneksel etanol ektraksiyon yöntemiyle Türkiye'den (Şırnak) alınan meyan kökünde %86,60 oranında görülmüştür. Çalışmada kullanılan bütün ekstratların DPPH süpürme güc BHT'ye göre daha yüksektir.

Key words: Meyan kökü, Antimikrobiyal, Antioksidan, bitki fito-kimyasalları

Kahramanmaraş Sütçü İmam Üniversitesi

Fen Bilimleri Enstitüsü

Biyomühendislik ve Bilimleri Bölümü, Haziran/2015

Danışman: Prof. Dr. M. Hakkı ALMA

Sayfa sayısı: 94

ANTIMICROBIAL, ANTIOXIDANT AND PHYTOCHEMICALS ANALYSES OF *Glcyrrhiza glabra L.* COLLECTED FROM NORTHERN OF IRAQ AND TURKEY

(M.Sc. THESIS)

Soran Kayfi NAJMALDIN

ABSTRACT

The present study was undertaken to find out the (DPPH) free radical scavenging and antimicrobial activities of *Glycyrrhiza glabra* L. which were collected from Northern of Iraq (Erbil, Shaqlawa locality, and Sulaymaniyah, Dokan locality,) and Turkey (Şırnak and Ağrı) by applying different extraction techiniques. Furthermore, analyses of phyto-chemicals of plant were done by using LC-MS/MS and also total yield extracs and total condensed tannin were also evaluated. Three methods were employed for extraction; accel-erated solvent extraction (ASE), microwave assisted extraction (MAE) and conventional extraction (CE) methods along with three solvents including methanol, ethanol and water. All the extractions showed different yield percentages in the case of different locations and methods, the highest yield percentages for the the all locations were detected for the accel-erated solvent extraction with methanol. Furthermore, the liquorice collected from Erbil exhibited the highest yield percentage of 22.32% at the same time, the highest amount of condensed tannin was detected for the same location of 20.83 mg/L. Antimicrobial activity of *Glycyrrhiza glabra* L. against four Gram-positive, four Gram-negative and three fungi were evaluated based on disc diffusion assay, different degrees of inhibition zones were noticed incase of different methods of study. In case of antibacterial screening, both Turk-ish and Iraqian liquorice showed notable antibacterial activity against tested bacteria except for water extraction for Turkish one which didn't exhibit good antimicrobial activity. The liquorice extracts showed good antifungal activity as well against *candida albicans* while showed almost no effect against both *Yarrowia lipolytica* and *Candida utilis*. Moreover, *Glycyrrhiza glabra* L. also exhibited good antioxidant activity for all the locations, DPPH free radical scavenging power of the whole exracts studied here were higher than that of BHT, as well.

Key words: *Glycyrrhiza glabra* L., Antimicrobial, Antioxidant, Plant phytochemicals

Kahramanmaraş Sütçü İmam University

Graduate School of Natural and Applied Sciences

Department of Bioengineering and Sciences, June/ 2015

Supervisor: Prof. Dr. M.Hakkı ALMA

Number of pages: 94

ACKNOWLEDGMENT

Firstly, I thank God for affording me the opportunities that I have received. It is through your grace that I have come this far in this incredible journey.

A great appreciation goes thank to my supervisor, Assoc. Prof. Dr. Prof. Dr. M. Hakkı ALMA (Supervisor) who was always there for me to listen and give advice. We had many insightful conversations during the development of ideas. He is the person who inspired me for this work and showed me different ways to approach a problem and how to deal with it in a fruitful manner.

I would like to express my special thanks to Prof. Dr. Metin DIĞRAK at the Department of Biology, at Kahramanmaras Sutcu Imam University for valuable advises and helping me during antimicrobial test.

I would like to express my thank and gratitude to all the staff of Department of the Forest Industry Engineering, at Kahramanmaras Sutcu Imam University, especially Mr. Eyyüp KARAOĞUL, Mr. Ali GÖÇERI and Mr. Tufan SALAN for their advices and teaching me the laboratory analysis.

Special appreciation is also to Mrs. H.Gülşah ÖZKAN and Nahide BEŞİKCİ. Thanks you for always helping and assisting me throughout my study and sharing your expertise whether in the lab. I also thank my classmates Maaroof ABDALRAHMAN and Shno ABDALQADIR for helping me whether in the lab, or in real life for the great support and kind help during the period of study.

A warm thanks to my colleagues Mr.Badr Surchi and Mr.Ali Galali for their advice, encouragement and support throughout the duration of the research.

Finally, I want to express my special thanks to my lovely mother and father for their encouragements, I am forever indebted to you and I wish to extend my final thanks to my brothers and sisters whom have been my pillar and a very great source of encouragement throughout this study, and also my life.

LIST OF CONTENTS

Page No

LIST OF FIGURES

Page No

LIST OF TABLES

Page No

LIST OF ABBREVIATIONS

WHO:	World Health Organization
CTX-M:	Cefotaxime hydrolyzing capabilities
UTI:	Urinary Tract Infection
DNA:	Deoxyribonucleic acid
RNA:	Ribonucleic acid
ROS:	Reactive Oxygen Species
DGL:	Deglycyrrhizinated licorice
LDL:	Low-density lipoprotein
SARS:	Severe Acute Respiratory Syndrome
GA:	Glycyrrhizic acid
MIC:	Minimum Inhibitory Concentration
BHT:	Butylated hydroxytoluene
DPPH:	1,1-diphenyl-2 picrylhydrazyl
HPLC:	High Performance Liquid Chromatography
LC:	Liquid Chromatography
MS:	Mass Spectrometry
CE:	Conventional Extraction
MAE:	Microwave Assisted Extraction
ASE:	Accelerated Solvent Extraction
ATCC:	American Type Culture Collection
G–ve:	Gram-negative bacteria
G+ve:	Gram-posative bacteria
ESBLs:	Extended-spectrum beta-lactamases
FRAP:	Ferric reducing antioxidant power
MHA:	Mueller-Hinton agar
SDA:	Sabouraud dextrose agar
DCM:	Dichloromethane

1. **INTRODUCTION**

Plants as a source of medicinal compounds have continued to play a leading role in the maintenance of human health since early times. According to the WHO Plant extracts or their active ingredients are used as folk medicine in tradtional therapies of 80% of the world's population. Over 50% of all the modern clinical drugs are of natural product origin (Kirbağ *et al*., 2009).

Plants used for traditional medicine contain a wide range of substances that can be used totreat chronic as well as infectious diseases. The substances that can inhibit the growth of micro-organisms or kill them are considered candidates for developing new drugs for treatment of various infectious diseases. The use of medicinal plants as a traditional medicine is well-known in the rural areas of many developing countries (Catalano *et al*., 1998; Martinez *et al*., 1996; Sundar, 1996; Taylor *et al*., 1996).

Herbal medicine is still the mainstay of about 75 - 80% of the world population, mostly in the developing countries, for primary health care (Kamboj, 2000). This is mainly because of the general belief that herbal drugs are without any side effects besides being cheap and locally available (Gupta and Raina, 1998).

Therefore, people are increasingly interested in alternative medicines, including herbal medicine, as they perceive these forms of healing as being both safe and effective. This trend in use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants (Balunas and Kinghorn, 2005).

In the last two decades, the community attention has been concentrated on medicinal and aromatic plants as they are a good candidate to be used as natural sources in pharmaceutical, food and cosmetic industries all around the world. The center of attention arises as the bioactive compounds present in those medicinal plants used as botanical drugs, functional foods and additives, dietary supplements and antimicrobial food packing materials, etc. Nowadays, many of the bioactive plant metabolites, which has been used as a cure for various diseases like hypertension, cancer, cold flue, eczema and cholesterol for centuries, has been identified and isolated to be used in ethnopharmacy (Aziz *et al*., 2003; Littleton *et al*., 2005).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952).

Plants are the richest sources of bioactive natural compounds. They have a wide diversity of metabolites in their seeds, barks, roots, leaves or fruits. The universal role of plants in the treatment of disease and health maintenance is demonstrated by their employment in all the major systems of medicine. Valuable knowledge concerning the medicinal and other properties of plants is propagated from generation to generation by tribal societies. Modern isolation techniques have made it possible for new plant drugs to find their way into medicine as purified substances rather than in the form of galenical preparations. The use of single pure compounds, including synthetic drugs, is not without its limitations, and in recent years there has been an immense revival in interest in the herbal system of medicine, which depends on plant sources (Evans, 2002).

Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey and Chowdhry, 2006). The use of both plant extracts and phytochemicals, with antimicrobial properties, can be of great significance in therapeutic actions. In the last few years, a number of studies have been conducted in different countries to prove such effectiveness. Many plants have been used because of their antimicrobial behaviors, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al*., 2000).

Early use of antimicrobial plant extracts (i.e. herbs and oils) was well documented in ancient Egypt. Plant extracts used in ancient Egyptians for for embalming the dead as well as preservation of food. Hippocrates,Virgil, and Pliny mentioned garlic as a treatment for a variety of illnesses such as indigestion, wounds, pneumonia, and infections. Although ancient civilizations recognized the antiseptic or antimicrobial potential of many plant extracts, it was only in the eighteenth century that the scientific documentation of the preservative effects of spices and spice-type extracts were developed or recorded (Conner, 1993).

The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Intensive care physicians consider antibiotic-resistant bacteria

a significant or major problem in the treatment of patients (Lepape *et al*., 2009). Increasing bacterial resistance is prompting a resurgence in research of the antimicrobial role of herbs against resistant strains (Alviano and Alviano, 2009; Hemaiswarya *et al*., 2008).

Multiple microbial resistances are growing problem and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, it is necessary to take measures to reduce microbial resistance and to explore alternative antimicrobial sources (Nascimento *et al*., 2000).

Because of the side effects and resistance that pathogenic micro-organisms build against the antibiotics, much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants may provide a natural and new source of antibacterial agents for use (Uzun *et al*., 2004).

For example *E. coli* is showing increased resistance to different antibiotics like amoxicillin and trimethoprim (Manges *et al*., 2001; Goettsch *et al*., 2000). Hence, searching for alternative and effective medicines from plants against such resistant bacteria has become an important concern all over the world (Kafaru, 1994).

The fabaceae or leguminosae commonly known as the legume, pea, or bean family, are a large and economically important family of flowering plants. The family includes shrubs, trees and herbaceous plants perennials or annuals, which are simply recognized by their fruits (legume) and their compound, stipulated leaves. The group is broadly distributed and is considered as the third-largest land plant family in terms of number of species, behind only the orchidaceae and asteraceae, with 730 genera and over 19,400 species (Judd *et al*., 2002).

The largest genera are Astragalus (over 2,400 species), Acacia (over 950 species), Indigofera (around 700 species), Crotalaria (around 700 species), and Mimosa (around 500 species), which contain around (9.4%) of all flowering plant species (Magalion and Sanderson, 2001). The Fabaceae is the most common family found in tropical rainforests and in dry forests in the Americans and Africa (Burnham *et al*., 2004).

Licorice (*Glycyrrhiza glabra* L.) is belonging to family Papilionaceae/Fabaceae is a traditional medicinal herb grows in the various parts of the world. It is a very sweet, moist, herb that detoxifies and protects the liver and is also a powerful anti-inflammatory finds applications in arthritis and mouth ulcers. Licorice is a hardy herb or under shrub, erect

grows to about 2 m height. The roots are long, cylindrical, thick and multibranched (Wealth of India, 1985).

The objective of this study was to determine the followings:

1. Evaluation of antibacterial and antifungal activeties of Turkish and Iraqian *Glycyrrhiza glabra* L.
2. Determination of (DPPH) free radical scavenging activity.
3. Estimation of antioxidant activity of plant extraction.
4. Find out the effect of different extraction methods and solvenst on antimicrobial activity.
5. Evaluation of total tannin content inside the plant.
6. Separation and identification of chemical ingredients by using (LC-MS/MS).

2. LITERATURE REVIEW

2.1. Antimicrobial Resistance

Webster's Third International Dictionary (1981) defines an antibiotic as ''a substance produced by a microorganism (as a bacterium or a fungus) and in dilute solution having the capacity to inhibit the growth of or kill another microorganism (such as a disease germ)''. Brock's well-known textbook of microbiology (Madigan *et al*., 2008). While, the term of an antimicrobial agent is a term that refers to a group of drugs that contains antibiotics, antifungals, antiprotozoals, and antivirals. Antimicrobial resistance is an immense and serious global challenge and could risk the lives of future generations. The phenomenon of antibiotic resistance was predicted by Alexander Fleming, since the discovery of penicillin in 1940s (Levy, 2002). Ironically, the misuse of antibiotics by Human, the employment of antibiotics in veterinary practices and the growing presence of antibiotics in water, soil and food are contribute to the problem of antibiotic resistance (Moshirfar *et al*., 2006).

Multidrug-resistant Enterobacteriaceae, mostly *Escherichia coli*, produces extended-spectrum β-lactamases (ESBLs) such as the CTX-M enzymes. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates such as ceftazidime, ceftriaxone, or cefepime have emerged within the community setting as an important cause of urinary tract infections (UTIs). Recent reports have also described ESBL-producing *Escherichia coli* as a cause of bloodstream infections associated with these community-onsets of UTI (Darwish and Aburjai, 2010).

2.1.1. Mechanisms of antimicrobial resistance

Bacteria own various mechanismes to develop antimicrobial rsistanse including first active efflux of the anti-microbial out the cell second, changes in the permeability of the bacterial cell wall, third, mutation in the target site; fourth, modification of the anti-microbial agent; and final acquisition of alternative metabolic pathways to those inhibited by the antimicrobial agent (Mc Dermott *et al*. 2003).

2.2. Phytochemicals

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999).

Table 2.1. Mechanisms of antimicrobial resistance

Mechanisms of resistance	Antimicrobial(s) affected
1- Alteration or protection of the target site	Aminoglycosides, chloramphenicol, and ß-lactams.
2- Modification of the antimicrobial agent	Aminoglycosides, ß-lactams, macrolides, quinolones, rifampicin, trimethoprim, and tetracycline.
3- Decreased antibiotic accumulation Decreased uptake Increased efflux	Many antibiotics. Tetracycline, macrolides, quinolones, and chloramphenicol.
4- Alternation of metabolic pathway	Sulfonamides, trimethoprim.

Plants are rich in a wide variety of secondary metabolites, the great majority of which do not appear to participate directly in growth and development (Croteau *et al*., 2000).

The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (Shakeri *et al*., 2012).

2.2.1. Major classes of phytochemicals

In resent year phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Hahn, 1998). Literature survey indicated that phenolics are the most numerous and structurally diverse plant phytocontituents. Based on their biosynthetic origins, phytochemicals can be classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds. Interestingly, an important classification has been depicted

by (Liu, 2004). These groups have also several subgroups and these were demonstrated in Figure 2.1.

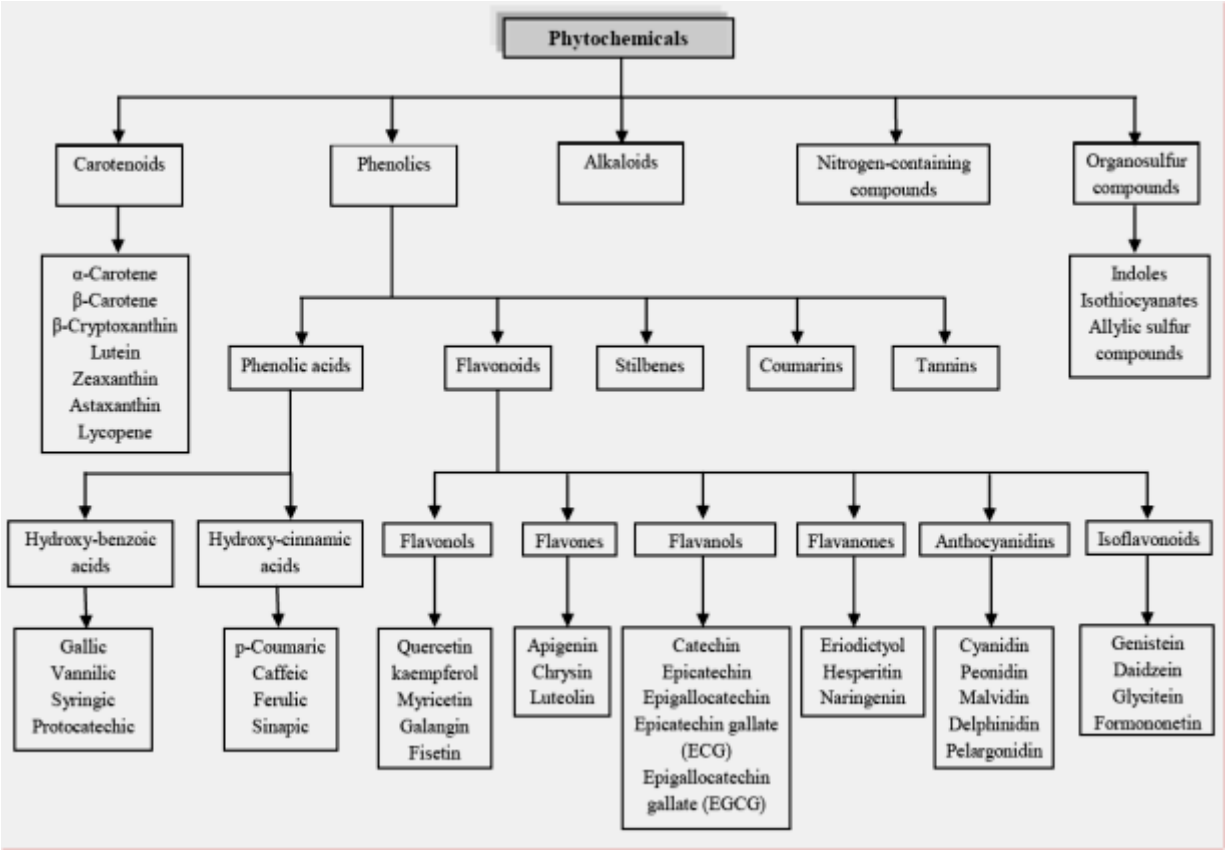

Figure 2.1. Classififcation of phytochemicals

2.2.2. Phytochemicals as antimicrobial agent

These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents. However, some medicinal herbs for some reasons have not found wider application and some times are referred as forgotten plants. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by micro-organisms has increased. Generally, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cheng *et al*., 2009).

The synthetic drugs have modest antimicrobial activity even they have been successfully designed and used as enzyme inhibitors, probably because of the complex structure of cell uptake of those drugs (Cushnie and lamb, 2005). Thus, scientist tended to look for more effective plant based antimicrobial sources as an alternative to synthetic ones and now it is estimated that more than 50% of the western drugs are plant derived, which have been once used in crude form in traditional or folk healing practices. Plant based drugs has

proven themselves as they serve relatively safer and more affordable treatments. Since this is the way it is, there has been an improved interest in natural products resulted from factors such as consumers displeasure from conventional medicines and boosted confidence in natural products as being superior. Changing law enforcements to supply structure-function claims, national concerns about health care cost or even changes in advertising have contributed to enhanced interest in natural products. Thus, it is fair to consider including antimicrobials into medicines is rewarding for both standpoints of drug development and phytomedicines (Ciocan and Bara, 2007).

2.2.2.1. Simple phenols and phenolic acids

Plant products are divided into two classes: phytoanticipins and phytoalexins. Molecules that are present in an inactive form (example: glucosides) belong to the first group; the second group is composed by molecules whose levels increase in response to microbial attack or is generated in response to a specific infection (Tegos *et al*., 2002). Phytoalexins are a large group of chemically diverse molecules, including simple-phenylpropanoids derivatives, alkaloids, glycosteroids, flavonoids, isoflavonoids, sulfur products, terpenes and polyketides (Hammerschmidt, 1999). The same molecule can be a phytoalexin or a phytoanticipin in different organs of the same plant. Examples of phytoanticipin are terprenoids, quinones and tannins (Abreu *et al*., 2012).

Simple phenols are the simplest bioactive phytochemicals contain a phenolic ring. The toxicity of phenols to micro-organisms was found to depend on the number of hydroxyl groups bound to phenol group since increased hydroxylation results in increased toxicity. Some other researchers have proposed that more highly oxidized phenols are inhibitorier. The toxicity to micro-organisms are bond to enzyme inhibition as a result of the reaction between oxidized compounds and sulfhydryl groups, or may it is just because of non - specific interactions of phenols with the proteins. Essential oils are phenolic compounds having a C_3 side chain at a lower level of oxidation and they are often referred as antimicrobial as well (Cowan, 1999).

2.2.2.2. Quinones

It has highly reactive characteristic which is unique in nature. They are composed of aromatic rings with two ketone substitutions. They are responsible from theprocess known as browning reaction seen in cut or injured fruits and vegetables. The antimicrobial property of quinines depends on their irreversible binding with nucleophilic amino acids in

proteins, which usually results in function loss of protei bound. Therefore, quinones are very popular as antimicrobial agents. Cellwall proteins, surface-exposed adhesins, and many membrane bound enzymes are targeted structures for quinones. Potential targets in the microbial cell are cell wall polypeptides, surface-exposed adhesins , and membrane-bound enzymes. Quinones may also deliver substrates unavailable to the micro-organism. (Ciocan and Bara, 2007).

2.2.2.3. Tannins

Tannins are water-soluble polyphenols that are commonly found in higher herbaceous and woody plants. Tannins have been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* (Scalbert, 1991).

Plant based tannin can be classified as hydrolyzable tannins, e.g Gallic acid and non- hydrolyzable tannins, e.g. flavones. Tanins may be formed from flavan derivatives located in woody tissues of plants or from quinine units by polymerization.Tanin is can binds to various organic compounds such as amino acids and proteins through hydrophobic effects or by hydrogen bonding or covalent bonding. So it is considered that the antimicrobial properties of tannin is due to their capability to inactivate microbial adhesins, transport proteins or enzymes. Besides their capability to form complexes with proteins, they were assigned to many other molecular activity in human such as host-mediated tumor activity, phagocytic cells stimulation or anti-infective actions (Cowan, 1999).

2.2.2.4. Flavonoids

One of the undoubted roles of flavonoids and related polyphenols in plants is protecting against microbial invasion (Harborne and Williams, 2000). Flavonoids are polyphenolic compounds containing 15 C atoms with 2 ring structure bound by a 3-C bridge. They are sub-classed into 6 groups as flavonols, flavan-3-ols, flavones, isoflavones, flavanones, and anthocyanidins according to different forms of central C-ring (Fraga, 2010).

Antibacterial property of flavonoids comes from the inhibitory effect of flavonoids on the RNA and DNA synthesis, which was proposed as intercalation or hydrogen bonding of the B ring of the flavonoids with the nucleic acid bases has an inhibitory action on nucleic acid formation. Furthermore, Another research claims that, flavonoids have an inhibitory effect on function of cytoplasmic membrane which was supported with evidence of reduced the fluidity of cell membranes when flavonoids are present.

Another mechanism of antimicrobial activity of flavonoids is associated with the inhibition of energy metabolism. The theory is that the licochalcones may be interfering with energy metabolism in a way that it affects the energy required for metabolite uptake or macromolecule biosynthesis (Cushnie and Lamb, 2005).

2.2.3. Mode of action of phytochemicals as antimicrobials

The traditional antibiotics have been recognized because they are able to kill bacteria or inhibit their growth, through inhibition of bacterial functions, including: DNA replication, RNA transcription, protein synthesis and cell wall synthesis, which are vital for cell growth (Clatworthy *et al*., 2007). It is necessary a more effective therapy to treat infections caused by antimicrobial resistant organisms, (Eliopoulos *et al*., 2003).

The antimicrobial susceptibility tests are divided into 3 principles: diffusion, dilution and diffusion and dilution. Kirby-Bauer, E Test, agar dilution, Stokes, microdilution and macrodilution are considered the most popular methods (Lalitha, 2004; Othman *et al*., 2011).The antimicrobial mode of action is associated with their ability to inactivate microbial adhesin, enzymes and cell envelope transport proteins (Ciocan and Bara, 2007).

Some experiments have been done to study the mode of action of several phytochemicals. The chemical structure and properties influence the site of action of phytochemicals. The mechanism of action of essential oils against bacteria involves membrane disruption through the lipophilic structure (Mendoza *et al*., 1997; Griffin *et al*., 1999).

Phenols act by interruption of energy production due to enzyme inhibition by the oxidized products, which react with sulfhydyl groups or non-specific interaction with proteins (Mason and Wasserman, 1987). In the case of flavonoids, they inhibit the synthesis of nucleic acids of Gram-negative and Gram-positive bacteria (Mori *et al*., 1987; Cushnie and Lamb, 2005). While, alkaloids, such as berberine and piperine, interact with bacterial cytoplasmic membrane, intercalate with DNA or inhibit efflux pumps in *S. aureus* (Khan *et al*., 2006). Other authors, shown that glycoside saponins are able to induce pore-like structures that change the membrane permeability, they can interfere with energy metabolism as well (Babu *et al*., 1997; Melzig *et al*., 2001; Mandal *et al*., 2005).

2.3. Antioxidants and Free radicals

2.3.1. Free radicals

Free radicals are atoms or molecules with unpaired electrons in the outermost shell, and may have positive, negative or neutral charge (Murthy *et al*., 2010).

Up to now, many studies have been revealed demonstrating the beneficial use of free radicals in body such as their role in physiological functions in metabolic pathways, cell signaling, immune response; but harmful effects of free radicals causing potential biological damage in living systems has also been a known fact such as their role in variety of pathophysiological conditions (Valko *et al.*, 2006).

Although oxygen is vital for aerobic bioprocesses up to 5% of inhaled oxygen is converted into reactive oxygen species (ROS). ROS can be classified into oxygen centered radicals and oxygen centered non radicals. The oxygen centered radicals are superoxide anion ($O^{\bullet -2}$), hydroxyl radicals (OH•) and alkoxyl radicals (RO•) and peroxyl radicals (ROO•). Oxygen centered non radicals are hydrogen peroxide (H_2O_2) and singlet oxygen ($1O_2$). Other radicals species are nitrogen species such as nitric oxide (NO•), nitric dioxide ($NO^{\bullet}_2$) and peroxynitrite ($OONO^-$) (Halliwell *et al*., 1995; Salama and El-Bahr, 2007).

2.3.2. Sources of free radicals

There are many sources for free radical formation. UV radiations, X-rays, gamma rays and microwave radiation, metal-catalyzed reactions and oxygen free radicals present in the atmosphere considered as pollutants are the major exogenous contributors. Among many environmental factors like interaction with chemicals, automobile exhausts fumes, industrial effluents, burning of organic matter, or some pesticides and herbicides, there are many endogenous substances serving as a source for formation of free radicals such as ROS generated by mitochondrial cytochrome oxidase, xanthine oxidases, neutrophils and by lipid peroxidation or by arachidonic acid, platelets and macrophages metabolism or mitochondria-catalyzed electron transport reactions (Sen *et al*., 2010).

2.3.3. Antioxidants and mechanism of action

An antioxidant is a molecule stable enough to give an electron to neutralize free radicals, thus reducing its ability to damage. These antioxidants delay or inhibit cellular

damage mainly through their free radical scavenging property (Halliwell, 1995). Beta-carotene, lycopene, vitamins C, E, A and other substances (Sies, 1997).

Antioxidants also can be defined as compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms including: (1) chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, (2) scavenging species that initiate peroxidation, (3) quenching O2 − preventing formation of peroxides, (4) breaking the autoxidative chain reaction, and/or (5) reducing localized O2 concentrations (Nawar, 1996). Chain-breaking antioxidants differ in their antioxidative effectiveness depending on their chemical characteristics and physical location within a food (proximity to membrane phospholipids, emulsion interfaces, or in the aqueous phase). Both chemical strength of an antioxidant and solubility in oil influence its accessibility to peroxy radicals particularly in membrane, micellar and emulsion systems, and the amphiphilic character required for effectiveness in these systems (Wanatabe *et al*., 2010).

2.4. *Glycyrrhiza glabra* L.

2.4.1. Botanical description

Glycyrrhiza glabra L. is a perennial herb, which is 3-5 feet in height, smooth rising from thick rhizome. Leaves are pinnate with 4 to 7 pairs of leaflets which are ovate in shape. Flowers are in axillary spikes, papilionaceous and lavender to violet in color (Figure 2.2). The floral structures and fruits of this family pose the mode of cross-pollination, mainly by insect (Vibha *et al*., 2009).

2.4.2. Distribution and classification of plant

This plant is distributed in the subtropical and warm temperate regions of the world, chiefly in the Mediterranean countries (Vibha *et al*., 2009). The plant is ondigenous to Greece, spain, Iraq, Turkey, Caucasian, Tanscaspian Russia and northern China (Roshan *et al*., 2012). The genus *Glycyrrhiza* (Leguminosae) consists of about 30 species including *G. glabra L, G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi* and *G. eurycarpa*, *G. glabra* L. also includes three varieties: Persian and Turkish licorices are assigned to *G. glabra* var. violacea, Russian licorice is *G. glabra* L. var gladulifera, and Spanish and Italian licorices are *G. glabra* L. var. typical (Nomura *et al*., 2002).

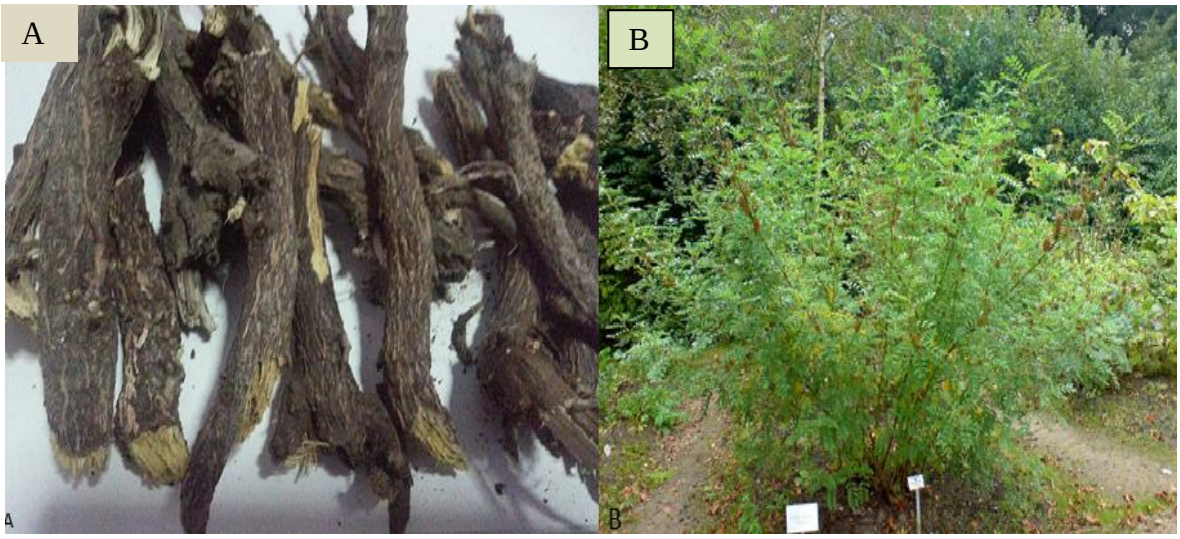

Figure 2.2. *Glycyrrhiza glabra* L. **Note**: A: root; B: aerial part

Table 2.2. Classification of plant

Kingdom	**Plantae-plants**
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Glycyrrhiza* L.

2.4.3. Active components of *Glycyrrhiza glabra* L.

2.4.3.1. Flavonoids and isoflavonoids

Flavonoids are hydroxylated phenolic substances that occur as a C6-C3 unit connected to an aromatic ring. Flavonoids are known to be synthesized by plants in response to microbial infection. Therefore, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide range of micro-organisms (Cowan, 1999). Flavonoids occur as aglycones, glycosides and methylated derivatives (Harborne, 2000).

In plants, flavonoids aglycones are flavonoids without attached sugar occur in a variety of structural forms. All contain fifteen carbon atoms in their basic nucleus: two

six-membered rings linked with a three carbon unit which may or may not be a part of a third ring (Middleton and Kandaswami, 1992).

More than 300 different flavonoids have been isolated from *Glycyrrhiza* species. These flavonoids including flavanones or flavanonols, isoflavans, chalcones, isoflavenes, flavones or flavonols, isoflavanones and isoflavones. Amongst them, flavanones and chalcones are the main types (Zhang and Ye, 2009). Furthermore, flavonoids are responsible for the yellow colour of *Glycyrrhiza glabra* L. They also include liquiritin, liquiritigenin, rhamnoliquiritin, neoliquiritin, isoliquiritigenin, isoliquiritin, glabrolide, licuraside and licoflavonol (Williamson, 2003).

Isoflavonoid derivatives present in licorice include glabridin, glabrone, galbrene, licoisoflavones, shinpterocarpin (A, B formononetin) and glyzarin kumatakenin (Williamson *et al*., 2003). Further, glabroiso flavanone A and B glabroiso-flavanone B (Kinoshita *et al*., 2005) and hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin and 3'-hydroxy-4'-O-methylglabridin have been found (Haraguchi, 2001; De Simone *et al*., 2001).

Glabridin is a polyphenolic flavonoid and a main constituent in the hydrophobic fraction of *Glycyrrhiza glabra* L. extract. It has been noticed to exhibit several pharmacological actions, such as antimicrobial activity, cytotoxic activity, estrogenic and anti-proliferative activity against human breast cancer cells. It also affects melanogenesis, low-density lipoprotein oxidation, inflammation and protection of mitochondrial functions from oxidative stresses (Choi, 2005; Tian *et al*., 2008).

Liqourice also contains (more than 3.8 %) glucose, sucrose (2.4 to 6.5 %), bitter principles, mannite, resins, asparagines (2 to 4%) and fat (0.8%) (Chopra and Chopra, 1958).

2.4.3.2. Vitamins

Glycyrrhiza glabra L. contain various types of vitamins including vitamin B1, B2, B3, B6, C, E, biotin, folic acid and pantothenic acid (Blach and Phylls, 1997).

2.4.3.3. Minerals

Glycyrrhiza glabra L. have many minerals compound mostly aluminum, calcium, magnesium, iron, zinc, phosphorus, sodium, silicone, cobalt, potassium, and stannous (Newall *et al*., 1997).

2.4.3.4. Saponins

Saponins are compounds with ‘soaplike’ behaviour in water, i.e. they produce foam upon shaking. On hydrolysis, an aglycone is produced, which is called sapogenin. Saponins are very poisonous, they cause heamolysis of blood and are known to cause cattle poisoning. They have a bitter taste, also causing irritation to mucosal membranes (Kar, 2007).

Saponins have Foaming and emulsifying properties (Heng, 2006). Licorice root contains (4–20%) of triterpenoid saponins, mostly glycyrrhizin, a mixture of potassium and calcium salts of glycyrrhizic acid (also known as glycyrrhizic or glycyrrhizinic acid, and a glycoside of glycyrrhetinic acid) (Blumenthal *et al*., 2000). Other triterpenes present are licorice acid, glabrolide, glycyrretol, isoglaborlide and liquiritic acid (Williamson, 2003).

The most studied active constituent of licorice is a sweet-tasting material. The constituent is 50 times sweeter than sugar, making it a widely used as a sweetening additive in the food industry (Acharya *et al*., 1993). In many countries, GA is used as a major therapeutic agent to treat chronic viral hepatitis and allergic dermatitis (Tanahashi *et al*., 2002). It is also known to have anti-inflammation (Fujisawa, 2000), anti-ulcer (Dehpour *et al*., 1995), anti-hepatotoxic (Ito *et al*., 1997) and antivirus activities (Cinatl *et al*., 2003; Fu *et al*., 2005).

Glycyrrhizin consists of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid (Mao *et al*., 2005). Glycyrrhizin used as a sweetening and flavoring agent due to its extreme sweetness (Zhang and Ye, 2009). It has been reported to exhibit multiple pharmacological activities, such as estrogenic, cytotoxic, antimicrobial activites and also anti-proliferative activity against human breast cancer cells. It also affects melanogenesis, low density lipoprotein oxidation and protection of mitochondrial functions from oxidative stresses (Choi, 2005).

2.4.4. Some medicinal effects of plant

2.4.4.1. Anti-inflamatory

Licorice constituents also exhibit steroid-like anti-inflammatory activity, similar to the action of hydrocortisone. This is due, in part, to inhibition of phospholipase A2 activity, an enzyme critical to numerous inflammatory processes (Okimasu *et al*., 1983). *In vitro* research has alsodemonstrated glycyrrhizic acid inhibits cyclooxygenase activity and pros-

taglandin formation as well as indirectly inhibiting platelet aggregation, all factors in the inflammatory process (Ohuchi *et al*., 1982; Okimasu *et al*., 1983).

Glabridin has effect in melanogenesis and inflammation by inhibiting the tyrosinase activity of melanocytes. Glycyhrritinic acid exhibits anti-inflamatory activity by inhibiting gulucocorticoid metabolism (Ohuchi *et al*., 1982; Okimasu *et al*., 1983; Akamatsu *et al*., 1992).

2.4.4.2. Peptic ulcer disease

Liquorice has been used as an antiulcer agent since early 1970's. The extracted glycyrrhizin, Deglycyrrhizinated licorice (DGL) is generally employed for the effective treatment of ulcers. Carbenoxolon from liquorice roots produce the anti-ulcerogenic effect by inhibiting the secretion of gastrin (Mendes-Silva *et al* 2003; Malek Jafarian and Ghazvini, 2010).

Liquorice can raise the concentration of prostaglandins in the digestive system that promote mucus secretion from the stomach, it was also reported that liquorice prolongs the life span of surface cells in the stomach and has an anti-pepsin effect (Aly *et al*., 2005).

Consequently, an *in vitro* study was performed by Fukai *et al*. (2002a; 2002b) which investigated the effects of licorice flavonoids on the growth of *Heliobacter pylori*. These flavonoids components showed promising anti- *Helicobacter pylori* activity against clarithromycin-and amoxicillin-resistant strains. As the antimicrobial property seems to be attributed to the flavonoid constituents of licorice, DGL preparations may provide therapeutic benefit for *H. pylori* infection. Other studies have demonstrated DGL's benefit in healing duodenal ulcers (Armanini *et al*., 2004).

2.4.4.3. Antiobesity action

The effects of hydrophobic flavonoids from *Glycyrrhiza glabra* L. on abdominal fat accumulation and blood glucose level in obese diabetic KK-A(y) mice, were investigated. The results indicated that licorice hydrophobic flavonoids have abdominal fat-lowering and hypoglycemic effects, possibly mediated via activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) (Nakagawa *et al*., 2004).

2.4.4.4. Antiatherogenic effects

In vitro and *in vivo* studies have demonstrated that liquorice extracts possess anti-atherogenic effects and can inhibit LDL oxidation. The administration of small amounts

(0.1 g daily for 1 month) of liquorice-root alcoholic extract (free of glycyrrhizin) may act as a potent antioxidant in atherosclerotic apolipoprotein E-deficient mice (Fuhrman *et al*. 1997) and in moderately hypercholesterolemic patients (Fuhrman *et al*., 2002).

2.4.4.5. Antitumor activity

Cancer is a multi-step disease incorporating environmental, chemical, physical, metabolic and genetic factors which play a direct and/or indirect role in the induction and deterioration of cancers (Fresco *et al*., 2006).Glycyrrhizin and other licorice components appear to possess anticarcinogenic properties as well. Although the exact mechanisms are still under investigation, research has demonstrated they inhibit abnormal cell proliferation, as well as tumor formation and growth in breast (Tamir *et al*., 200), liver (Shiota *et al*., 1999) and skin cancer (Nishino *et al*., 1984; Liu *et al*., 1998).

Khan *et al*. (2013) indicated that the glycyrrhizin obviously attenuated the level of TNF-α and it also declined the depletion of the mucous layer and the shifting of sialomucin to sulphomucin. Their findings suggested that glycyrrhizin had strong chemopreventive potential against DMH-induced colon carcinogenesis.

2.4.4.6. Learning and memory

Due to probable anti-inflammatory and antioxidant properties of liquorice about memory enhancement effect, *Glycyrrhiza glabra* L. showed that it promise as a memory enhancing agent in all the laboratory models employed (Dhingra *et al*., 2004).

Glabridin was isolated from the roots of *Glycyrrhiza glabra* L. and it's effects on cognitive functions and cholinesterase activity were investigated in mice. Glabridin and piracetam were administered daily for 3 successive days to different groups of mice. Both remarkably reduced the brain cholinesterase activity in mice compared to the control group. Therefore, glabridin appeared to be a promising candidate for memory improvement (Cui *et al*., 2008).

2.4.4.7. Antiviral activity

Glycyrrhizin has a prominent antiviral activity, as it does not allow the virus cell binding. It has been reported as HIV-1, Japanese encephalitis virus and yellow fever virus. Recently antiviral activities of ribavirin, 6-azauridine, pyraziofurin, mycophenolic acid and glycyrrhizin against two clinical isolates of SARS (Severe Acute Respiratory Syndrome) virus (FFM-1 and FFM-2) from patients with SARS, admitted to clinical center of Frank-

furt University, Germany were evaluated and it was observed that glycyrrhizin was the most effective in controlling viral replication and could be used as a prophylactic measure; glycyrrhizin has been previously used to treat patients suffering from HIV-1 and chronic hepatitis C virus (Lalita, 1994; Badam, 1997; De Clercq, 2000).

It has been suggested that glycyrrhizin has an effect on viral growth, possibly through an inhibition of viral particle to cell membrane binding, replication mechanisms, or through cellular signal transduction mechanisms (Crance *et al*., 2003).

2.4.5. Biological effects

2.4.5.1. Antimicrobial activity

Active componant of *Glycyrrhiza glabra* L., 18-beta glycyrrhetinic acid (18-beta GA) was studied by Pellati *et al.* (2009). They showed that the *in vitro* growth of the *Candida albicans* strains was markedly reduced, in a pH- dependent manner, by relatively low doses (6.2 µg/mL) of 18-beta GA.

Messier *et al*. (2011) also reported that the Glabridin and licochalcone A of *Glycyrrhiza glabra* L. Both licochalcone A and glabridin prevented yeast-hyphal transition in *C. albicans*. These results suggested a therapeutic potential of licochalcone A and glabridin for *C. albicans* oral infections.

Glabridin componant of *Glycyrrhiza glabra* L was studied in Amphotericin B resistant *C. Albicans*. The ethanol root extract of *Glycyrrhiza glabra* L. showed a wider spectrum of activity against various *C. albicans* strains (Fatima *et al.*, 2009).

Hojo and Sato (2002) determined the antibacterial activities of Licorice root extract (ether, chloroform and acetone) by using the well diffusion method. The extracts were shown significant antibacterial activities against two Gram-positive (*Bacillus subtilis* and *Stapphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.

Streptococcus mutans, *Streptococcus sanguis*, *Actinomyces viscosus*, and *Enterococcus faecalis* were used as oral pathogens in this *in vitro* study. *In vitro* antibacterial activity of *Glycyrrhiza glabra* L. was assessed quantitatively and qualitatively by determining the inhibition zone diameter and MIC. *Glycyrrhiza glabra* L. extract showed good antibacterial activity against all oral pathogens (Sedighinia and Afshar, 2012).

2.4.5.2. Antioxidant action

A group of neolignan lipid esters and phenolic compounds isolated from the roots and stolons of liquorice (*Glycyrrhiza glabra* L.) were found to have chemopreventive properties. These compounds, hispaglabridin B isoliquiritigenin, and paratocarpin B were found to be the most potent anti-oxidant agents (Chin *et al*., 2007).

The metal chelating activity of *Glycyrrhiza glabra* L. along with *Syzygium aromaticum*, licorice mace (aril of *Myristica fragans*) and greater cardamom (*Amomum subulatum*) were tested for their antioxidant properties *in vitro* by Yadav *et al*. (2007) bleomycin dependent DNA oxidation, diphenyl-p-picryl hydrazyl glabridin is reported to be a potent antioxidant towards LDL oxidation. Moreover, (DPPH) radical scavenging activity and the ferric reducing antioxidant power (FRAP) were measured in rat liver . All the results were obtained by species including *Glycyrrhiza glabra* L. which mentioned above showed the strong antioxidant activities and may have beneficial effects on human health (2007).

Another study by Kanimozhi *et al*. (2011) determined that the ethanolic extract of *G. glabra* L. leaves showed potent antioxidant activity in 1,4-dichlorobenzene induced liver carcinogenesis in albino rats. Also, the essential oil which obtained by hydrodistillation and its phytochemical composition was determined by Gas Chromatography–Mass Spectrometry (GC-MS) analysis.

Li *et al*. (2011) evaluated the role of glycyrrhizin on lipid peroxidation and antioxidant status in the blood and nasal mucosa of allergic rhinitis (AR) mice, the results demonstrated that glycyrrhizin, which is the main component of *Glycyrrhiza glabra* L., treatment enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation and improved immunity activities in the blood and nasal mucosa of AR mice

2.5. The Bacteria and Fungi Used in This Study

2.5.1. *Staphylococcus aureus*

Gram-positive and non-spore forming spherical shape bacterium that belongs to the *Staphylococcus* genus. *S. aureus* has ability to produce a kind of toxin which known as staphylococcal enterotoxin (SE) and is responsible for nearly all staphylococcal food poisoning (Montville and Matthews 2008; FDA, 2012).

S. aureus is regarded as facultative an-aerobic which can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart and Hocking, 2003).

2.5.2. *Micrococcus luteus*

Gram-positive cocci bacteria, they are generally strict aerobes and can generally reduce nitrate. The diameter of bacteria is between 0.5 to 3.5 µm and usually arranged in tetrads or irregular clusters. *Micrococcus* species are oxidase-positive, which can be used to differentiate them from other bacteria like most *Staphylococcus* species, which are generally oxidase-negative. *M. luteus* has ability to oxidize carbohydrates to CO_2 and H_2O and it does not produce acid from glucose besides it does not make arginine dihydrolase or b-galactosidase. Some Micrococcus is pigmented bacteria; for example, M. roseus produces redish colonies and *M. luteus* produces yellow colonies (Smith *et al*., 1999).

Micrococcus spp. is generally considered as harmless saprophytes that contaminate the skin, mucosa, and also the oropharynx; however, they can be opportunistic pathogens for the immunocompromised patients (Bannerman *et al*., 2006; Kocur *et al*., 2006). They have been related with several infections, such as bacteremia, continuous ambulatory peritoneal dialysis peritonitis, and infections related with ventricular shunts and central venous catheters (Bannerman *et al*., 2006).

2.5.3. *Bacillus subtilis*

B. subtilis is Gram-positive, aerobic, endospore-forming bacterium, it is opportunistic pathogen and the virulence characteristics of the micro-organism are low (Sietske and Diderichsen, 1991). They are common soil inhabitants and may commonly contaminate foods and broadly distributed in hospital environments. The ability of some bacteria to form resistant spores allows it to endure extreme conditions such as heat and desiccation in the environment promotes their survival in many instances like hospitals making problems for cleaning and disinfection (Garcia-Arribas, 1985).

Airborne spread has been linked to the development of a cluster of symptoms, mainly in immunocompromised patients, including irritation of eye and sinus, sore throat, headache, fatigue, and dizziness (Weber *et al*., 1989). Furthermore, producing of subtilisin is another characteristic of *B. subtilis* which is known as a proteinaceous compound capa-

ble of causing allergic and hypersensitivity reactions in persons who are frequently exposed to it (Gill, 1982).

2.5.4. *Bacillus megaterium*

Gram- positive bacterium which was first described by de Bary more than one century ago. megat(h)erium is greek word means big animal with its lare size of 1.5 x 4 µm, this micro-organism belongs to the larger bacteria. Due to the dimension of the vegetative form and spores, *B. megaterium* is well suited for morphological research, for example on cell-wall and cytoplasmic membrane biosynthesis, on sporulation, spore structure and organisation (Vary, 2007).

2.5.5. *Pseudomonas aeruginosa*

Gram-negative bacteria, non-spore forming, capsulated, motile, bacillus shape, oxidase positive, no glucose fomenter and opportunistic micro-organism (Al-Mathkhury *et al*., 2011).Infected individually was suffering from with immunocompromsied system, cystic fibrosis, and cancer. Multi resistance drugs due to it have numerous virulence factors like flagellum and pili both of them consist of exopolysaccharide play role for producing biofilm therefore difficult to eradicate and protected them self from phagocytes cell, antibody, antibiotic, and detergent substance,thus therapeutic treatment of it more difficult but antibiotic my able to infected capacity of *P.aerugenosa*. 50% colonization reproved in the human body organs. Infection occur with compromised host condition such as disrupting physical skin barriers lead to invasion bacteria especially during burn injuries (Valdez *et al.*, 2005).

2.5.6. *Klebsiella pneumoniae*

Gram-negative bacteria, lactose fermentation, rod shape, encapsulated ,found in mouth and skin, frequently habitat in the intestine flora, pharyngeal flora of the human, under Enterobacteriaceae family, opportunistic pathogen, can be isolated in varies part of the body, nasocomial pathogen infected human with the immune compromised hosts. UTI, cholecystitis, thrombophlebitis, wound infection and bacteremia (Komatsu *et al*., 2001).

K. pneumonia regarded as a second Gram-negative bacteria type comes to after *E.coli* may cause intrauterine infection ,neonatal sepsis,and vaginosis despite high concentration posses of lactobacilli available because able to produce biofilm in which prevent high acidic condition environment (Donders *et al.*, 2011).

Member of this genus are also a well-known food-poisoning organism producing diarrheal enterotoxins. Subsequently, ingestion of contaminated food may be a risk of setting outbreak case (Oguntoyinbo and Sanni, 2007).

2.5.7. *Escherichia coli*

E. coli is a Gram-negative bacteria, facultative anaerobic, rod shape, motile, spore forming, habitat in the lower intestine and worm blood organisms, produce vitamin-k in the intestine by metabolic activity, most strain of *E. coli* are harmless (Jann *et al*., 1992).

It belong to the Entrobactreiaceae family may causes of many disease in the lower intestine such as gastroenteritis, urinary tract infection, mastitis, vaginosis, septicemia, peritonitis, and it is enterotoxigenic bacterium commonly causes diarrhea in the child (Hossain *et al*., 2012).

That abounded type of Gram-negative bacteria which studies in the microbiology, 50% are nosocomial accompanied with the intestinal and extra intestinal infection lead to infected human and many type of animal (Jann *et al*., 1992).

2.5.8. *Enterococcus faecium*

E. faecium is a Gram-positive bacterium has a thick peptidogycan layer along with teichoic and lipoteichoic acids. It has circular DNA as well as several plasmids. It is capable of conjugation through the release of sex pheromones and secretes aggregation substances and also forms biofilms. The cell has pili and flagella (Murray, 1998; Van Wamel *et al*., 2007; Gülhan *et al*., 2007).

2.5.9. *Candida albicans*

It is a member of the normal flora of the mucous membranes in the gastrointestinal, upper respiratory, and female genital tracts (Nobel, 1980; Kaufman , 1986; Clayton and Noble, 1966)

A number of virulence factors including the adherence to the tissue, formation of a biofilm, secretion of hydrolytic enzymes, phenotypic switching and morphological dimorphism play a role in the pathogenesis of this fungus. One characteristic of *C. albicans* is producing adhesions which help with adherence to host cells to switch between yeast cells, pseudohyphae and septate hyphae to increase its virulence during adhesion and infection establishment as well as to secrete enzymes such as aspartyl phospholipases, proteinases,

farnesol, superoxide dismutases and catalases which helps with nutrient uptake, tissue invasion, adherence and dissemination and prevention of oxidative damage among others (Spicer, 2000; Karkowska-Kuleta *et al*., 2009; Khan *et al*., 2010).

2.5.10. *Candida utilis*

C. utilis is anindustrially important yeast, as it is capable of several useful noneth-anolic fermentation reactions that result in the production of various organics such as, acet-aldehyde. The organism is also capable of using alcohols as a carbon source (Prior *et al*., 1980). As a pathogen, *C. utilis* has been reported as a rare agent of fungemia (Alsina *et al*., 1988; Bougnoux *et al*., 1993).

2.5.11. *Yarrowia lipolytica*

Y. lipolytica is one of the most widely studied non-conventional yeasts, a strictly aerobic micro-organism capable of producing important metabolites and having an intense secretory activity, which justifies efforts to use it in industry (as a biocatalyst), in molecu-lar biology and in genetics studies. This yeast is mainly adapted to hydrophobic substrates and in the last years it became a reference in research dealing with non-polar substrate metabolism. Many industrial applications of *Y. lipolytica* have been proposed. (Gonçalves *et al*., 2009).

Transient recurrent catheter-related fungemia attributable to this organism in a leukemic patient has been observed. The fungemia and accompanying fever subsided spontaneously. The data suggest that it might be possible to withhold specific treatment for *Y. lipolytica* fungemia even in an immunocompromised patient (Chang *et al*., 2001).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Plants collection and preparation

The roots of *Glycyrrhiza glabra* L. were collected in four different locations from Northern of Iraq (Erbil, Shaqlawa locality, and Sulaymaniyah, Dokan locality,) and Turkey (Şırnak and Ağrı) during august 2014. After removing dust, the plant materials were dried at 40 °C for 72 h in an oven and then ground to fine powders by using electric grinder and passed through the No. 20 mesh sieve and stored in plastic bags until use.

3.1.2. Micro-organisms used in this study

All the strains of bacteria *Staphylococcus aureus* Cowan 1, *Micrococus luteus* LA 2971, *Bacillus subtilis* IMG22, *Bacillus megaterium* DSM 32, *Klebsiella pnemoniae* FMC 5, *Escherichia coli* DM, *Pseudomonas aeruginosa* DSM 50071, *Enterococcus faecium* Clinic izolate and fungi *Candida albicans* ATCC1023, *Candida utilis* NRRL-Y-900, *Yarrowia lipolytica* NRRL-Y-900 obtained from microbiology department in Kahramanmaras Sutcu Imam University.

Table 3.1. List of chemicals and reagents used in this study

Chemical and apparatus	Purpose of usage
Ampicillin 10 µg/mL	Used in antibacterial test
BHT	Used as reference in antioxidant assay
Distilled water	Used for Extraction
DPPH	To evaluate antioxidant
Ethanol	Used for Extraction
Fe2SO4	To determination Condensed tannin
Gentamicin 10 µg/mL	Used in antibacterial test
Hcl	To determination Condensed tannin
Methanol	Used for Extraction
MHA	Used in antimicrobial assay
Mimosa	Standard reference for determination total condensed tannin
N-butanol	To determination Condensed tannin
Nutrient Broth	Used for the cultivation of micro-organisms

Nystatin syrup	Used in antifungal test
Petri dish	Used to culture bacteria and fungi
SDA	Used in antimicrobial assay
Test tube	Hold the extracts

Table 3.2. List of Instruments and machines

Instruments and machines	Purpose of useage
Autoclave OT O12	Sterilization
Dionex ASE 350	Extraction the sample
Electric blender	Crushing the sample
Gravimeter	Weighting the sample
Heating stireer	Extraction the sample
Hiedolph evaporator	Evaporation of solvents after extraction
Incubator	Growing micro-organisms
Microwave NEOS system	Extraction the sample
Nexera model Shimadzu UHPLC	Identification of phenolic compound
Oven	Drying
SHIMADZU UV-vis 1240 spec-trophotometer	Measurement Condensed tannin Measurement antioxidant

3.2. Methods

3.2.1. Plant extraction

Three different methods were applied for extraction of plant roots (e.g conventional extraction (CE), microwave assisted extraction (MAE) and accelerated solvent extraction (ASE)) methods along with three solvents such as methanol, ethanol and water except that for ASE method just methanol and ethanol were used.

3.2.1.1. Conventional extraction

Five gram of *Glycyrrhiza glabra* L. roots in powder were extracted by mixing with 50 mL of water, methanol and ethanol separately at 50 °C for 3 hrs using a magnetic stirrer where is conducted with refluxing system (Figure 3.1). At the end of the extraction cycle the liquid extract was separated from the solid residue by filtration through Wattmann No.1

filter paper for removing of particles. The extract was pooled and concentrated under vacuum at 60 °C by rotary evaporator.

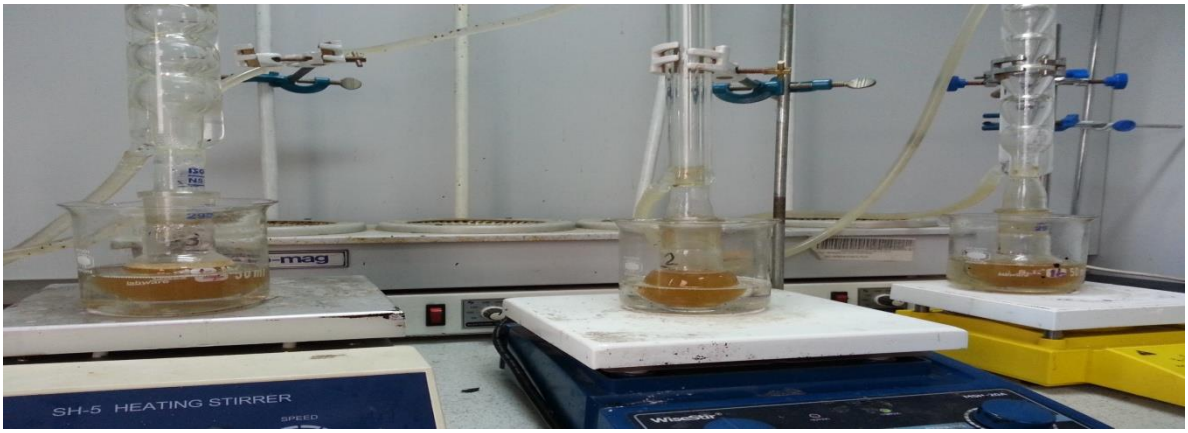

Figure 3.1. Heating system with stirrer (conventional extraction)

3.2.1.2. Microwave assisted extraction method

Is the process utilizing microwave energy to facilitate partition analytes from the sample matrix into the solvent. The main advantage of this technique is the reduced extraction time and solvent volume as compared to conventional extraction techniques (Weidner *et al*., 1999). Opened system microwave milestone NEOS was used for extraction, 5 g of samples from each locations were mixed with 50 mL of each solvents that mentioned above separately then transferred to a round bottom flask connected with reflux condenser, extraction was carried out for 30 min and the different temperature were standard for solvents and different powers**.** After extraction of roots, all the extractions were passed through Whatman filter paper No.1 to separate extractions.

3.2.1.3. Accelerated solvent extraction

Accelerated Solvent Extraction (ASE) is a relative new technology for extraction of phytochemicals under high temperature and pressure (Benthin *et al*., 1999).

Dried root samples were extracted by accelerated solvent extraction (Dionex ASE 350) under the following conditions: temperature 60 ºC and pressure. The extracts of the 2 extraction cycles were combined and the solvent was evaporated under reduced pressure. Dried extracts were stored at 4 ºC until analysis.

3.2.2. Determination of extraction yield

The the yield percentages of plant extracts were calculated gravimetrically using following formula:

$$\text{Yield percentage (\%)} = \frac{X}{Y} \times 100 \quad (3.1)$$

where,
X is the oven dry weight of extract (g),
Y is the oven dry weight of the sample (g).

3.2.3. Determination of total condensed tannin

Extraction solution was prepared by mixing 0.05 g of Fe_2SO_4, 95 mL N-butanol and 5 mL HCl 35% .Then, weighed 0.01 g form *Glycyrrhiza glabra* L. samples and mimosa tannin, then put in small plastic tube separately, and added 10 mL of the extraction solution and placed in water bath for heated during 1 h. Finally, 1 mL from extraction solution was taken and placed into a spectrophotometer quartz cuvette and read at 580 nm wavelength (Makkar *et al*., 1995).

3.2.4. Phytochemicals analysis in methanolic extraction of *Glycyrrhiza glabra* L. root by Liquid chromatography mas-spectrometry LC–MS/MS

3.2.4.1. Instruments and chromatographic conditions for LC–MS/MS

LC–MS/MS analyses of the phenolic compounds were performed by using a Nexera model Shimadzu UHPLC coupled to a tan-dem MS instrument. The liquid chromatography was equipped with LC-30AD binary pumps, DGU-20A3R degasser, CTO-10 ASvp-column oven and SIL-30AC autosampler. The chromatographic separation was performed on a C18 reversed-phase Inertsil ODS-4 (150 mm × 4.6 mm, 3 m) analytical column. The column temper-ature was fixed at 40 $^{\circ}$C. The elution gradient consisted of mobilephase A (water, 5 mM ammonium formate and 0.1% formic acid) and mobile phase B (methanol, 5 mM ammonium formate and 0.1%formic acid). The solvent flow rate was maintained at 0.5 mL/min and injection volume was settled as 4 µl (Ertas *et al*., 2015).

3.2.4.2. MS instrumentation

MS detection was performed using Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer equipped with an ESI source operating in both positive and

negative ionization modes. LC–MS/MS data were collected and processed by Lab Solutions soft-ware (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation.

3.2.4.3. Optimization of LC–MS/MS method

After different combinations of trials, in order to have richionization and the separation of the molecules, gradient elutionwas achieved using two solvents as (A) water (5 mM ammonium formate and 0.1% formic acid) and (B) methanol (5 mM ammonium formate and 0.1% formic acid). Among the most com-monly used atmospheric pressure ionization sources such as ESI (Electrospray ionization), APCI (atmospheric pressure chemicalionization) and APPI (atmospheric pressure photoionization), ESI source was chosen since the phenolic compounds were small and relatively polar molecules. Furthermore, liquid chromatography tandem mass spectrometry was decided to be used for the current-study due to its ion fragmentation stability (Ertas *et al*., 2014a; 2014b).

The optimum ESI conditions were determined as interface temperature; 350 °C, DL temperature; 250 °C, heat block temperature; 400°C, nebulizing gas flow (nitrogen); 3 L/min and drying gas flow or nitrogen of 15 L/min.

3.2.4.4. Method validation parameters for LC–MS/MS

In this study, twenty-four phenolic and three non-phenolic organic acids which are widespread inplant materials were qualified and quantified in *Glycyrrhiza glabra* L. Rectilinear regression equations and the linearity ranges of the studied standard compounds are given in Table 3.3 (Ertas *et al*., 2014a). Correlation coefficients were found to be higher than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of thereported analytical method were shown in Table 3.3. For the studied compounds, LOD ranged between 0.05 and 25.8 g/L and LOQ ranged between 0.17 and 85.9 g/L. Moreover, the recoveries of the phenolic compounds ranged from 96.9% to 106.2%. The results were calculated by the equation below:

Quantification of compound $(\mu g/g) = \frac{R \times U^f}{100}$ (3,2)

where,
R is the result from LC–MS/MS (µg),
U^f is the percent relative uncertainty at 95% confidence level (%).

Table 3.3. Analytical parameters of LC–MS/MS method

No.	Analyses	RT[a]	Parent ion (m/z)[b]	Ionization Mode	R^{2c}	RSD %[d]	Linearity Range (mg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)	U[f]
1	Quinic acid	3.32	190.95	Neg	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	4.8
2	Malic acid	3.54	133.05	Neg	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	5.3
3	trans-Aconitic acid	4.13	172.85	Neg	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	4.9
4	Gallic acid	4.29	169.05	Neg	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	5.1
5	Chlorogenic acid	5.43	353	Neg	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	4.9
6	Protocatechuic acid	5.63	152.95	Neg	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	5.1
7	Tannic acid	6.46	182.95	Neg	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	5.1
8	trans- Caffeic acid	7.37	178.95	Neg	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	5.2
9	Vanillin	8.77	151.05	Neg	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	4.9
10	p-Coumaric acid	9.53	162.95	Neg	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	5.1
11	Rosmarinic acid	9.57	358.9	Neg	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	4.9
12	Rutin	10.18	609.1	Neg	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	5.0
13	Hesperidin	9.69	611.1	Poz	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	4.9
14	Hyperoside	10.43	463.1	Neg	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	4.9
15	4-OH Benzoic acid	11.72	136.95	Neg	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	5.2
16	Salicylic acid	11.72	136.95	Neg	0.9904	0.6619	25-1000	4 / 13.3	106.2	5.0
17	Myricetin	11.94	317	Neg	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	5.9
18	Fisetin	12.61	284.95	Neg	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	5.5
19	Coumarin	12.52	146.95	Poz	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	4.9
20	Quercetin	14.48	300.9	Neg	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	7.1
21	Naringenin	14.66	270.95	Neg	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	5.5
22	Hesperetin	15.29	300.95	Neg	0.9961	1.0164	25-1000	3.3/ 11.0	102.4	5.3
23	Luteolin	15.43	284.95	Neg	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	6.9
24	Kaempferol	15.43	284.95	Neg	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	5.2
25	Apigenin	17.31	268.95	Neg	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	5.3
26	Rhamnetin	18.94	314.95	Neg	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	6.1
27	Chrysin	21.18	253	Neg	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	5.3

Note: RT[a]: Retention time, parent ion (*m/z*) [b]: Molecular ions of the standard compounds (mass to charge ratio) , R^{2c}: coefficient of determination, RSD[d]: relative standard deviation, LOD/LOQ (µg/L)[e]: Limit of detection/Limit of quantification, U[f] (%): Percentage of relative uncertainty at 95% confidence level.

3.2.5. Determination of antimicrobial activity

3.2.5.1. Preparation for antimicrobial assay

Table 3.4. Mueller-Hinton agar conistiuents and conditions

Conistituents/conditions	Values
Agar	17.0 g/L
Beef infusionSolids	2 g/L
Casein hydrolysate	17.5 g/L
Starch	1.5 g/L
pH	7.4 ±0.2 at 25 °C

Mueller-Hinton agar was prepared by adding 13.6 g of the medium with 400 mL of distilled water, the suspention was mixed well and heated with frequent agitation then boiled for one minute and sterilized at 121 °C (15 lbs. of pressure) for 15 min, finaly cooling to 40-45 °C and pouring into petri dishes.

Table 3.5. Sabouraud dextrose agar conistiuents and conditions

Conistituents/conditions	Values
Agar	17.0 g/L
peptone	10.0 g/L
D(+) Glucose	20 g/L
pH	5.6 ±0.2 at 25 °C

Sabouraud dextrose agar was prepared by adding 18.8 g of the medium with 400 mL of distilled water, the suspention was mixed well and heated with frequent agitation then boiled for one minute and sterilized at 121 °C (15 lbs. of pressure) for 15 min, then cooling to 40-45 °C and pouring into petri dishes.

3.2.5.2. Disc diffusion test

The disc diffusion method (CLSI, 2012) was used to test antimicrobial activity of the plant extracts against 4 Gram-positive, 4 Gram-neagative and 3 fungi (mentioned in 3.1.2). Solutions of known concentrations of the test samples were made by dissolving measured amount of the samples in solvents. Filter paper discs (10 mm diameter) were then impregnated with 100 µl with concentration of 25 mg/disc of the test substances using micropipette and the solvents were completely evaporated. Discs containing the test mate-

rials were fixed on nutrient agar medium cultured with the test micro-organisms. Blank discs impregnated with solvents followed by evaporation were used as negative control. These plates were then kept at low temperature 4 °C for 1 h to allow diffusion of the test materials. Standard antibiotic discs of ampicillin (10 µg/disc) and gentamicin (10 µg/disc) were used for bacteria. While, the disc of 10 mm in diameter was impregnated with 100 µl Nystatin unit/disc as antifungal was used. Then the plates were transferred to refrigerator at 4 °C for 40 minutes to allow maximum diffusion of extractions then incubated at 37 °C for 24 h for bacteria and 72 h for fungi to allow maximum growth of the micro-organisms. The test material having antimicrobial activity inhibited the growth of the micro-organisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in (mm).

3.2.6. DPPH radical scavenging activity

The DPPH method is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of foods and beverages (Kedare and Singh, 2011).

The free radical scavenging activities of ethanolic and methanolic extracts of *Glycyrrhiza glabra* L. roots were determined by the DPPH free radical scavenging activity assay described by Blois (1958) with slight modification. In its radical form, 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 517 nm, but upon reduction by an antioxidant its absorption decreases.

Briefly, 0.1 mM solution of DPPH for ethanol and methanol were prepared. Then, 0.1 mL, 0.2 mL and 0.3 mL of the sample solution mixed with methanol and ethanol up to 3 mL in test tube, separately. Then, 1 mL of DPPH was added. The mixture was shaken vigorously and placed in the dark at room temperature for 30 min. Later, the absorbance was measured by using (Shimadzu UV-vis 1240 spectrophotometer) at 517 nm. Butylated hydroxyltoluene (BHT) was used as a reference. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following equation (Gülçın *et al*., 2003).

$$\text{Inhibition of DPPH radical scavenging activity (\%)} = \frac{A-B}{A} x100 \quad (3.3)$$

where,

A is the absorbance of DPPH,

B is the absorbance of the presence of sample and BHT.

3.3. Statistical Analyses

The data for antimicrobial activity were analysed with ANOVA using SPSS (version 18) statistical program. The mean differences were compared as significant ($P< 0.05$) or non-significant ($P > 0.05$).

4. RESULTS AND DISCUSSION

4.1. Determination of Extraction Yield of *Glycyrrhiza glabra* L.

The yield percentages of plant extracts were shown in Figure 4.1. Among the extraction methods, accelerated solvent extraction showed the highest yield percentages followed by conventional and microwave extraction methods, this might be due to fact that ASE operates under nitrogen at high temperature and pressure, which helps the solvent penetrate rapidly into the plant cells and prevents degradation of phenolic compounds. Compared to conventional methods, the amount of solvent and extraction time are dramatically lower (Richter *et al*., 1996). ASE with methanol showed the highest yield for the all locations with 22.87, 22.32, 20.78 and 19.33% for Erbil, Sulaymaniyah, Şırnak and Ağrı, respectively. This gave a good indicator for the probability using these new techniques for licorice.

The variation in the extraction yields might be due to the different availability of extractable components, resulting from the varied chemical composition and the polarity of the solvents (Sultana *et al*., 2009). The effects of solvents plolarity on extraction yield both qualitatively and quantitatevly was confirmed by Franco *et al*. (2008).

Table 4.1. Extraction yield percentages of *Glycyrrhiza glabra* L.

Locations	CE			MAE			ASE	
	M	E	W	M	E	W	M	E
Sulaymaniyah	16.53 ±0.960	14.23 ±0.50	14.16 ±0.68	15.73 ±0.53	14.3 ±1.05	12.67 ±0.47	22.32 ±0.94	18.28 ±0.75
Ağrı	15.66 ±1.15	12.66 ±058	12.33 ±0.58	12.66 ±0.58	11.33 ±058	10.33 ±0.58	19.33 ±0.58	15.23 ±0.48
Erbil	15.92 ±0.58	14.45 ±0.93	12.96 ±0.68	16.04 ±0.77	13.87 ±0.4	12.79 ±0.52	22.87 ±0.95	19.53 ±1.13
Şırnak	16.10 ±0.49	14.99 ±0.44	13.68 ±0.56	15.18 ±0.56	14.21 ±0.73	13.22 ±0.87	20.78 ±0.39	15.72 ±0.63

Note: M: methanol; E:ethanol; W: water, Values presented as mean ± SD of three.

4.2. Determination Total Condensed Tannin of *Glycyrrhiza glabra* L.

Table 4.2. illustrates tannins concentration of *Glycyrrhiza glabra* L. roots chemically were analysed with mimosa calibration for calculating total tannin (Figure 4.1). The reesults indicated that *Glycyrrhiza glabra* L. which were collected from Erbil showed the highest concentration of tannin with concentration of 20.83 mg/L while the lowest concentration of tannin was detected for the liquorice from Ağrı with concentration of 14,74 mg/L.

The presence of tannin in *Glycyrrhiza glabra* L. was previouly proven by Al-Obaidi, (2013) with 13.2 % of plant. Condensed tannin is good antimicrobial activity against certain bacteria and yeasts, and also has good enough DPPH radical scavenging activity (Sulaiman *et al*., 2011).

Table 4.2. Condensed tannin concentration of *Glycyrrhiza glabra* L. grown in different locations

Locations	Condensed tannin concentration (mg/L)			Average (X)	STD	Variation (V)
Sulaymaniyah	19,92	20,05	19,99	19,99	0,07	0,33
Ağrı	14,88	14,74	14,61	14,74	0,13	0,90
Erbil	20,05	20,45	21,98	20,83	1,02	4,88
Şırnak	18,46	18,46	18,79	18,57	0,19	1,03

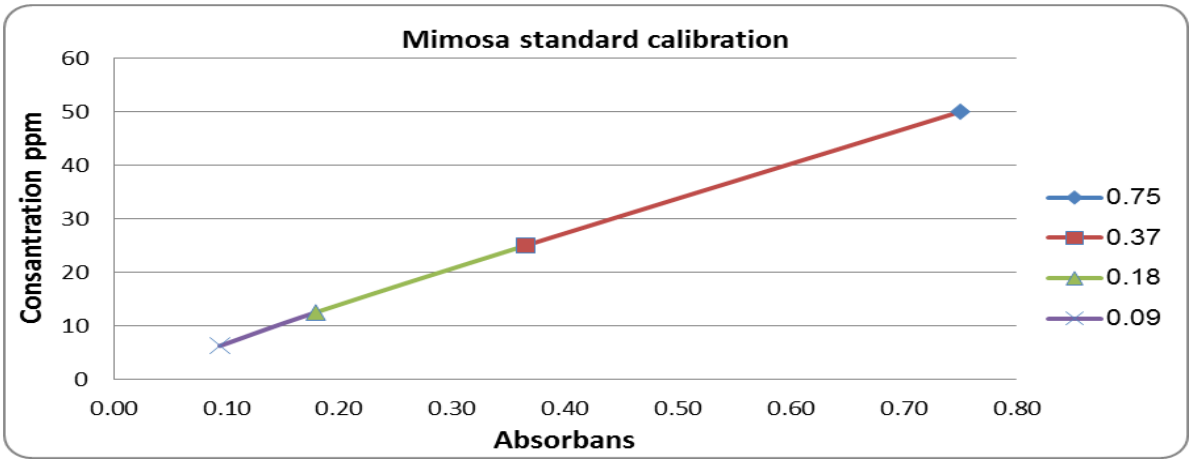

Figure 4.1. Mimosa tannin standard calibration curve

4.3. Quantitative and Qualitative Analysis of Phytochemicals by LC–MS/MS

Compound analyses were carried ou for *Glycyrrhiza glabra* L. liquid chromatography with mas spectrometry (LC-MS/MS). The flavonoid compounds have been identified according to their retention times and quantified according to their respective

standard calibration curves. In the current study, 27 compunds were quantitatively analaysed by LC-MS/MS including plant non-phenolic compound and phenolic compound.

4.3.1. Quantitative and qualitative of plant non-phenolic phytochemicals

Table 4.3. illustrates the amount of plant non-phenolic acids which are widespread inplant materials were qualified and quantified in *Glycyrrhiza glabra* L.

Three non-phenolic organic acids were identified which were quinic acid, malic acid and trans-aconitic acid, the liquorice which were collected from Sulaymaniyah showed the highest quinic acid among all locations with concentration of 2939,153 µg/g followed by the samlpes from Erbil with 1907.908 µg/g whereas the Şırnak's one showed the lowest amount of quinic acid with 293.829 µg/g. Whereas, in the term of malic acid, the liquorice which were collected from Sulaymaniyah also showed the highest amount of malic acid with concentration of 12055.991 µg/g followed by Erbil's one with 9647.358 µg/g while the liquorice from Şırnak again showed the lowest concentration with 220.835 µg/g.

Trans-aconitic acid showed the lowest concentration among non-phenolic acids for all locations and the Şırnak's liquorice exhibited the highest amount of it with concentration of 42.243 µg/g.

Table 4.3. Non-phenolic phytochemicals detected for *Glycyrrhiza glabra* L. roots grown in four locations

No.	compounds	concentration (µg/g)			
		Sulaymaniyah	Ağrı	Erbil	Şırnak
1	Quinic acid	2939,153 ±141,08	555.120 ±26.64	1907.908 ±91.58	293.829 ±14.10
2	Malic acid	12055.991 ±638,97	670.004 ±35.51	9647.358 ±511.31	220.835 ±12
3	trans-Aconitic acid	10.804 ± 0.53	2.852 ± 0.14	19.660 ± 0.96	42.243 ±2.06

4.3.2. Quantitative and qualitative plant phenolic phytochemicals

4.3.2.1. Flavonoid phytochemicals

LC–MS/MS results were examined interms of the flavonoids. Eleven flavonoids were qualified and quantified for all locations. The highest amount of flavonoids which was detected for *Glycyrrhiza glabra* L. from Sulaymaniyah was fisetin with concentration of 331.118 µg/g. However, oher flavonoids like hyperoside, rutin and hesperidin were also

detected with concentrations of 43.402, 32.026 and 15.458 µg/g, respectively. But if we look at liquorice from Erbil we can see that naringenin, fisetin, apigenin, kaempferol and luteolin showed the highest concentrations among all flavonoids with concentrations of 791.063, 332.738, 313.139, 94.080 and 89.477 µg/g, respectively (Table 4.4).

On the other hand, the most flavonoid which has been detected for Ağrı's liquorice was hyperoside with concentration of 237.317 µg/g. The most flavonoid which was detected for Şırnak's liquorice was apigenin with concentration of 148.648 µg/g. The presence of chlorogenic acid, rutin, myricitin, quercetin and kaempferol in *Glycyrrhiza glabra* L. was also confirmed by Gupta *et al*. (2013)

Table 4.4. Flavanoid phytochemicals detected for *Glycyrrhiza glabra* L. roots grown in four locations

No.	Compounds	Concentration (µg/g)			
		Sulaymaniyah	Ağrı	Erbil	Şırnak
1	Rutin	32.026± 1.60	21.889± 1.09	3.107± 0.16	4.096± 0.20
2	Hesperidin	15.458± 0.76	24.219± 1.19	2.039± 0.099	1.998 ±0.1
3	Hyperoside	43.402± 2.13	237.317±11.63	31.929± 1.56	20.930 ±1.02
4	Myricetin	1.634±0.10	4.204± 0.25	4.811± 0.28	0.526 ±0.03
5	Fisetin	331.118±1.82	25.995± 1.43	332.738± 18.30	54.097 ±2.9
6	Quercetin	0.313± 0.02	0.643± 0.05	1.121± 0.08	0.244 ±0.02
7	Naringenin	9.837± 0.54	5.676± 0.31	791.063± 43.50	66.111 ±3.63
8	Hesperetin	0.731± 0.04	1.834± 0.10	0.489± 0.03	0.367 ±19.45
9	Luteolin	1.438± 0.10	0.853± 0.06	89.477± 6.17	50.956 ±3.52
10	Kaempferol	0.659± 0.03	0.327± 0.02	94.080± 4.89	60.212±3.13
11	Apigenin	0.853± 0.05	1.532± 0.08	313.139± 16.60	148.648±708
12	Rhamnetin	2.241± 0.14	0.839± 0.05	2.480± 0.15	1.130 ±0.07
13	Chrysin	ND	0.857± 0.03	1.307± 0.07	2.482 ±0.13

Note: ND: not detected

4.3.2.2. Non-flavonoids phytochemicals

Twelve non- flavonoid phenolics were analysed by LC-MS/MS, the highest amount of non-flavonoid phenolics were detected for the liquorice from Sulaymaniyah which were tannic acid, p-coumaric acid, 4-OH Benzoic acid, salicylic acid, vanillin, trans-caffeic acid and gallic acid with concentrations of 1757.76, 612.773, 462.189, 444.727, 339.388, 277.951 and 110.220 µg/g, respectively. However, the most non-flavonoids phenolics from

Erbil's liquorice were tannic acid, p-cpumaric acid, 4-OH Benzoic acid, trans- caffeic acid and Salicylic acid with concentrations of 1471.641, 584.923, 304.688, 116.796 and 111.813 µg/g, respectively.

On the other hand, most Non-flavonoids phenolics from Şırnak (Turkish) liquorice were tannic acid, p-coumaric acid, vanillin, 4-OH benzoic acid and salicylic acid with concentrations of 2928.333, 465.036, 212.490, 80.699 and 78.432 µg/g, respectively. Finally, the most non-flavonoid phenolics from Ağrı (Turkish) liquorice were tannic acid and p-coumaric acid with concentrations of 353.044 and 118.772 µg/g.

Table 4.5. Non-flavonoids phenolics detected for *Glycyrrhiza glabra* L. roots grown in four locations

No.	Compounds	Concentration (µg/g)			
		Sulaymaniyah	Ağrı	Erbil	Şırnak
1	Gallic acid	110.220	34.111	36.742	8.948
2	Chlorogenic acid	27.103	1.312	1.433	0.852
3	Protocatechuic acid	60.060	41.57	38.386	38.386
4	Tannic acid	1757.76	353.044	1471.641	2928.333
5	trans- Caffeic acid	277.951	48.798	304.688	11.279
6	Vanillin	339.388	35.315	311.273	212.490
7	p-Coumaric acid	612.773	118.772	584.923	465.036
8	Rosmarinic acid	2.250	0.690	1.272	ND
9	4-OH Benzoic acid	462.189	27.665	116.796	80.699
10	Salicylic acid	444.727	24.464	111.813	78.432
11	Coumarin	2.556	1.647	2.443	1.520

Note: ND: not detected

Results showed the variation of active components concentration between all locations, this is might be due to numerous factors affect the polyphenol content of plants these include degree of ripeness at the time of harvest and environmental factors.

Polyphenolic content of the foods are greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall etc. The degree of ripeness considerably affects the concentrations and proportions of various polyphenols (Manach *et al.*, 2004).

Variation of phenolic concentration in licorice root affirms the influence of both harvest time and climate factors on production and release of these metabolites. The variation in content of phenolic compounds correlates with the metabolic balance between biosynthesis and further catabolism of plants and external environmental disturbances (Liu *et al*., 2010; Gao *et al*., 2011).

The variation of chemical components in *Glycyrrhiza glabra* L. between two locations was also confirmed by Astaf'eva and Sukhenko (2014). Many polyphenols, especially phenolic acids, are directly involved in the response of plants to different types of stress, they contribute to healing by lignifications of damaged areas possess antimicrobial properties, and their concentrations may increase after infection (Parr *et al*., 2000).

Oloumi and Hassibi (2011) also indicated that the variation of phytochemical concentrations might be due to several environmental factors, they Studied the correlation between some climate parameters and the content of phenolic compounds in roots of the content of flavonoids, anthocyanins and sugar in liquorice roots was correlated to soil pH. Total phenolic compounds, glycyrrhizin and sugar content showed the direct correlation with soil texture. Based on the results, it seems that tannins content is correlated with mean annual temperature.

4.4. Antimicrobial Activity of *Glycyrrhiza glabra* L. Root Extracts

The antimicrobial activity of *Glycyrrhiza glabra* L. was examined against four gram positive, four Gram-negative bacteria and three fungal species by using disc diffusion method. The various extracts of the of *Glycyrrhiza glabra* L. were statically significant by the differnet diameters of inhibition zones ($P < 0.05$) and statistically significant differences in inhibition zones were observed between some locations.

4.4.1. Antimicrobial effect on Gram-positive bacteria

4.4.1.1. Antimicrobial effect against *Staphylococcus aureus*

Glycyrrhiza glabra L. showed good anti-*staphylococcus* activity with inhibition zones of 12.33-19.33 mm. The effect of *Glycyrrhiza glabra* L. against *Staphylococcus aureus* were shown in Figure 4.2.

- **Conventional extraction**

The highest antimicrobial activity against *Staphylococcus aureus* was detected from Erbils liquorice with an inhibition zone of 19.00 mm while the lowest inhibitory zone was shown by ethanolic extract of the plant from Ağrı with an inhibition zone of 14.12 mm.

- **Microwave assisted extraction**

Like conventional extraction, the highest inhibition zone to ward *Staphylococcus aureus* was noticed by methanolic extract of sample from Şırnak with an inhibition zone of 18.66 mm followed by those from Sulaymaniyah and Erbil with inhibition zones of 18.00 and 15.33 mm respectively while the lowest inhibitory zone was detected for the ethanolic extract of liquorice from Ağrı with an inhibition zone of 12.67 mm. However, the aqueous extract of liquorice from both Şırnak and Ağrı didn't show inhibition zone whereas the liquorice from both Sulaymaniyah and Erbil showed inhibition zones of 12.33 and 13.66 mm, respectively.

- **Accelerated solvent extraction**

Only methanol and ethanol were employed with accelerated solvent extraction, with methanol, the highest inhibition zone was detected for the liquorice from Şırnak with inhibition zone of 19.33 mm followed by Sulaymaniyah with 18.67 mm while, the ethanolic extract of liquorice from Ağrı showed the lowest inhibition zone with 12.33 mm.

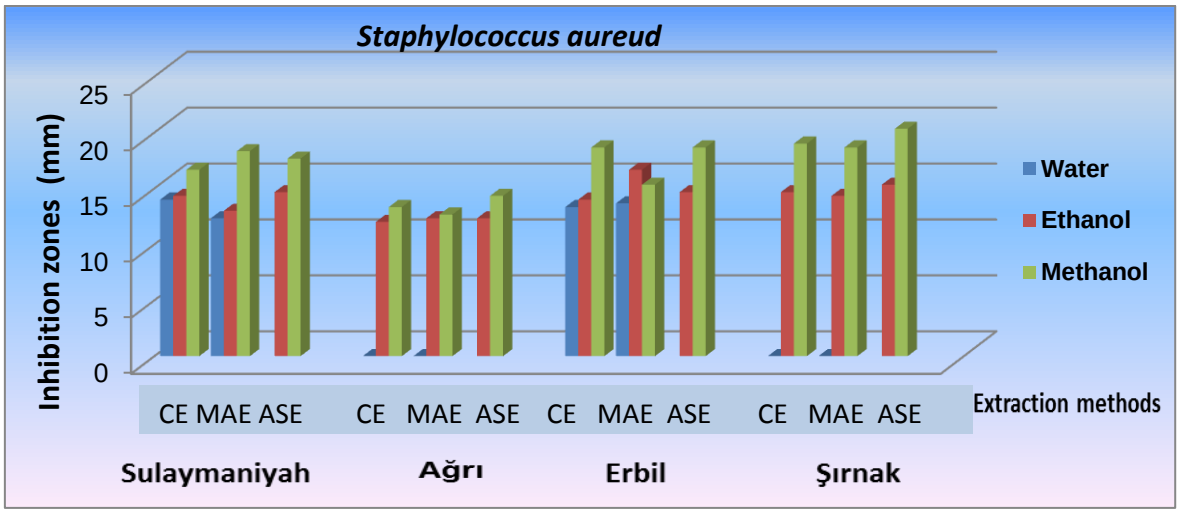

Figure 4.2. Effect of *Glycyrrhiza glabra* L. against *Staphylococcus aureus*

4.4.1.2. Antimicrobial effect against *Micrococcus luteus*

The effect of *Glycyrrhiza glabra* L. against *Micrococcus luteus* was illustrated in the Figure 4.3.

- **Conventional extraction**

Among methanolic extracts, the highest inhibition zone against *Micrococcus luteus* was detected for the liquorice from Şırnak with an inhibition zone of 22.33 mm. However, the aqueous extract of Turkish liquorice didn't show any inhibition zones whereas the samples from both Sulaymaniyah and Erbil exhibited inhibition zones of 16.33 and 15.33 mm, respectively.

- **Microwave assisted extraction**

The highest inhibition zone with microwave assisted extraction was noticed by methanolic extract of *Glycyrrhiza glabra* L. from Sulaymaniyah with an inhibition zone of 19.00 mm while the lowest inhibition zone was detected ethanolic extract of liquorice from Ağrı with an inhibition zone of 13.00 mm. On the other hand, the aqueous extract showed low inhibition zone compared with that of methanol and ethanol.

- **Accelerated solvent extraction**

The highest inhibition zone showed by methanolic extract of liquorice from Sulaymaniyah with an inhibition zone of 20.00 mm while the lowest inhibition zone was detected for the ethanolic extract of liquorice from Ağrı with an inhibition zone of 13.00 mm.

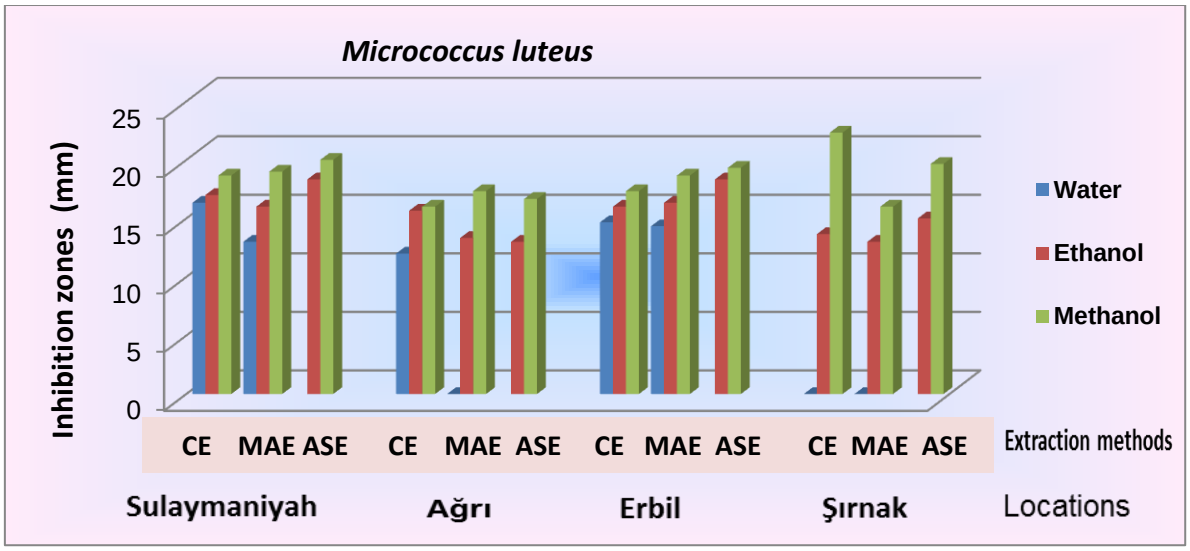

Figure 4.3. Effect of *Glycyrrhiza glabra* L. against *Micrococcus luteus*

4.4.1.3. Antimicrobial effect against *Bacillus subtilis*

Antimicrobial activity of *Glycyrrhiza glabra* L. against *Bacillus subtilis* were shown in Figure 4.4.

- **Conventional extraction**

The highest antimicrobial activity to ward *Bacillus subtilis* was detected for the methanolic extract of Şırnak's liquorice with an inhibition zone of 21.00 mm while the lowest inhibition zone was shown by ethanolic extract of liquorice collected from Ağrı with an inhibition zone of 12.66 mm. On the other hand, the aqueous etract of liquorice from both Erbil and Sulaymaniyah showed good anti-*Bacillus* activity with inhibition zones of 16.33 and 13.66 mm. However, the Turkish liquorice exhibited no inhibition zones against *Bacillus subtilis.*

- **Microwave assisted extraction**

The highest antimicrobial activity was detected for the methanolic extract of Şırnak's liquorice with an inhibition zone of 20.67 mm while the lowest inhibition zone was shown by methanolic extract of liquorice from Ağrı with inhibition zone of 14.33 mm.

- **Accelerated solvent extraction**

The liquorice from both Şırnak and Erbil exhibited the highest antimicrobial activity against *Bacillus subtilis* by ASE with inhibition zones of 20.33 and 19.00 mm. The antimicrobial activity of *Glycyrrhiza glabra* L. against *Micrococcus luteus* was shown in this figure.

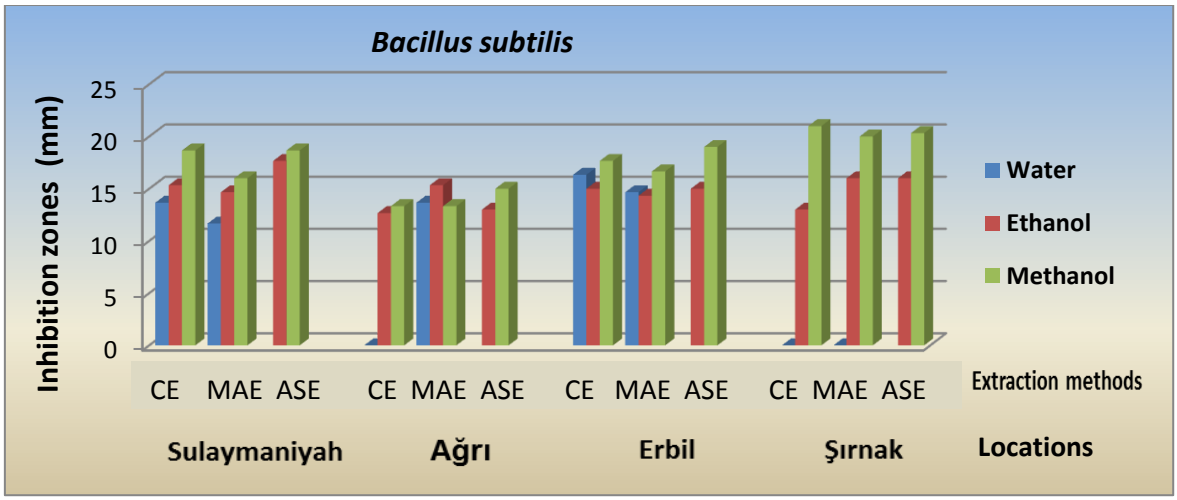

Figure 4.4. Effect of *Glycyrrhiza glabra* L. against *Bacilus subtilis*

4.4.1.4. Antimicrobial effect against *Bacillus megaterium*

Glycyrrhiza glabra L. showed good antimicrobial activity against *Bacillus megaterium* as shown in the Figure 4.5

- **Conventional extraction**

The highest antimicrobial activity against *Bacillus megaterium* was dtermined by methanolic extract of Şırnak's liquorice with an inhibition zone of 20.00 mm while the lowest inhibition zone was shown by ethanolic extract of liquorice from Ağrı with an inhibition zone of 12.33 mm. Moreover, the aqueous extract of liquorice from both Erbil and Sulaymaniyah also showed good activity against *Bacillus megaterium* with 15.33 and 13.67 mm, respectively.

- **Microwave assisted extraction**

The highest antimicrobial activity was detected for the methanolic extract of Şırnak's liquorice with an inhibition zone of 20.33 mm while the lowest inhibition zone was shown by ethanolic extract of liquorice from Ağrı with an inhibition zone of 12.67 mm.

- **Accelerated solvent extraction**

Methanolic extract of liquorice from both Şırnak and Sulaymaniyah exhibited the highest antimicrobial activity by ASE with inhibition zones of 20.00 and 19.00 mm, respectively.

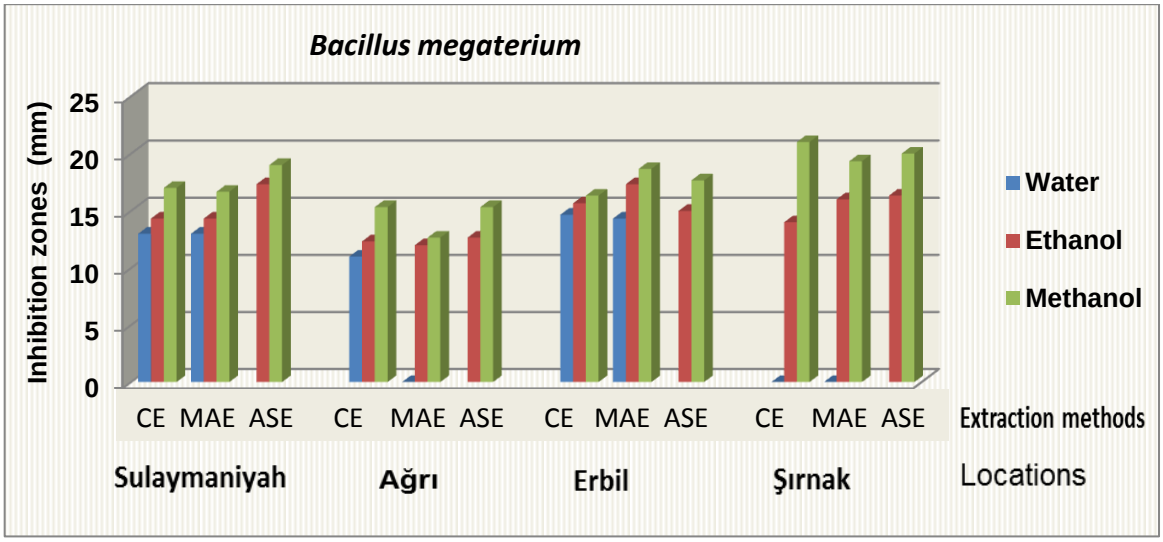

Figure 4.5. Effect of *Glycyrrhiza glabra* L. against *Bacilus megaterium*.

4.4.2. Antimicrobial effect on Gram-negative bacteria

4.4.2.1. Antimicrobial effect against *Klebsiella pneumiae*

The antimicrobial activity of *Glycyrrhiza glabra* L. to ward *Klebsiella pneumiae* is presented in Figure 4.6

- **Conventional extraction**

The highest antimicrobial activity to ward *Klebsiella pneumiae* was detected for the methanolic extract of liquorice from sulaymaniyah with an inhibition zone of 19.33 mm while the lowest inhibition zone was shown by aqueous extract of liquorice from Erbil with an inhibition zone of 11.33 mm. However, the water extraction of Turkish *Glycyrrhiza glabra* L. didn't show any effect to ward *Klebsiella pneumiae*.

- **Microwave assisted extraction**

The highest antimicrobial activity was detected for the methanolic extract of Şırnak's liquorice with an inhibition zone of 18.66 mm while the lowest inhibition zone was shown by ethanolic extract of liquorice from the same location with an inhibition zone of 12.00 mm.

- **Accelerated solvent extraction**

Glycyrrhiza glabra L. from Sulaymaniyah exhibited the highest antimicrobial activity by ASE with an inhibition zone of 21.00 mm.

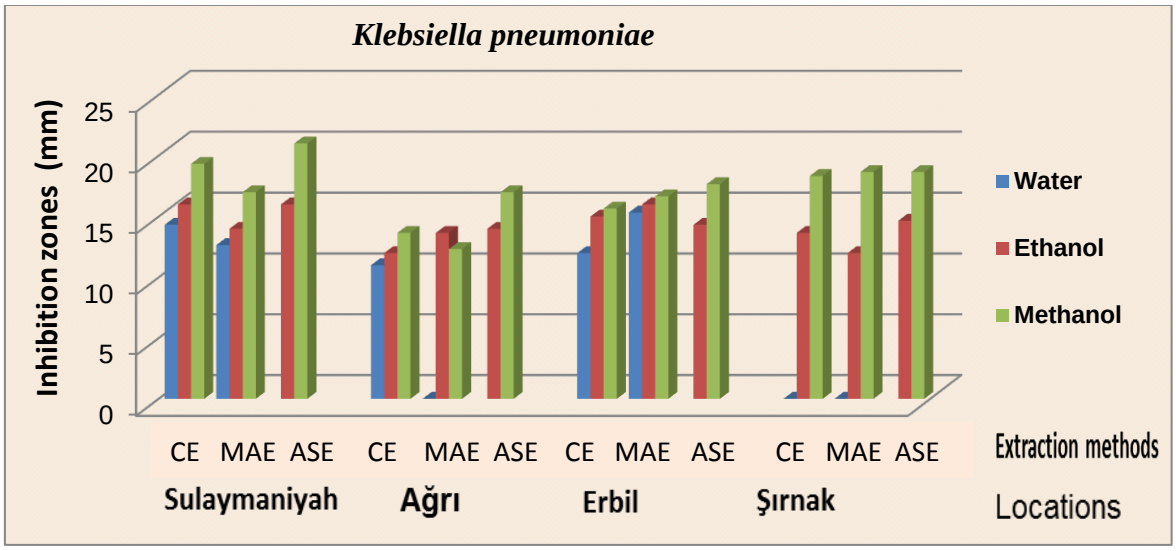

Figure 4.6. Effect of *Glycyrrhiza glabra* L. against *Klebsiella pneumoniae*

4.4.2.2. Antimicrobial effect against *Escherichia coli*

The inhibition zones against *Escherichia coli* were between 12.66 to 19.66 mm as shown in Figure 4.7.

- **Conventional extraction**

The highest antimicrobial activity against *Escherichia coli* with conventional extraction method was detected for the methanolic extract of liquorice from both Şırnak and Sulaymaniyah with inhibition zones of 19.00 and 18.33 mm, while the lowest inhibiton zone was shown by the ethanolic extract of *Glycyrrhiza glabra* L. from Ağrı with an inhibition zone of 13.66 mm

- **Microwave assisted extraction**

The highest antimicrobial activity was detected for the methanolic extract of liquorice from Sulaymaniyah with an inhibition zone of 18.66 mm while the lowest inhibition zone was shown by ethanolic extract of plant from Şırnak with an inhibition zone of 12.66 mm. However, the Turkish sample with aqueous extraction did not show any inhibition zones**.**

- **Accelerated solvent extraction**

The methanolic extract *Glycyrrhiza glabra* L. from Sulaymaniyah exhibited the highest antimicrobial activity against *E. coli* by ASE with an inhibition zone of 19.66 mm.

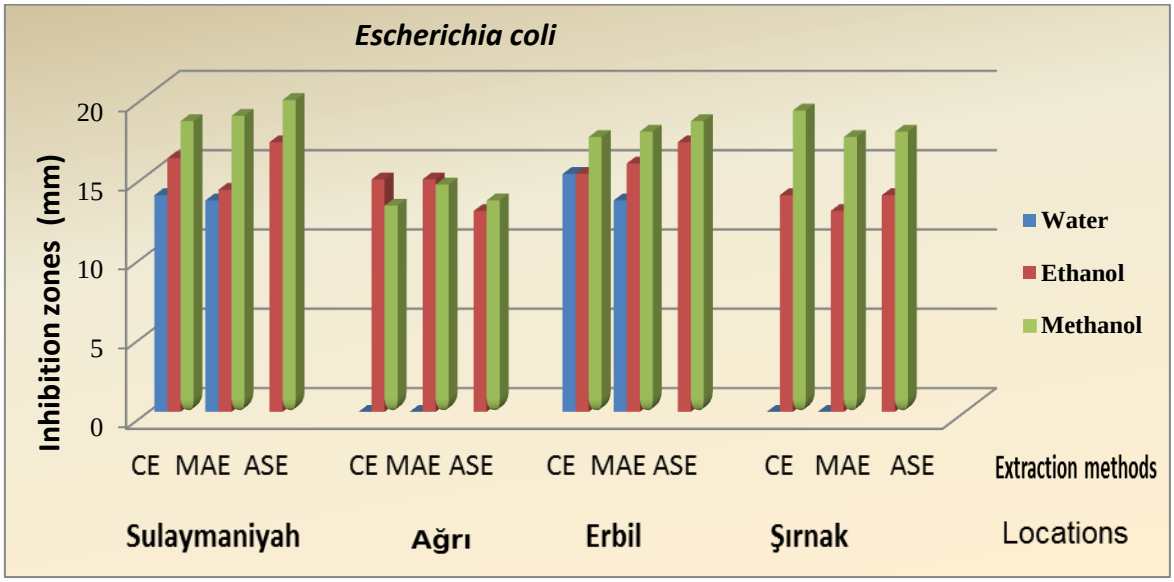

Figure 4.7. Effect of *Glycyrrhiza glabra* L. against *Escherichia coli*

4.4.2.3. Antimicrobial effect against Pseudomonas aeruginosa

The inhibition zones against *Pseudomonas aeruginosa* were between 12.66 -19.67 mm as illustrated in the Figure 4.8.

- **Conventional extraction**

The highest antimicrobial activity of *Glycyrrhiza glabra* L. against *Pseudomonas aeruginosa* with conventional extraction method was detected for the liquorice from Şırnak with an inhibition zone of 19.66 mm by using methanol as solvent while the lowest inhibiton zone was shown by ethanolic extract of liquorice from Ağrı with an inhibition zone of 12.66 mm.

- **Microwave assisted extraction**

The highest antimicrobial activity was detected for the methanolic extract of liquorice from Şırnak with an inhibition zone of 18.00 mm while the lowest inhibition zone was shown by aqueous extract of liquorice from both Erbil and Sulaymaniyah with an inhibition zone of 12.33 mm. However, the Turkish sample with aqueous extraction did not show any inhibition zone.

- **Accelerated solvent extraction**

Glycyrrhiza glabra L. from Şırnak exhibited the highest antimicrobial activity by ASE and methanol with an inhibition zone of 18.66 mm.

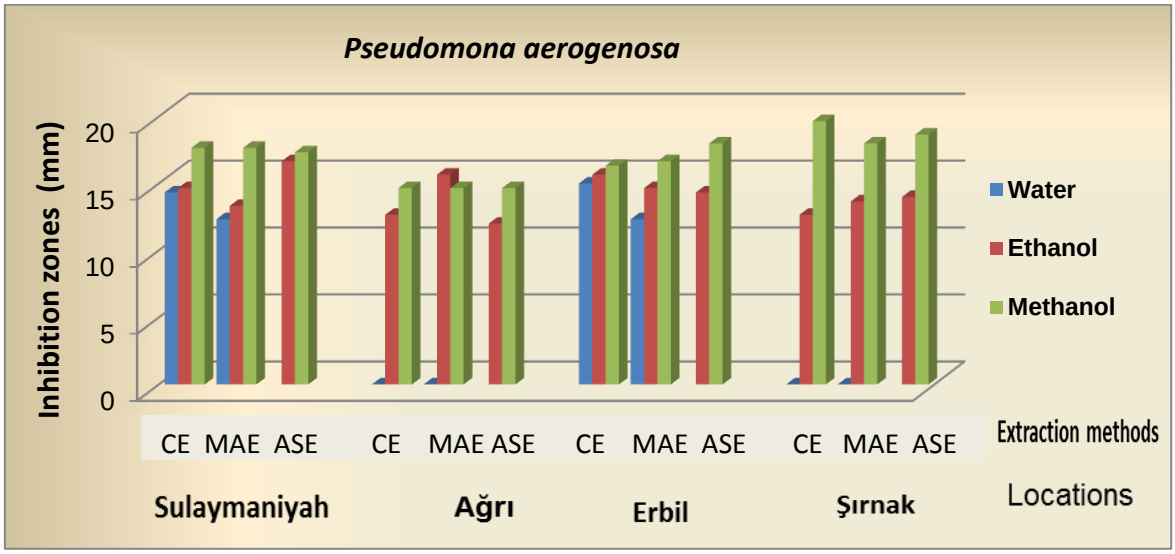

Figure 4.8. Effect of *Glycyrrhiza glabra* L. against *Pseudomonas aerugenosa*

4.4.2.4. Antimicrobial effect against *Enterococcus faecium*

The Figure 4.9. illustrates the inhibition zones against *Pseudomonas aeruginosa* were between 12.66 -19.33 mm

- **Conventional extraction**

The highest antimicrobial activity against *Pseudomonas aeruginosa* with conventional extraction method was detected for the methanolic extracts of *Glycyrrhiza glabra* L. from both Şırnak and Sulaymaniyah with inhibition zones of 19.66 and 17.33 mm while the lowest inhibitory zone was shown by ethanolic extract of plant from Ağrı with an inhibition zone of 12.66 mm.

- **Microwave assisted extraction**

The highest antimicrobial activity was detected from the methanolic extract of liquorice from Şırnak with an inhibition zone of 18.00 mm while the lowest inhibition zone was shown by ethanolic extract of liquorice from Şırnak with an inhibition zone of 13.66 mm. However, the liquorice from both Ağrı and Şırnak with aqueous extraction did not show any inhibition zones to ward *Enterococcus faecium*.

- **Accelerated solvent extraction**

The methanolic extract of *Glycyrrhiza glabra* L. from Şırnak exhibited the highest antimicrobial activity with an inhibition zone of 18.66 mm.

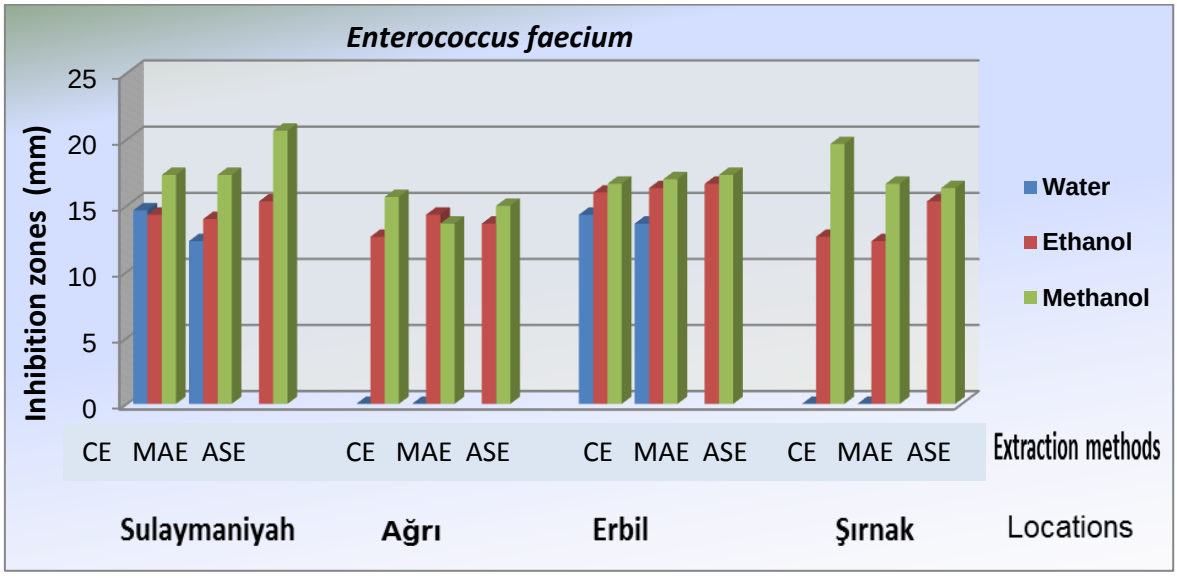

Figure 4.9. Effect of Glycyrrhiza glabra L. against Enterococcus fecium

The activity of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different places in the world. The antimicrobial activity of

Glycyrrhiza glabra L. is well known (Demizu *et al*., 1988; Okada *et al*., 1989; Haraguchi *et al*., 1998) and glabridin has been reported to possess antibacterial activities against some strains (Mitscher *et al*., 1980; Fukai *et al*., 2002b) and also presence of many phytochemical compounds like tannins, flavonoids, alkaloids, saponins, terpenes and glycosides in *G. glabra* make the plant good antimicrobial plant (Syed *et al*., 2013).

In our study the different solvents showed different inhibition zones this was confirmed by Kushwah *et al*. (2014) which investigated antimicrobial activity of extracts of *Glycyrrhiza glabra* L. against *Staphylococcus aureus* using petrolium ether, acetone, ethanol and water as solvents, the result of water extraction showed the lowest anti-*Staphylococcus* activity, this was parallel with the current study while the ethanolic extract showed the high inhibition zone. Furthermore, it was observed that methanolic extract exhibited the highest antimicrobial and antifungal effects.

In the present study, the alcoholic extract of *Glycyrrhiza glabra* L. exhibited higher inhibition zone comparing with the extraction with water. This was agreed with the previous study reported by Ajagannanavar *et al*. (2014) and the reason may be better solubility of the liquorice compounds in alcohol.

On the other hand, the effect of *Glycyrrhiza glabra* L. against both Gram-positive and Gram-negative bacteria was confirmed by Nitalikar *et al*. (2010), they had studied the antibacterial activity of *Glycyrrhiza glabra* L. root extract against two Gram-positive and two Gram-negative bacteria using well diffusion and by using different solvennts (ethanol, aceton and chloroform. Their results of the inhibition zones with ethanolic etract were higher than the results of current study. Moreover, he reported that there were an important effect of solvent on inhibition zone, as well.

Antimicrobial activity of methanolic extract of *Glycyrrhiza glabra* L. was also confirmed by Sultana *et al*. (2010) against *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus subtilis* and *Escherichia coli* the result of inhibition zones were close to our results.

4.4.3. Antifungal effect

Candida albicans, Candida utilis and Yarrowia lipolytica were used for evaluating antifungal activity of *Glycyrrhiza glabra* L in the current study.

4.4.3.1. Antifungal effect against *Candida albicans*

All extraction methoads and solvents exhibited high inhibition zones against *Candida albicans* except the water extraction by Şırnak's liquorice. The inhibition zones against *Candida albicans* were between 14.00 to 29.33 mm (Figure 4.10).

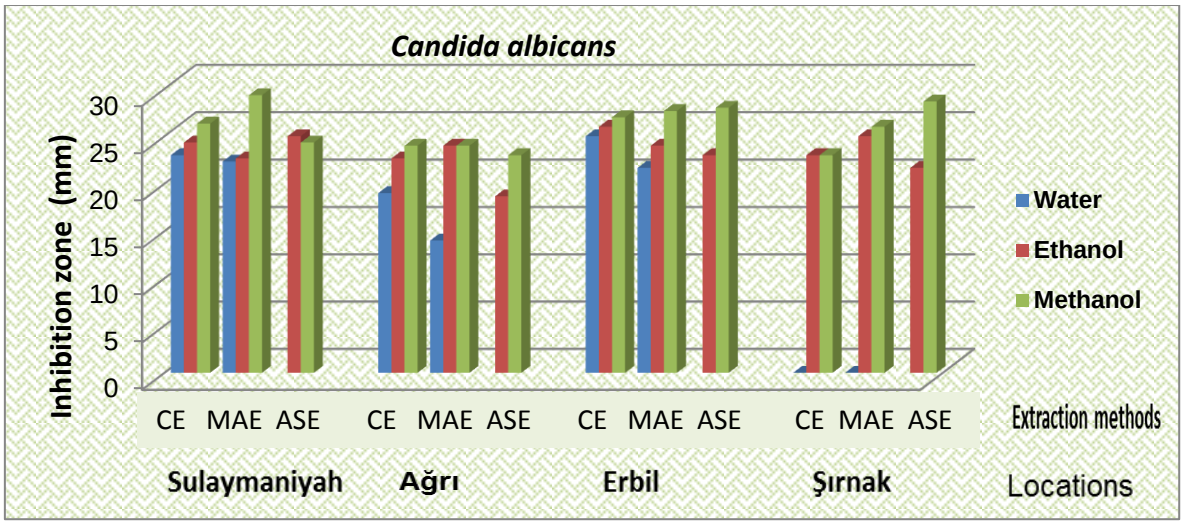

Figure 4.10. Effect of *Glycyrrhiza glabra* L. against *Candida albicans*

4.4.3.2. Antifungal effect against *Candida utilis* and *Yarrowia liplytica*

As shown in Figure 4.11. and Figure 4.12. Turkish *Glycyrrhiza glabra* L. didn't show any effect on *Candida utilis* and *Yarrowia lipolytica* while the methanolic extract of Iraqian *Glycyrrhiza glabra* L showed low inhibition zones.

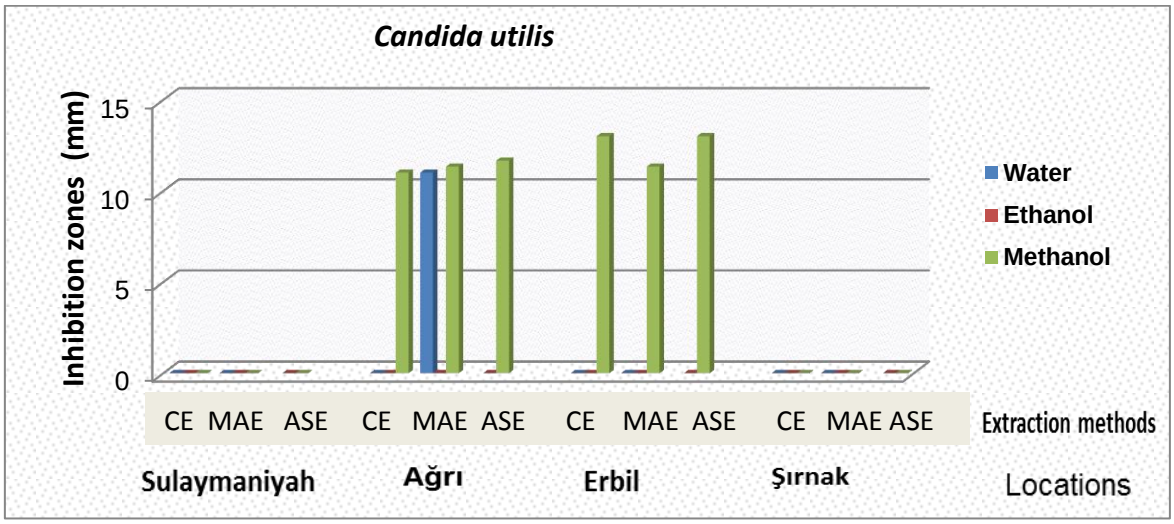

Figure 4.11. Effect of *Glycyrrhiza glabra* L. against *Candida utilis*

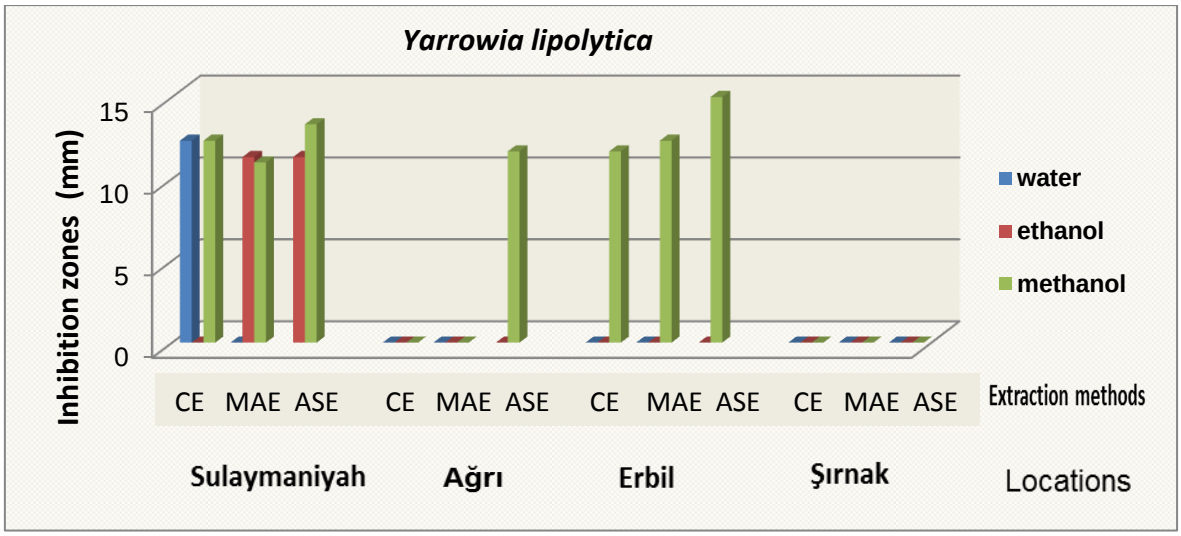

Figure 4.12. Effect of *Glycyrrhiza glabra* L. against *Yarrowia lipolytica*

The inhibition zones of positive and negative controls against micro-organisms (bacteria and fungi) were also illustrated in the Table 4.6.

Over the past two decades fungal infections have evolved into important cause of morbidity and mortality in modern medicine. The prevalence of resistance to antifungal agents has significantly increased. So, it makes necessary to discover new classes of antifungal compounds to treat fungal infections. The research on natural products derived compounds has accelerated in recent years due to their importance in drug discovery (Da Silva *et al*., 2011).

In our study, methanolic and ethanolic extracts of *Glycyrrhiza glabra* L. exhibited the highest antifungal activity against *Candida albicans* and this was proven by Tharkar *et al*. (2010) which investigated the hydro alcoholic extract of *Glycyrrhiza glabra* L. for anti-fungal activity against *Candida albicans* conventionally and by direct bioautography. Zone of inhibition for *G. glabra* L. was almost the same of our study with inhibition zone of 23.83 mm. Geetha and Roy (2013) also investigated mycotic activity of ethanolic extract of *Glycyrrhiza glabra* L. with the highest concentration showed an inhibitory zone of 20.00 mm against *Candida albicans*.

Table 4.6. Inhibition zones (mm) detected for the negative and positive controls

Micro-organisms	Negative controls			Positive controls		
	Water	Ethanol	Methanol	Gentamycin	Ampicillin	Nystatin
S. aureus cowan1	ND	ND	ND	37.33±0.58	8.67±0.56	ND
M. luteus LA2971	ND	ND	ND	34.33±0.58	6.33±0.58	ND
B. subtilis IMG 22	ND	ND	ND	41.66±1.52	5.67±0.58	ND
B. megaterium DSM32	ND	ND	ND	31.33±1.55	ND	ND
K. pneumonia FMC5	ND	ND	ND	44.66±1.15	ND	ND
E. coli DM	ND	ND	ND	36.33±1.53	ND	ND
P. aeruginosa DSM50071	ND	ND	ND	37.33±1.53	6.33±0.57	ND
E. faecium Clinic isolate	ND	ND	ND	33.66±1.15	6.33±1.52	ND
C. albicans ATCC1023	ND	ND	ND	ND	ND	15.67±0.58
C. utilis NRRL-Y-900	ND	ND	ND	ND	ND	18.66±0.58
Y. lipolytica	ND	ND	ND	ND	ND	13.66±0.58

Note: The values presented as mean ± SD of three replication; ND: not detected

4.4. Free Radical Scavenging Acctivity of *Glycyrrhiza glabra* L.

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is considered as a quick method used by most of the researchers to determine antioxidant activities.

DPPH is a free radical that keeps its stability in aqueous or ethanolic solutions. It accepts an electron or hydrogen ion to become a stable diamagnetic molecule. Thus, DPPH is occasionally used as a substrate to determine antioxidant activity of compounds. In an experiment, change in absorbance of DPPH radicals was monitored that was indicated by

discoloration from purple to yellow through free radical scavenging in various extracts of *Glycyrrhiza glabra* L. as shown in Table 4.7. The DPPH scavenging power of the whole exracts studied here were higher than that of BHT, this indicated a pewrful free radical scavenging activity of the plant.

The highest DPPH scavenging activity value for *Glycyrrhiza glabra* L. root with 0.1 mL was detected for the methanol extract by ASE method (98.57%) of Sulaymaniyah (Iraqian) *Glycyrrhiza glabra* L. root and the lowest value was detected from Şırnak (Turkish) one with (86.60%), which was obtained by using conventional extraction with ethanol.

The mechanism of radical scavenging activity of *Glycyrrhiza glabra* L. could be due to the presence of polyphenolic compounds. Polyphenolic compounds are considered as responsible for radical scavenging activity, due to the ability of their hydrogen atom donation to active free radicals (Ho *et al*., 1994).

Literatury studies confirmed the antioxidant power of *Glycyrrhiza glabra* L. by Lateef *et al*. (2012) showed that Crude methanolic extract of *Glycyrrhiza glabra* L. at the concentration of 62.5 µg, exhibited (91.3%) scavenging activity as compared to standard (BHA) with (58.8%) activity at the same concentration.

Another study by Asan-Ozusaglam and Karakoca (2014) monitored the change in absorbance of DPPH radicals and at the concentration of 400 µg/mL, the DCM extract of the root of *G. glabra* showed higher DPPH scavenging activity (92.58%) than n-hexane extract (37.53%). The radical scavenging of BHT was determined as (91.19%) at 200 µg/mL concentration. Polyphenolic compounds are considered as responsible for radical scavenging activity, due to the ability of their hydrogen atom donation to active free radicals (Ho *et al*., 1994).

Table 4.7. DPPH free radical scavenging ability of root extracts of *Glycyrrhiza glabra* L. compared with the standard BHT

			DPPH Radical Scavenging (%)					
			Glycyrrhiza glabra L. vol. (mL)			BHT vol. (mL)		
Locs.	Solvents	Methods	0.1	0.2	0.3	0.1	0.2	0.3
Sulaymaniyah (Iraq)	Methanol	ASE	98.01	97.77	97.69	90.47	91.90	90
	Methanol	MAE	98.49	98.33	98.17	90.47	91.90	90
	Methanol	CE	98.57	98.09	97.85	90.47	91.90	90
	Ethanol	ASE	96.65	97.67	97.39	67.44	65.67	78.32
	Ethanol	MAE	97.39	97.30	93.30	67.44	65.67	78.32
	Ethanol	CE	97.11	94.60	88.65	67.44	65.67	78.32
Ağrı (Turkey)	Methanol	ASE	97.77	97.61	96.19	90.47	91.90	90
	Methanol	MAE	97.77	97.30	96.58	90.47	91.90	90
	Methanol	CE	98.25	97.22	96.58	90.47	91.90	90
	Ethanol	ASE	96.93	96.37	95.53	67.44	65.67	78.32
	Ethanol	MAE	96.18	95.53	94.60	67.44	65.67	78.32
	Ethanol	CE	96.93	96.46	95.90	67.44	65.67	78.32
Erbil (Iraq)	Methanol	ASE	98.01	97.22	97.22	90.47	91.90	90
	Methanol	MAE	98.35	97.62	98.25	90.47	91.90	90
	Methanol	CE	98.25	98.10	97.56	90.47	91.90	90
	Ethanol	ASE	97.21	96.93	96.93	67.44	65.67	78.32
	Ethanol	MAE	96.28	95.26	95.26	67.44	65.67	78.32
	Ethanol	CE	97.12	96.74	95.53	67.44	65.67	78.32
Şırnak (Turkey)	Methanol	ASE	93.25	91.20	89.29	90.47	91.90	90
	Methanol	MAE	92.86	89.69	87.70	90.47	91.90	90
	Methanol	CE	93.25	90.87	88.50	90.47	91.90	90
	Ethanol	ASE	89.95	86.79	83.72	67.44	65.67	78.32
	Ethanol	MAE	89.49	87.53	84.93	67.44	65.67	78.32
	Ethanol	CE	86.60	84.93	83.72	67.44	65.67	78.32

Note: CE: conventional extraction; MAE: microwave assisted extraction; ASE: accelerated solvent extraction;BHT : butylated hydroxytoluene; DPPH

5. CONCLUSIONS

The present study was undertaken to find out antimicrobial and antioxidant activities of *Glycyrrhiza glabra* L. root along with determination of yield percentage, total condensed tannin and identification phenolic compounds constitution.

Extractions showed different yield percentages among methods, solvents and locations. Accelerated solven extraction with methanol exhibited the highest yield percentages among methods and solvents for all locations. Further, methanolic extract of Erbil's liquorice showed the highest yield percentages using ASE, at the same time the highest amount of condensed tannin detected for the same location location .

This plant has a great medicinal value as it has been reported to have useful phytochemical constituents including of non-phenolic, phenolic acid and flavanoids. In this study 27 phytochemicals were isolated as plant phenolics and non-phenolics but the amount of these phytochemicals were vrious ammong locations and this is might be due to internal and environmental factors.

Antioxidant activity of *Glycyrrhiza glabra* L. root extracts were detected by means of (DPPH) free radical scavenging assays. The plants contain high phenol & flavonoid which indicates that the roots have antioxidant effects. The DPPH scavenging power of the whole exracts studied here were higher than that of BHT, this indicated a pewrful free radical scavenging activity of the plant.

The antimicrobial activity of *Glycyrrhiza glabra* L. was evaluated based on the inhibition zone using the disc diffusion assay, the plant contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. The extracts possessed high inhibitory effect against tested micro-organisms. Among all the extracts, the methanolic extract had a better antibacterial and antifungal activity against micro-organisms. Our results support the idea that *Glycyrrhiza glabra* L. extracts are candidate for treatment of infectious diseases, clinical trials will be required to confirm its antimicrobial action and general safety.

Depending on our results, the extracts were obtained by the ASE method from the roots of *Glycyrrhiza glabra* L. contained larger amount of bioactive compound (total phenols and flavonoid). Also, it showed stronger antimicrobial activity.

Therefore, it could be concluded that methanolic extract of *Glycyrrhiza glabra* L. roots could be used as an available source of natural antioxidants and antimicrobial. But it was interesting that aqueous extract of Turkish *G. glabra* L. root did not show any antibacterial activity in contrast with Iraqian *G. glabra.* Additionally, we obtained different amount of phytochemicals and different yield percentages among the locations, showing that geographical locations might affects the plant production of phytochemicals.

REFERENCES

Abreu, A. C., McBain, A. J., Simoes, M., 2012. Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Reports*, 29(9), 1007-1021.

Acharya, S. K., Dasarathy, S., Tandon, A., Joshi, Y. K., Tandon, B. N., 1993. A preliminary open trial on interferon stimulator (SNMC) derived from *Glycyrrhiza glabra* L. in the treatment of subacute hepatic failure. *The Indian Journal of Medical Research*, 98, 69-74.

Akamatsu, H., Komura, J., Asada, Y., Niwa, Y., 1991. Mechanism of anti-inflammatory action of glycyrrhizin: effect on neutrophil functions including reactive oxygen species generation. *Planta Medica*, 57(2), 119-121.

Akao, T., Akao, T., Hattori, M., Kanaoka, M., Yamamoto, K., Namba, T., Kobashi, K., 1991. Hydrolysis of glycyrrhizin to 18β-glycyrrhetyl monoglucuronide by lysosomal β-D-glucuronidase of animal livers. *Biochemical Pharmacology*, 41(6), 1025-1029.

Ajagannanavar, S. L., Battur, H., Shamarao, S., Sivakumar, V., Patil, P. U., Shanavas, P. 2014. Effect of aqueous and alcoholic licorice (*Glycyrrhiza glabra* L.) root extract against *Streptococcus mutans* and *Lactobacillus acidophilus* in comparison to chlorhexidine: an in vitro study. *Journal of International Oral Health*: *JIOH*, 6(4), 29.

Al-Mathkhury, H. J. F., Ali, A. S., Ghafil, J. A., 2011. Antagonistic effect of bacteriocin against urinary catheter associated *Pseudomonas aeruginosa* biofilm. *North American Journal of Medical Sciences*, 3(8), 367.

Al-Obaidi, O. H. S., 2013. Aqueous and alcohol extraction of plant (*Glycyrrhiza glabra* L.) and activity study against bacteria and cancer (Cell Line RD). *European Chemical Bulletin*, *3*(2), 133-137.

Alsina, A., Mason, M., Uphoff, R. A., Riggsby, W. S., Becker, J. M., Murphy, D., 1988. Catheter-associated Candida utilis fungemia in a patient with acquired immunodeficiency syndrome: species verification with a molecular probe. *Journal of Clinical Microbiology*, 26(4), 621-624.

Alviano, D. S., Alviano, C. S., 2009. Plant extracts: search for new alternatives to treat microbial diseases. *Current Pharmaceutical Biotechnology*, 10(1), 106-121.

Aly, A. M., Al-Alousi, L., Salem, H. A., 2005. Licorice: a possible anti-inflammatory and anti-ulcer drug. *American Association of Pharmaceutical Scientists*, 6(1), E74-E82.

Armanini, D., Mattarello, M. J., Fiore, C., Bonanni, G., Scaroni, C., Sartorato, P., Palermo, M., 2004. Licorice reduces serum testosterone in healthy women. *Steroids*, 69(11), 763-766.

Asan-Ozusaglam, M., Karakoca, A. N. D. K., 2014. Evaluation of biological activity and antioxidant capacity of Turkish licorice root extracts. *Romanian Biotechnological Letters*, 19, 8994-9005.

Astaf'eva, O. V., Sukhenko, L. T., 2014. Comparative analysis of antibacterial properties and chemical composition of *Glycyrrhiza glabra* L. from Astrakhan region (Russia) and Calabria region (Italy). *Bulletin of Experimental Biology and Medicine*, 156(6), 829-832.

Aziz, R. A., Sarmidi, M. R., Kumaresan, S., Taher, Z. M., Foo, D. C. Y., 2003. Phytochemical Processing: The Next Emerging Field in Chemical Engineering–Aspects and Opportunities. *Jurnal Kejuruteraan Kimia Malaysia*, 3, 45-60.

Babu, S. P. S., Sarkar, D., Ghosh, N. K., Saha, A., Sukul, N. C., Bhattacharya, S., 1997. Enhancement of membrane damage by saponins isolated from *Acacia auriculiformis*. *The Japanese Journal of Pharmacology*, 75(4), 451-454.

Badam, L., 1997. *In vitro* antiviral activity of indigenous glycyrrhizin, licorice and glycyrrhizic acid (Sigma) on Japanese encephalitis virus. *The Journal of Communicable Diseases*, 29(2), 91-99.

Balunas, M. J., Kinghorn, A. D., 2005. Drug discovery from medicinal plants. *Life Sciences*, 78(5), 431-441.

Bannerman, T. L., Peacock, S. J., Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L., Pfaller, M. A., 2006. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. *Manual of Clinical Microbiology*: Volume 1, (Ed. 9), 390-411.

Benthin, B., Danz, H., Hamburger, M., 1999. Pressurized liquid extraction of medicinal plants. *Journal of Chromatography A*, 837(1), 211-219.

Blach, J.F., Phyllis, R.G., 1997. Prescription for Nutrition Healing. 2nd edition. Avery publishing Group, N.Y.USA. P73 (Cited by Langer. 1998).

Blumenthal, M., Goldberg, A., Brinckmann, J., 2000. Herbal Medicine. Expanded Commission E monographs. *Integrative Medicine Communications*, 233–236.

Bougnoux, M. E., Gueho, E., Potocka, A. C., 1993. Resolutive *Candida utilis* fungemia in a nonneutropenic patient. *Journal of Clinical Microbiology*, 31(6), 1644-1645.

Burnham, R. J., Johnson, K. R., 2004. South American palaeobotany and the origins of neotropical rainforests. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359(1450), 1595-1610.

Catalano, S., Cioni, P. L., Panizzi, L., Morelli, I., 1998. Antimicrobial activity of extracts of Mutisia acuminata var. acuminata. *Journal of Ethnopharmacology*, 59(3), 207-209.

Chang, C. L., Park, T. H., Lee, E. Y., Lim, Y. T., Son, H. C., 2001. Recurrent self-limited fungemia caused by *Yarrowia lipolytica* in a patient with acute myelogenous leukemia. *Journal of Clinical Microbiology*, 39(3), 1200-1201.

Cheng, S. S., Huang, C. G., Chen, Y. J., Yu, J. J., Chen, W. J., Chang, S. T., 2009. Chemical compositions and larvicidal activities of leaf essential oils from two *Eucalyptus* species. *Bioresource Technology*, 100(1), 452-456.

Chin, Y. W., Jung, H. A., Liu, Y., Su, B. N., Castoro, J. A., Keller, W. J., Pereira, M. A., Kinghorn, A. D., 2007. Anti-oxidant constituents of the roots and stolons of licorice (*Glycyrrhiza glabra* L.). *Journal of Agricultural and Food Chemistry*, 55(12), 4691-4697.

Choi, E. M., 2005. The licorice root derived isoflavan glabridin increases the function of osteoblastic MC3T3-E1 cells. *Biochemical Pharmacology*, 70(3), 363-368.

Chopra, R. N., Chopra, I. C., 1958. Indigenous drugs of India. Academic publishers.

Cinatl, J., Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H., Doerr, H. W., 2003. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *The Lancet*, 361(9374), 2045-2046.

Ciocan, I. D., Bara, I. I., 2007, Plant products as antimicrobial agents, Molecular Biology, TOM VIII, p: 151-156.

Clatworthy, A. E., Pierson, E., Hung, D. T., 2007. Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology*, 3(9), 541-548.

Clinical and Laboratory Standards Institute (CLSI)., 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-Ninth Edition. CLSI document M07-A9. Wayne, PA.

Clayton, Y. M., Noble, W. C.,1966. Observations on the epidemiology of *Candida albicans*. *Journal of Clinical Pathology*, 19(1), 76.

Conner, D. E., 1993. Naturally occurring compounds. Food science and technology-New York-Marcel Dekker-, 441-441.

Cowan, M. M., 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.

Crance, J. M., Scaramozzino, N., Jouan, A., Garin, D., 2003. Interferon, ribavirin, 6-azauridine and glycyrrhizin: antiviral compounds active against pathogenic flaviviruses. *Antiviral Research*, 58(1), 73-79.

Croteau, R., Kutchan, T. M., Lewis, N. G., 2000. Natural products (secondary metabolites). *Biochemistry and Molecular Biology of Plants*, 24, 1250-1319.

Cui, Y. M., Ao, M. Z., Li, W., Yu, L. J., 2008. Effect of glabridin from *Glycyrrhiza glabra* L. on learning and memory in mice. *Planta Medica*, 74(4), 377-380.

Cushnie, T. T., Lamb, A. J., 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, 26(5), 343-356.

Da Silva, C. M., da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, C. V., de Fátima, Â., 2011. Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced research*, 2(1), 1-8.

Darwish, R. M., Aburjai, T. A., 2010. Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli*. *BMC Complementary and Alternative medicine*, 10(1), 9.

De Clercq, E., 2000. Current lead natural products for the chemotherapy of human immunodeficiency virus (HIV) infection. *Medicinal Research Reviews*, 20(5), 323-349.

De Simone, F., Aquino, R., De Tommasi, N., Mahamood, N., Piacente, S., Pizza, C., 2001. Anti-HIV aromatic compounds from higher plants in bioactive compounds from natural sources. Corrado Tringali.

Dehpour, A. R., Zolfaghari, M. E., Samadian, T., Kobarfard, F., Faizi, M., Assari, M., 1995. Antiulcer activities of liquorice and its derivatives in experimental gastric lesion induced by ibuprofen in rats. *International Journal of Pharmaceutics*, 119(2), 133-138.

Demizu, S., Kajiyama, K., Takahashi, K., Hiraga, Y., Yamamoto, S., Tamura, Y., okada, K., Kinoshita, T., 1988. Antioxidant and antimicrobial constituents of licorice: Isolation and structure elucidation of a new benzofuran derivative. *Chemical and Pharmaceutical Bulletin*, 36(9), 3474-3479.

Dhingra, D., Parle, M., Kulkarni, S. K., 2004. Memory enhancing activity of *Glycyrrhiza glabra* L. in mice. *Journal of Ethnopharmacology*, 91(2), 361-365.

Donders, G. G. G., Bellen, G., Rezeberga, D., 2011. Aerobic vaginitis in pregnancy. BJOG: *An International Journal of Obstetrics & Gynaecology*, 118(10), 1163-1170.

Eliopoulos, G. M., Cosgrove, S. E., Carmeli, Y., 2003. The impact of antimicrobial resistance on health and economic outcomes. *Clinical Infectious Diseases*, 36(11), 1433-1437.

Ertaş, A., Boğa, M., Yılmaz, M. A., Yeşil, Y., Haşimi, N., Kaya, M. S., Temel, H., Kolak, U., 2015. Chemical compositions by using LC-MS/MS and GC-MS and biological activities of Sedum sediforme (Jacq.) Pau. *Journal of Agricultural and Food Chemistry*, *62*(20), 4601-4609.

Ertaş, A., Gören, A. C., Boğa, M., Yeşil, Y., Kolak, U., 2014b. Essential oil compositions and anticholinesterase activities of two edible plants *Tragopogon latifolius* var. angustifolius and *Lycopsis orientalis*. *Natural Product Research*, 28(17), 1405-1408.

Ertaş, A., Yilmaz, M. A., Firat, M., 2014a. Chemical profile by LC–MS/MS, GC/MS and antioxidant activities of the essential oils and crude extracts of two *Euphorbia* species. *Natural Product Research*, 29(6), 529-534.

Evans, W. C., 2009. Trease and Evans' pharmacognosy. Elsevier Health Sciences. ISBN:978-0-7020-2933-2.

Fatima, A., Gupta, V. K., Luqman, S., Negi, A. S., Kumar, J. K., Shanker, K., Saikia, D., Srivastava, S., Darokar, P. M., Khanuja, S. P., 2009. Antifungal activity of *Glycyrrhiza glabra* L. extracts and its active constituent glabridin. *Phytotherapy Research*, 23(8), 1190-1193.

FDA, 2012 Bad bug book: Foodborne pathogenic micro-organisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring, p. 87–92. URL (access date: 27. 03. 2013) http://www.fda.gov/Food/FoodborneIllness Contaminants/CausesOfIllnessBadBugBook/ucm2006773.htm. Accessed 27 March 2013.

Fraga, C. G., 2010. Plant Phenolics and Human Health: Biochemistry.*Nutrition, and Pharmacology. Wiley, Hoboken, NJ*.

Franco, D., Sineiro, J., Rubilar, M., Sánchez, M., Jerez, M., Pinelo, M., Costoya, N., Núñez, M. J., 2008. Polyphenols from plant materials: extraction and antioxidant power. *The Electronic Journal of Environmental, Agricultural and Food Chemistry*, 7, 3210-3216.

Fresco, P., Borges, F., Marques, M. P. M., Diniz, C., 2010. The anticancer properties of dietary polyphenols and its relation with apoptosis. *Current Pharmaceutical Design*, 16(1), 114-134.

Fu, B., Liu, J., Li, H., Li, L., Lee, F. S., Wang, X., 2005. The application of macroporous resins in the separation of licorice flavonoids and glycyrrhizic acid. *Journal of Chromatography* A, 1089(1), 18-24.

Fuhrman, B., Buch, S., Vaya, J., Belinky, P. A., Coleman, R., Hayek, T., Aviram, M., 1997. Licorice extract and its major polyphenol glabridin protect low-density lipoprotein against lipid peroxidation: *in vitro* and ex vivo studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *The American Journal of Clinical Nutrition*, 66(2), 267-275.

Fuhrman, B., Volkova, N., Kaplan, M., Presser, D., Attias, J., Hayek, T., Aviram, M., 2002. Antiatherosclerotic effects of licorice extract supplementation on hypercholesterolemic patients: increased resistance of LDL to atherogenic modifications, reduced plasma lipid levels, and decreased systolic blood pressure. *Nutrition*, 18(3), 268-273.

Fujisawa, Y., Sakamoto, M., Matsushita, M., Fujita, T., Nishioka, K., 2000. Glycyrrhizin inhibits the lytic pathway of complement—possible mechanism of its anti-inflammatory effect on liver cells in viral hepatitis. *Microbiology and Immunology*, 44(9), 799-804.

Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., Nomura, T., 2002b. Anti-*Helicobacter pylori* flavonoids from licorice extract. *Life Sciences*, 71(12), 1449-1463.

Fukai, T., Ali, M., Kaitou, K., Kanda, T., Terada, S., Nomura, T., 2002a. Anti-*Helicobacter pylori* flavonoids from licorice extract. Life Sciences 71, 1449–1463.

Gao, C. Y., Lu, Y. H., Tian, C. R., Xu, J. G., Guo, X. P., Zhou, R., Hao, G., 2011. Main nutrients, phenolics, antioxidant activity, DNA damage protective effect and microstructure of *Sphallerocarpus gracilis* root at different harvest time. *Food Chemistry*, 127(2), 615-622.

Garcia-Arribas, M. L., de la Rosa, M. C., Mosso, M. A., 1985. Characterization of the strains of *Bacillus* isolated from orally administered solid drugs. *Pharmaceutica acta Helvetiae*, 61(10-11), 303-307.

Gardam, M. A., 2000. Is methicillin-resistant *Staphylococcus aureus* an emerging community pathogen? A review of the literature. *The Canadian Journal of Infectious Diseases*, 11(4), 202.

Gill, D. M., 1982. Bacterial toxins: a table of lethal amounts. *Microbiological Reviews*, 46(1), 86-94.

Goettsch, W., Van Pelt, W., Nagelkerke, N., Hendrix, M. G. R., Buiting, A. G. M., Petit, P. L., Sabbe, L. J. M., van Griethuysen, A. J. A., De Neeling, A. J., 2000. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. *Journal of Antimicrobial Chemotherapy*, 46(2), 223-228.

Gonçalves, C., Lopes, M., Ferreira, J. P., Belo, I., 2009. Biological treatment of olive mill wastewater by non-conventional yeasts. *Bioresource Technology*, 100(15), 3759-3763.

Griffin, S. G., Wyllie, S. G., Markham, J. L., Leach, D. N., 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Fragrance Journal*, 14(5), 322-332.

Gülçın, İ., Okay, M., Kıreççı, E., Küfrevıoğlu, Ö.İ., 2003. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. *Food Chemistry*, 83: 371–382.

Gülhan, T., Aksakal, A., Ekin, I. H., Savaşan, S., Boynukara, B., 2007. Virulence factors of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from humans and pets. *Turkish Journal of Veterinary and Animal Sciences*, 30(5), 477-482.

Gupta, A., Maheshwari, D. K., Khandelwal, G., 2013. Antibacterial activity of *Glycyrrhiza glabra* roots against certain Gram-positive and Gram-negative bacterial strains. *Journal of Applied and Natural Science*, 5(2), 459-464.

Gupta, L. M., Raina, R., 1998. Side effects of some medicinal plants. *Current Science*, 75(9), 897-900.

Hahn, N. I., 1998. Are phytoestrogens nature's cure for what ails us? A look at the research. *Journal of the American Dietetic Association*, 98(9), 974-976.

Halliwell, B., Murcia, M. A., Chirico, S., Aruoma, O. I. 1995. Free radicals and antioxidants in food and *in vivo*: what they do and how they work. *Critical Reviews in Food Science & Nutrition*, 35(1-2), 7-20.

Halliwell, B.,1995, January. How to characterize an antioxidant: an update. *Biochemical Society Symposia*, 61, 73-101.

Hammerschmidt, R., 1999. Phytoalexins: What have we learned after 60 years?. *Annual Review of Phytopathology*, 37(1), 285-306.

Haraguchi, H., 2001. Antioxidative plant constituents. In Bioactive Compounds from Natural Sources: Isolation, Characterization and Biological Properties, Tringali C (ed.). Taylor and Francis: New York, pp: 348-352.

Haraguchi, H., Tanimoto, K., Tamura, Y., Mizutani, K., Kinoshita, T., 1998. Mode of antibacterial action of retrochalcones from Glycyrrhiza inflata. *Phytochemistry*, 48(1), 125-129.

Harborne, J. B., Williams, C. A., 2000. Advances in flavonoid research since 1992. *Phytochemistry*, 55(6), 481-504.

Hasler, C. M., Blumberg, J. B., 1999. Introduction. *The Journal of Nutrition*, 129(3), 756S-757S.

Hattori M, Sakamoto T, Yamagishi T, Sakamoto, K., Konishi, K., Kobashi, K., Namba, T., 1985. Metabolism of glycyrrhizin by human intestinal flora. II. Isolation and characterization of human intestinal bacteria capable of metabolizing glycyrrhizin and related compounds. *Chemical & Pharmaceutical Bulletin*, 33(1), 210-217.

Hemaiswarya, S., Kruthiventi, A. K., Doble, M., 2008. Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*, 15(8), 639-652.

Heng, L., Vincken, J. P., van Koningsveld, G., Legger, A., Gruppen, H., van Boekel, T., Roozen, j., Voragen, F., 2006. Bitterness of saponins and their content in dry peas. *Journal of the Science of Food and Agriculture*, 86(8), 1225-1231.

Hill A. F., 1952. Economic Botany. A textbook of useful plants and plant products. 2 nd edn. McGarw-Hill Book Company Inc, New York, USA.

Ho, C. T., Osawa, T., Huang, M.T., Rosen, R.T., 1994. Food Phytochemicals for Cancer Prevention II. Teas, Spices, and Herbs. *American Chemical Society*. Washington, 547: 132–143.

Hojo, H., Sato, J., 2002. Antifungal activity of licorice (*Glycyrrhiza glabra* L.) and potential applications in beverage. *Foods and Food Ingredients Journal of Japan*, 27-33.

Hossain, F.M.A., Hossain, M.D.T., Hossain, M.D.M., Buying, M.D.E., 2012. Characterized-ion of *Escherichia coli* isolated from migratory water fowls in Hakuluki Haor, Bangladesh, 2:30

Ito, A., Hayashi, N., Katayama, K., Hagiwara, H., Kasahara, A., Kashiwagi, T., Yoshihara, H., Fusamoto, H., Kamada, T., 1997. Effect of glycyrrhizin on viral replication and quasispecies in patients with type C chronic hepatitis. *International Hepatology Communications*, 6(5), 233-238.

Jann, K., Jann, B., 1992. Capsules of *Escherichia coli*, expression and biological significance. *Canadian Journal of Microbiology*, *38*(7), 705-710.

Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F., Donoghue, M. J., 1999. Plant systematics: a phylogenetic approach. *Ecologia Mediterranea*, 25(2), 215.

Kafaru, E., 1994. Immense help formative workshop. Essential. Pharmacology, 1st Ed. Elizabeth Kafaru Publishers, Lagos, Nigeria, 11-14.

Kamboj, V. P., 2000. Herbal medicine. *Current Science-Bangalore-*, 78(1), 35-38.

Kanimozhi, P., Karthikeyan, J., 2011. *Journal of Chemical and Pharmaceutical Research*. J. Chem, 3(6), 288-292.

Kar A. Pharmaocgnosy and Pharmaco-biotechnology, 2007. New Delhi: New Age International Limited Publishers, p 332.

Karkowska-Kuleta, J., Rapala-Kozik, M., Kozik, A., 2009. Fungi pathogenic to humans: molecular bases of virulence of *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*. *Acta Biochimica Polonica*, 56(2), 211-224.

Kaufman R. H., 1986. Vulvovaginal candidiasis: A symposium. *J. Reprod. Med*. 31: 639-72.

Kedare, S. B., Singh, R. P., 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4), 412-422.

Khan, I. A., Mirza, Z. M., Kumar, A., Verma, V., Qazi, G. N., 2006. Piperine, a phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 50(2), 810-812.

Khan, M. S. A., Ahmad, I., Aqil, F., Owais, M., Shahid, M., Musarrat, J., 2010. Virulence and pathogenicity of fungal pathogens with special reference to *Candida albicans*. In Combating Fungal Infections (pp. 21-45). Springer, Berlin Heidelberg.

Khan, R., Khan, A. Q., Lateef, A., Rehman, M. U., Tahir, M., Ali, F., Hamiza, O. O., Sultana, S., 2013. Glycyrrhizic acid suppresses the development of precancerous lesions via regulating the hyperproliferation, inflammation, angiogenesis and apoptosis in the colon of Wistar rats. *PloS One*, 8(2), e56020.

Kinoshita, T., Tamura, Y., Mizutani, K., 2005. The isolation and structure elucidation of minor isoflavonoids from licorice of *Glycyrrhiza glabra* L. origin. *Chemical & Pharmaceutical Bulletin*, 53(7), 847-849.

Kirbağ, S. E. V. D. A., Zengin, F., Kursat, M., 2009. Antimicrobial activities of extracts of some plants. *Pakistan Journal of Botany*, 41(4), 2067-2070.

Kocur, M., Kloos, W. E., Schleifer, K. H., 2006. The genus *Micrococcus*. In the prokaryotes (pp. 961-971). Springer, New York, USA.

Komatsu, M., Ikeda, N., Aihara, M., Nakamachi, Y., Kinoshita, S., Yamasaki, K., Shimakawa, K., 2001. Hospital outbreak of MEN-1-derived extended spectrum β-

lactamase-producing *Klebsiella pneumoniae*. *Journal of Infection and Chemotherapy*, 7(2), 94-101.

Kushwah, P., Vir, D. K., Kayande, N., Patidar, R., 2014. Phytochemical screening and evaluation of antimicrobial activity of *Glycyrrhiza glabra* L.. *Pharma Tutor*, 2(5), 144-147.

Lalita, B., 1994. *In vitro* studies on the effect of glycyrrhizin from Indian *Glycyrrhiza glabra* L. on some RNA and DNA viruses. *Indian Journal of Pharmacology*, 26(3), 194.

Lalitha, M. K., 2004. Manual on antimicrobial susceptibility testing. Performance standards for antimicrobial testing: *Twelfth Informational Supplement*, 56238, 454-6.

Lepape, A., Monnet, D. L., 2008. Experience of European intensive care physicians with infections due to antibiotic-resistant bacteria, 2009. Euro surveillance: bulletin européen sur les maladies transmissibles= *European Communicable Disease Bulletin*, 14(45), 429-433.

Levy, S. B., 2002. The antibiotic paradox: how the misuse of antibiotics destroys their curative powers. Da Capo Press. ISBN:0-7382-0440-4.

Li, X. L., Zhou, A. G., Zhang, L., Chen, W. J., 2011. Antioxidant status and immune activity of glycyrrhizin in allergic rhinitis mice. *International journal of molecular sciences*, 12(2), 905-916.

Littleton, J., Rogers, T., Falcone, D., 2005. Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation. *Life Sciences*, 78(5), 467-475.

Liu, R. H., 2004. Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of Nutrition*, 134(12), 3479S-3485S.

Liu, W., Kato, M. A. S. A. S. H. I., Akhand, A. A., Hayakawa, A., Takemura, M., Yoshida, S., Suzuki, H., Nakashima, I., 1998. The herbal medicine sho-saiko-to inhibits the growth of malignant melanoma cells by upregulating Fas-mediated apoptosis and arresting cell cycle through downregulation of cyclin dependent kinases. *International Journal of Oncology*, 12(6), 1321-1327.

Liu, W., Zu, Y. G., Fu, Y. J., Kong, Y., Ma, W., Yang, M., Li, J., Wu, N., 2010. Variation in contents of phenolic compounds during growth and post-harvest storage of pigeon pea seedlings. *Food Chemistry*, 121(3), 732-739.

Madigan, M. T., Martinko, J. M., Dunlap, P. V., Clark, D. P., 2008. Brock Biology of microorganisms 12th edn. *International Microbiology*, *11*, 65-73.

Magallón, S., Sanderson, M. J., 2001. Absolute diversification rates in angiosperm clades. *Evolution*, 55(9), 1762-1780.

Makkar, H.P., Bummel, M.S., Becker, K., 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in vitro techniques. *The British Journal of Nutrition*, 7 (6): 897–913.

Malek Jafarian, M., Ghazvini, K., 2010. *In vitro* susceptibility of *Helicobacter pylori* to licorice extract. *Iranian Journal of Pharmaceutical Research*, 6(1),69-72.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., 2004. Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*(5), 727-747.

Mandal, P., Babu, S. S., Mandal, N. C., 2005. Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia*, 76(5), 462-465.

Manges, A. R., Johnson, J. R., Foxman, B., O'Bryan, T. T., Fullerton, K. E., Riley, L. W., 2001. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *New England Journal of Medicine*, 345(14), 1007-1013.

Mao, S. J., Hou, S. X., He, R., Zhang, L. K., Wei, D. P., Bi, Y. Q., Jin, H., 2005. Uptake of albumin nanoparticle surface modified with glycyrrhizin by primary cultured rat hepatocytes. *World Journal of Gastroenterology*, 11(20), 3075.

Martinez, M. J., Betancourt, J., Alonso-Gonzalez, N., Jauregui, A., 1996. Screening of some Cuban medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology*, 52(3), 171-174.

Mason, T. L., 1987. Inactivation of red beet β-glucan synthase by native and oxidized phenolic compounds. *Phytochemistry*, *26*(8), 2197-2202.

Mc Dermott, P. F., Walker, R. D., White, D. G., 2003. Antimicrobials: modes of action and mechanisms of resistance. *International Journal of Toxicology*, 22(2), 135-143.

Melzig, M. F., Bader, G., Loose, R., 2001. Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Medica*, 67(1), 43-48.

Mendes-Silva, W., Assafim, M., Ruta, B., Monteiro, R. Q., Guimarães, J. A., Zingali, R. B., 2003. Antithrombotic effect of Glycyrrhizin, a plant-derived thrombin inhibitor. *Thrombosis Research*, 112(1), 93-98.

Mendoza, L. E. O. N. O. R. A., Wilkens, M. A. R. C. E. L. A., Urzúa, A. L. E. J. A. N. D. R. O., 1997. Antimicrobial study of the resinous exudates and of diterpenoids and

flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). *Journal of Ethnopharmacology*, 58(2), 85-88.

Messier, C., Grenier, D., 2011. Effect of licorice compounds licochalcone A, glabridin and glycyrrhizic acid on growth and virulence properties of *Candida albicans*. *Mycoses*, 54(6), e801-e806.

Middleton, E., Kandaswami, C., 1992. Effects of flavonoids on immune and inflammatory cell functions. *Biochemical pharmacology*, 43(6), 1167-1179.

Mitscher, L. A., Park, Y. H., Clark, D., Beal, J. L., 1980. Antimicrobial agents from higher plants. Antimicrobial isoflavanoids and related substances from *Glycyrrhiza glabra* L. var. typica. *Journal of Natural Products*, 43(2), 259-269.

Montville, T. J., Matthews, K. R., 2005. Food microbiology: an introduction. ASM Press.

Mori, A., Nishino, C., Enoki, N., Tawata, S., 1987. Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. *Phytochemistry*, 26(8), 2231-2234.

Moshirfar, M., Mirzaian, G., Feiz, V., Kang, P. C., 2006. Fourth-generation fluoroquinolone-resistant bacterial keratitis after refractive surgery. *Journal of Cataract & Refractive Surgery*, 32(3), 515-518.

Murray, B. E., 1998. Diversity among multidrug-resistant enterococci. *Emerging Infectious Diseases*, 4(1), 37.

Murthy, S. N., Pryor, W. A., Parinandi, N. L. (Eds.)., 2010. Free radicals and antioxidant protocols (pp. 170-186). Humana Press.

Nakagawa, K., Kishida, H., Arai, N., Nishiyama, T., Mae, T., 2004. Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A^ y mice. *Biological and Pharmaceutical Bulletin*, 27, 1775-1778.

Nascimento, G. G., Locatelli, J., Freitas, P. C., Silva, G. L., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247-256.

Nawar WF. 1996. Lipids. In: Fennema O, editor. Food chemistry. 3rd ed. New York: Marcel Dekker, Inc. p 225–320.

Newall, C.A.; Anderson, L.A., Phillipson, J. D., 1997. Herbal Medicines 1st published The Pharmaceutical press. London .PP.183- 186.

Nishino, H., Kitagawa, K., Iwashima, A., 1984. Antitumor-promoting activity of glycyrrhetic acid in mouse skin tumor formation induced by 7,12-dimethylbenz [a] anthracene plus teleocidin. *Carcinogenesis*, 5(11), 1529-1530.

Nitalikar, M. M., Munde, K. C., Dhore, B. V., Shikalgar, S. N., 2010. Studies of antibacterial activities of *Glycyrrhiza glabra* L. root extract. *International Journal of Pharm Tech Research*, 2(1), 899-901.

Nobel W. C., 1980. Microbiology of Human skin. 2nd ed. London: Lioyd-Luke, 263-90.

Nomura, T., Fukai, T., Akiyama, T., 2002. Chemistry of phenolic compounds of licorice (*Glycyrrhiza* species) and their estrogenic and cytotoxic activities. *Pure and Applied Chemistry*, 74(7), 1199-1206.

Oguntoyinbo, F. A., Sanni, A. I., 2007. Determination of toxigenic potentials of *Bacillus* strains isolated from Okpehe, a Nigerian fermented condiment. *World Journal of Microbiology and Biotechnology*, 23(1), 65-70.

Ohuchi, K., Tsurufuji, A., 1982. A study of the anti-inflammatory mechanism of glycyrrhizin. *Mini Reviews in Medicinal Chemistry*, 27, 188-193.

Okada, K., Tamura, Y., Yamamoto, M., Inoue, Y., Takagaki, R., Takahashi, K., Demizu, S., Kajiyama, K., Hiraga, Y., Kinoshita, T., 1989. Identification of antimicrobial and antioxidant constituents from licorice of Russian and Xinjiang origin. *Chemical and Pharmaceutical Bulletin*, 37(9), 2528-2530.

Okimasu, E., Moromizato, Y., Watanabe, S., Sasaki, J., Shiraishi, N., Morimoto, Y. M., Miyahara, M., Utsumi, K., Utsumi, K., 1983. Inhibition of phospholipase A2 and platelet aggregation by glycyrrhizin, an anti-inflammation drug. *Acta Medica Okayama*, 37(5), 385-391.

Oloumi, H., Hassibi, N., 2011. Study the correlation between some climate parameters and the content of phenolic compounds in roots of *Glycyrrhiza glabra* L. *Journal of Medicinal Plants Research*, 5(25), 6011-6016.

Othman, M., San Loh, H., Wiart, C., Khoo, T. J., Lim, K. H., Ting, K. N., 2011. Optimal methods for evaluating antimicrobial activities from plant extracts. *Journal of Microbiological Methods*, 84(2), 161-166.

Pandey, A.K, Chowdhry, P.K, 2006 Propagation techniques and harvesting time on productivity and root quality of *Withania somnifera*. *Journal of Tropical Medicinal Plants* Vol.7:79-81.

Parr, A. J., Bolwell, G. P., 2000. Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture*, 80(7), 985-1012.

Pellati, D., Fiore, C., Armanini, D., Rassu, M., Bertoloni, G., 2009. *In vitro* effects of glycyrrhetinic acid on the growth of clinical isolates of *Candida albicans*. *Phytotherapy Research*, 23(4), 572-574.

Prior, B., Kilian, S., Lategan, P., 1980. Growth of *Candida utilis* on ethanol and isopropanol. *Archives of Microbiology*, 125(1-2), 133-136.

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N., Pohl, C., 1996. Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, 68(6), 1033-1039.

Roshan, A., Verma, N. K., Kumar, C. S., Chandra, V., Singh, D. P., Panday, M. K., 2012. phytochemical constituent, pharmacological activities and medicinal use through the millennia of *Glycyrrhiza glabra* L.: A review. *International Research Journal of Pharmacy*, 3(8), 45-55.

Salama, A. F., El-Bahr, S. M., 2007. Effect of curcumin on cadmium-induced oxidative testicular damage in rats. *Journal of Medical Research Institute*, 28(2), 167-73.

Scalbert, A., 1991. Antimicrobial properties of tannins. *Phytochemistry*, 30(12), 3875-3883.

Sedighinia, F., Afshar, A. S., 2012. Antibacterial activity of *Glycyrrhiza glabra* L. against oral pathogens: an *in vitro* study. *Avicenna Journal of Phytomedicine*, 2(3), 118.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R., De, B., 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *International Journal of Pharmaceutical Sciences Review and Research*, 3(1), 91-100.

Shakeri, A., Hazeri, N., Vlizadeh, J., Ghasemi, A., Tavallaei, Z. F., 2012. Phytochemical screening, Antimicrobial and antioxidant activities of *Anabasis aphylla* L. extracts. *Kragujevac Journal of Science*, 34(34), 71-78.

Shiota, G., Harada, K. I., Ishida, M., Tomie, Y., Okubo, M., Katayama, S., Ito, H., Kawasaki, H., 1999. Inhibition of hepatocellular carcinoma by glycyrrhizin in diethylnitrosamine-treated mice. *Carcinogenesis*, 20(1), 59-63.

Sies, H., 1997. Oxidative stress: oxidants and antioxidants. *Experimental Physiology*, 82(2), 291-295.

Sietske, A., Diderichsen, B.,1991. On the safety of *Bacillus subtilis* and B. amyloliquefaciens: a review. *Applied Microbiology and Biotechnology*, 36(1), 1-4.

Smith, K. J., Neafie, R., Yeager, J., Skelton, H. G., 1999. *Micrococcus folliculitis* in HIV-1 disease. *British Journal of Dermatology*, 141, 558-561.

Spicer, W. J., 2000. Clinical bacteriology, mycology and parasitology. Churchill Livingstone.

Stewart, C. M., Hocking, A. D., 2003. *Staphylococcus aureus* and staphylococcal enterotoxins. *Foodborne Microorganisms ofP Health Significance*, (Ed. 6), 359-379.

Sulaiman, S., Ibrahim, D., Kassim, J., Sheh Hong, L., 2011. Antimicrobial and antioxidant activities of condensed tannin from *Rhizophora apiculata* barks. *Journal of Chemical and Pharmaceutical Research*, 3(4), 436-444.

Suliman, B., Anwar, F., Ashraf, M., 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14 (6): 2167–2180.

Sultana, B., Anwar, F., Ashraf, M., 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 14(6), 2167-2180.

Sultana, S., Haque, A., Hamid, K., Urmi, K. F., Roy, S., 2010. Antimicrobial, cytotoxic and antioxidant activity of methanolic extract of *Glycyrrhiza glabra* L. *Agriculture and Biology Journal of North America*, 1(5), 957-960.

Sundar Rao, K., 1996. Antibacterial activity of some medicinal plants of Papua New Guinea. *Pharmaceutical Biology*, 34(3), 223-225.

Syed, F., Jahan, R., Ahmad, A., Khan, S., 2013. *In vitro* antimicrobial activities of *Glycyrhiza glabra* L. and *Fagonia arabica*. *Journal of Medicinal Plants Research*, 7(10), 2265-2270.

Tamir, S., Eizenberg, M., Somjen, D., Stern, N., Shelach, R., Kaye, A., Vaya, J., 2000. Estrogenic and antiproliferative properties of glabridin from licorice in human breast cancer cells. *Cancer Research*, 60(20), 5704-5709.

Tanahashi, T., Mune, T., Morita, H., Tanahashi, H., Isomura, Y., Suwa, T., Daido, H., Gomez-Sancehz E. C., Yasuda, K., 2002. Glycyrrhizic acid suppresses type 2 11β-hydroxysteroid dehydrogenase expression *in vivo*. *The Journal of Steroid Biochemistry and Molecular Biology*, 80(4), 441-447.

Taylor, R. S. L., Edel, F., Manandhar, N. P., Towers, G. H. N., 1996. Antimicrobial activities of southern Nepalese medicinal plants. *Journal of Ethnopharmacology*, 50(2), 97-102.

Tegos, G., Stermitz, F. R., Lomovskaya, O., Lewis, K., 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrobial Agents and Chemotherapy*, 46(10), 3133-3141.

Tharkar, P. R., Tatiya, A. U., Shinde, P. R., Surana, S. J., Patil, U. K., 2010. Antifungal activity of *Glycyrrhiza glabra* L . And *Emblica officinalis* Gaertn. by direct bioautography method. *International Journal of Pharm Tech Research*, 2, 1547-1549.

Tian, M., Yan, H., Row, K. H., 2008. Simultaneous extraction and separation of liquiritin, glycyrrhizic acid, and glabridin from licorice root with analytical and preparative chromatography. *Biotechnology and Bioprocess Engineering*, 13(6), 671-676.

Uzun, E., Sariyar, G., Adsersen, A., Karakoc, B., Ötük, G., Oktayoglu, E., Pirildar, S., 2004. Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. *Journal of Ethnopharmacology*, 95(2), 287-296.

Valdez, J. C., Peral, M. C., Rachid, M., Santana, M., Perdigon, G., 2005. Interference of *Lactobacillus plantarum* with *Pseudomonas aeruginosa in vitro* and in infected burns: the potential use of probiotics in wound treatment. *Clinical Microbiology and Infection*, 11(6), 472-479.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160(1), 1-40.

Van Wamel, W. J., Hendrickx, A. P., Bonten, M. J., Top, J., Posthuma, G., Willems, R. J., 2007. Growth condition-dependent Esp expression by *Enterococcus faecium* affects initial adherence and biofilm formation. *Infection and Immunity*, 75(2), 924-931.

Vary, P. S., Biedendieck, R., Fuerch, T., Meinhardt, F., Rohde, M., Deckwer, W. D., Jahn, D., 2007. *Bacillus megaterium*—from simple soil bacterium to industrial protein production host. *Applied Microbiology and Biotechnology*, 76(5), 957-967.

Vibha, J. B., Choudhary, K., Singh, M., Rathore, M. S., Shekhawat, N. S., 2009. A study on pharmacokinetics and therapeutic efficacy of *Glycyrrhiza glabra*: a miracle medicinal herb. *Bot Res Int*, *2*(3), 157-63.

Wanatabe Y, Nakanashi H, Goto N, Otsuka K, Kimura T, Adachi S., 2010. Antioxidative properties of ascorbic acid and acyl ascorbates in mL/W emulsion. *Journal of the American Oil Chemists*, 85:1475–80

Wealth of India, 1985. Publication and Information Directorate, vol. I–XI. CSIR, New Delhi, India.

Weber, D. J., Saviteer, S. M., Rutala, W. A., Thomann, C. A., 1989. Clinical significance of *Bacillus* species isolated from blood cultures. *Southern Medical Journal*, 82(6), 705-709.

Weidner, S., Amarowicz, R., Karamać, M., Dąbrowski, G., 1999. Phenolic acids in caryopses of two cultivars of wheat, rye and triticale that display different resistance to pre-harvest sprouting. *European Food Research and Technology*, 210(2), 109-113.

Williamson, E. M., 2003. Liquorice. Potter's Cyclopedia of Herbal Medicines, 269-271.

Yadav, A. S., Bhatnagar, D., 2007. Free radical scavenging activity, metal chelation and antioxidant power of some of the Indian spices. *Biofactors*, 31(3-4), 219-227.

Zhang, Q., Ye, M., 2009. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice). *Journal of Chromatography* A, 1216(11), 1954-1969.

APPENDIX

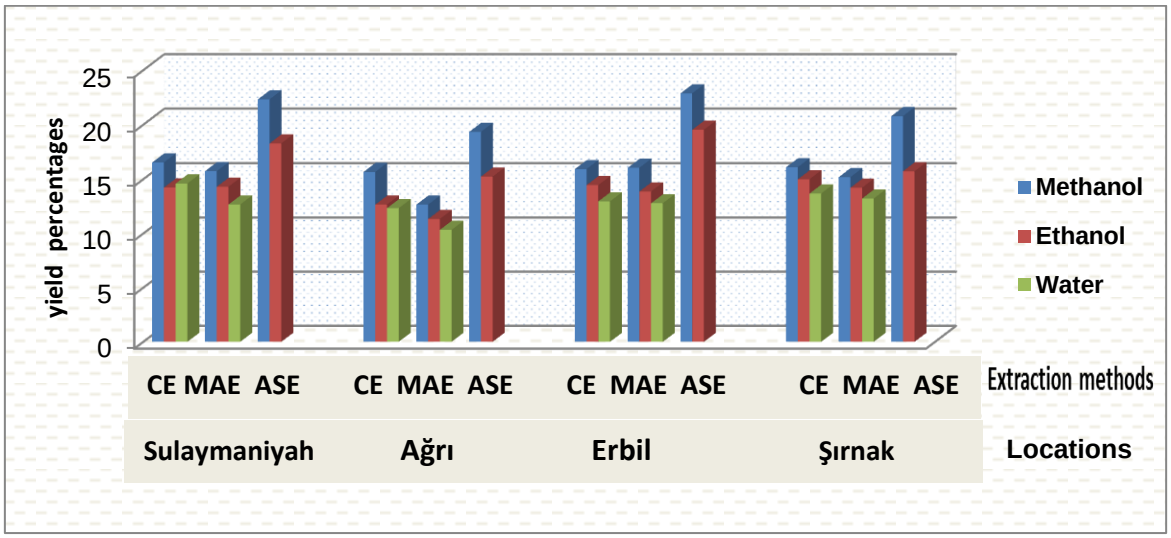

Figure A1. Extraction yield percentages of *Glycyrrhiza glabra* L. roots collected from four locations

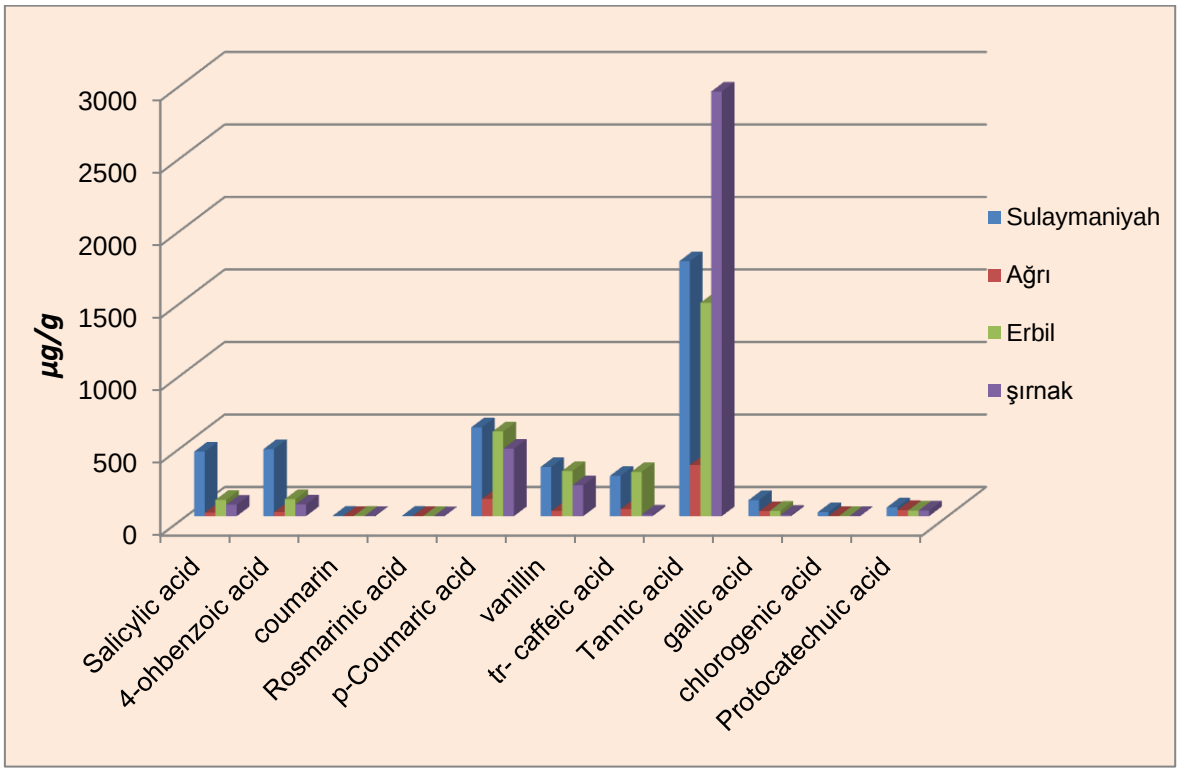

Figure A2. Non-flavonoid phenolics in *Glycyrrhiza glabra* L. roots . roots collected from four locations

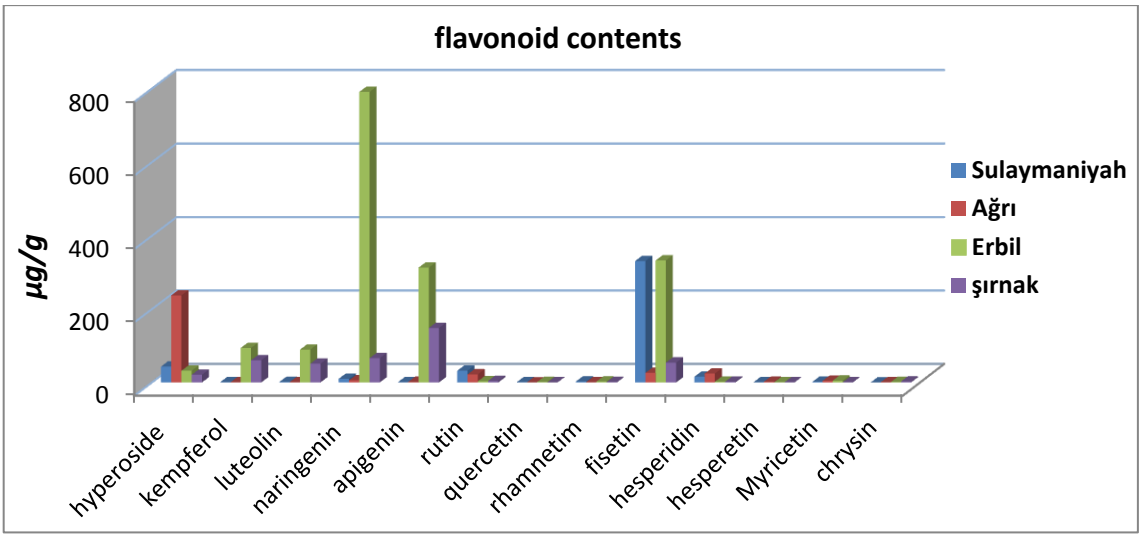

Figure A3. Flavonoids contents in *Glycyrrhiza glabra* L.root . roots collected from four locations

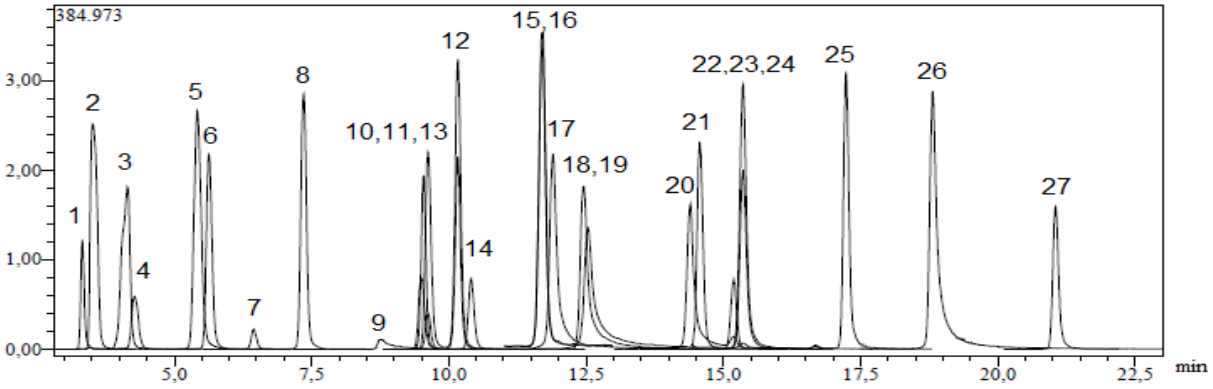

Figure A4. LC-MS/MS chromatogram of the standard calibration

Note: (1): Quinic acid; (2): Malic acid; (3): trans-Aconitic acid;(4): Gallic acid; (5): Chlorogenic acid; (6): Protocatechuic acid; (7): Tannic acid; (8): tr-caffeic acid; (9): Vanillin; (10): p-Coumaric acid; (11): Rosmarinic acid; (12): Rutin; (13): Hesperidin; (14): Hyperoside; (15): 4-OH Benzoic acid; (16): Salicylic acid; (17): Myricetin; (18): Fisetin; (19): Coumarin; (20): Quercetin; (21): Naringenin; (22): Hesperetin; (23): Luteolin; (24): Kaempferol; (25): Apigenin; (26): Rhamnetin; (27): Chrysin

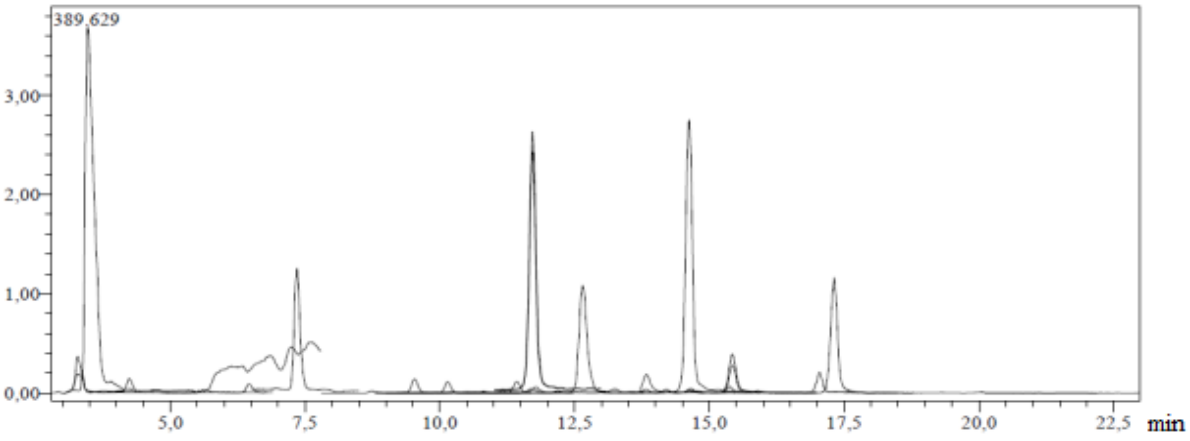

Figure A5. LC-MS/MS chromatogram of the roots of *Glycyrrhiza glabra* L. from Sulaymaniyah (Iraq)

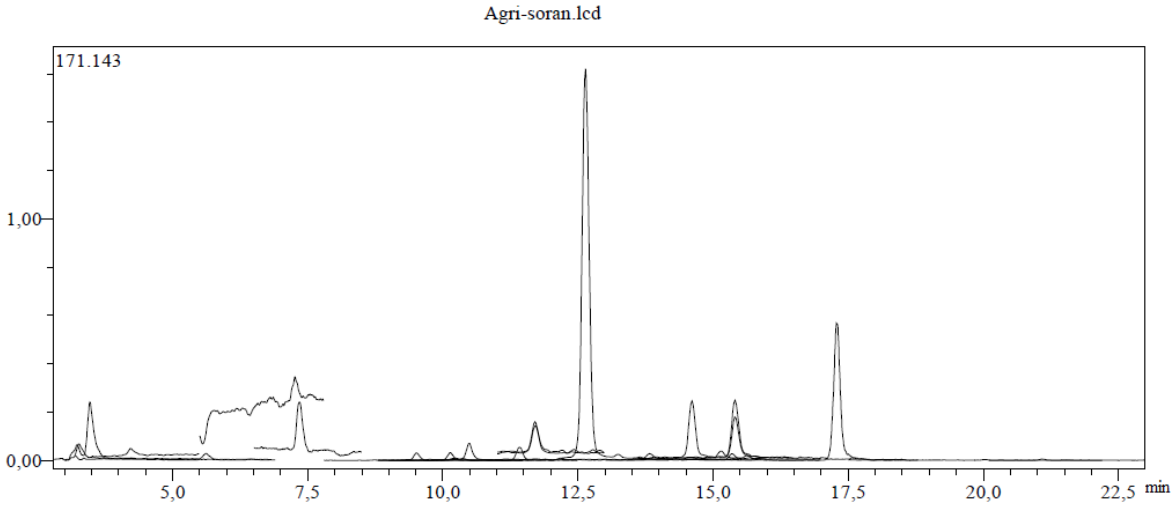

Figure A6. LC-MS/MS chromatogram of the roots of *Glycyrrhiza glabra* L. from Ağrı (Turkey)

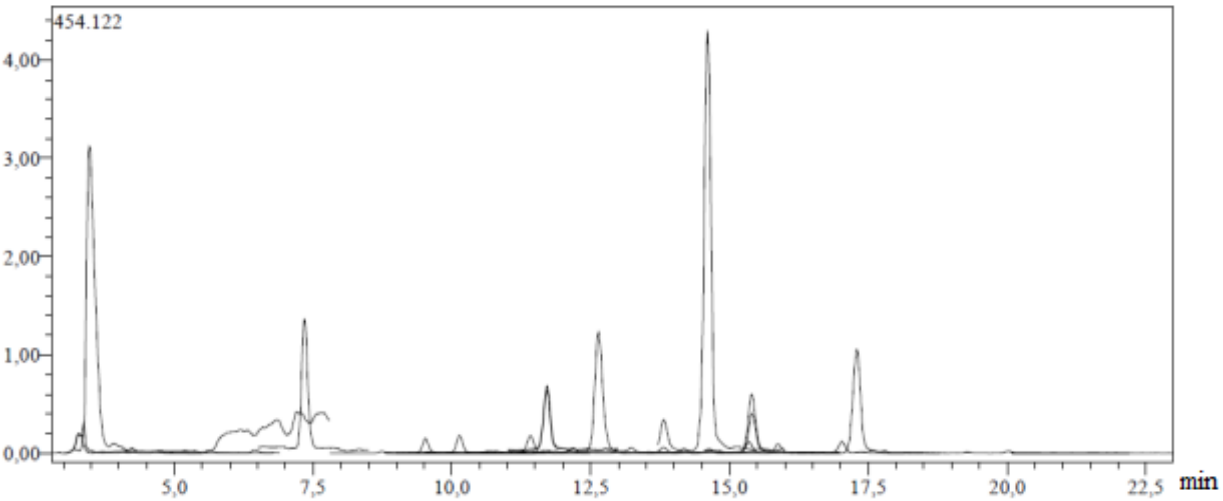

Figure A7. LC-MS/MS chromatogram of the roots of *Glycyrrhiza glabra* L. from Erbil (Iraq)

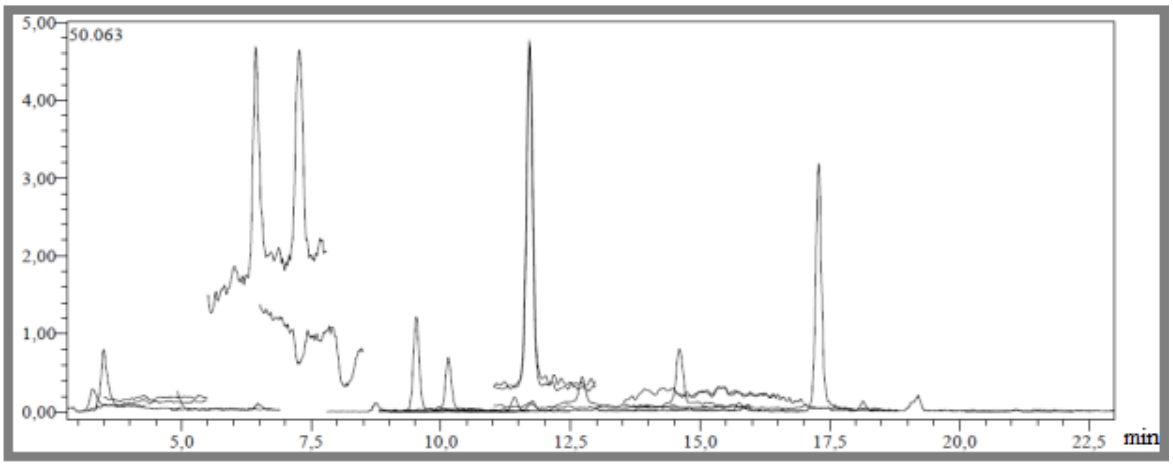

Figure A8. LC-MS/MS chromatogram of the roots of *Glycyrrhiza glabra* L. from Şırnak (Turkey)

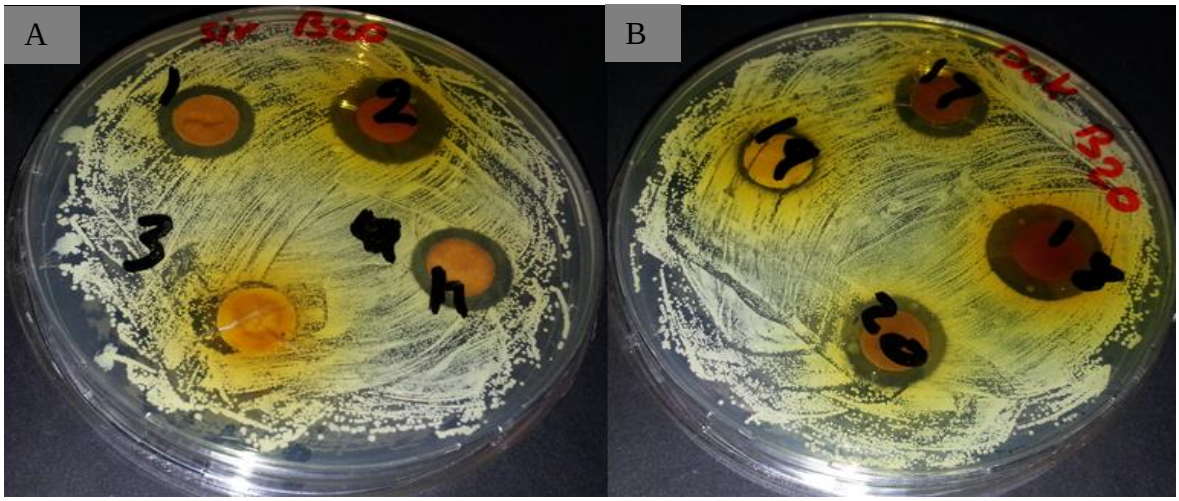

Figure A9. Effect of *Glycyrrhiza glabra* L. against *Staphylococcus aureus*
Note : A: liquorice from Şırnak ; B: liquorice from Sulaymaniyah

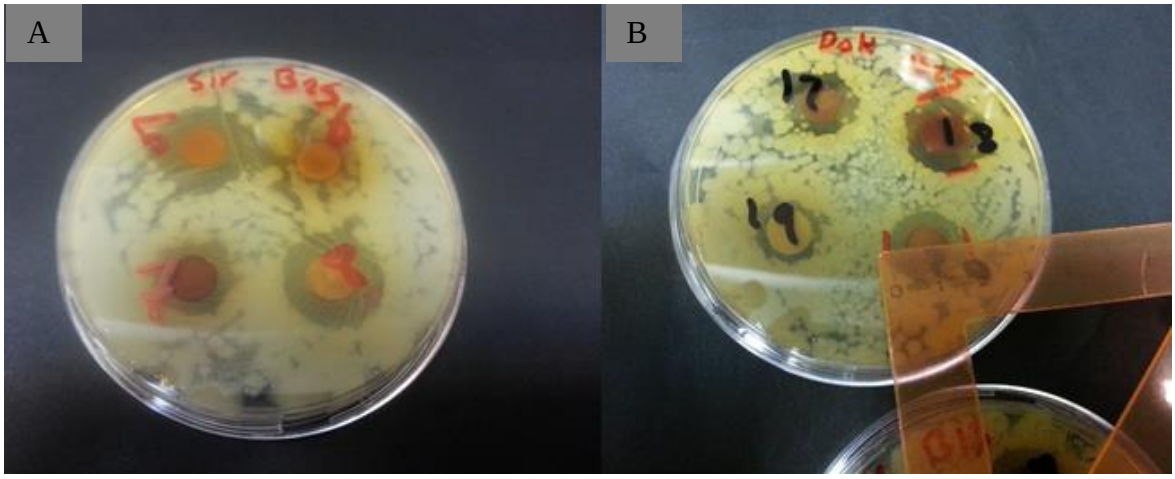

Figure A10. Effect of *Glycyrrhiza glabra* L. against *Micrococcus luteus*
Note : A: liquorice from Şırnak ; B: liquorice from Sulaymaniyah

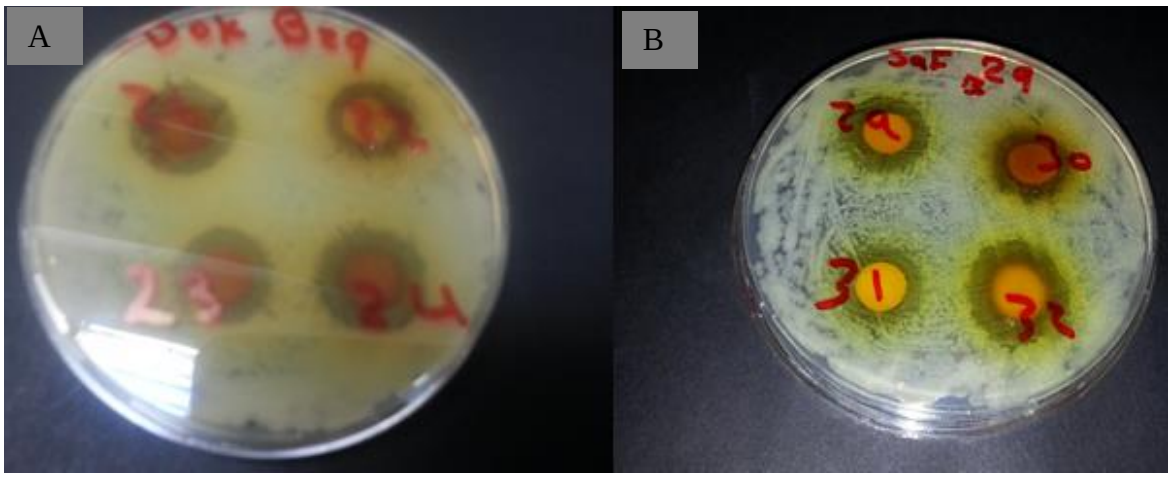

Figure A11. Effect of *Glycyrrhiza glabra* L. against *Bacillus subtilis*
Note : A: liquorice from Sulaymaniyah; B: liquorice from Erbil

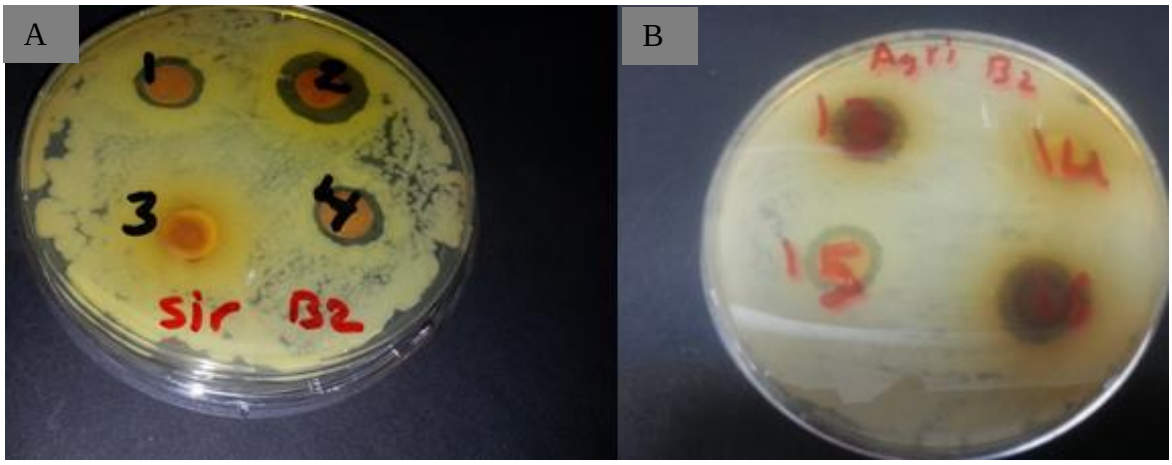

Figure A12. Effect of *Glycyrrhiza glabra* L. against *Bacillus megaterium*
Note : A: liquorice from Şırnak; B: liquorice from Ağrı

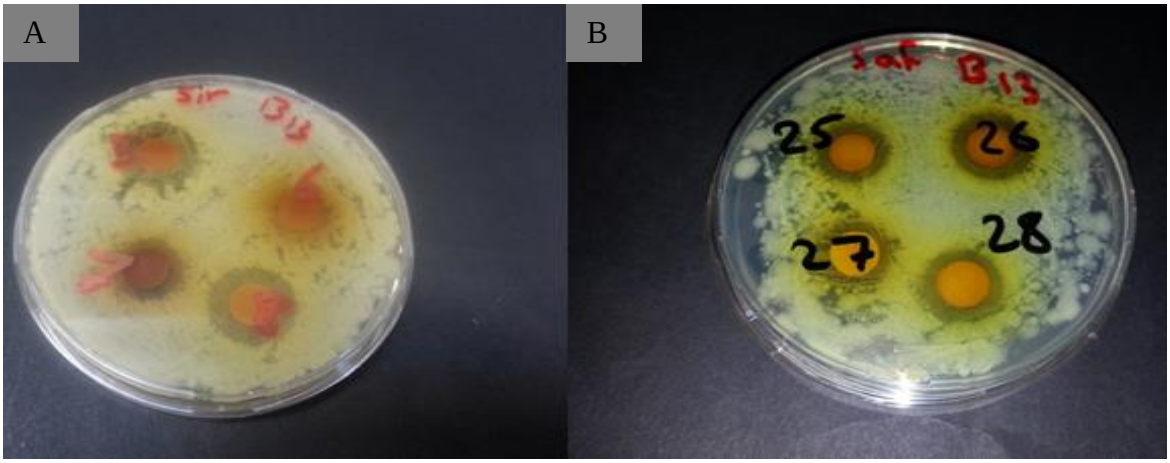

Figure A13. Effect of *Glycyrrhiza glabra* L. against *Escherichia coli*
Note : A: liquorice from Şırnak; B: liquorice from Erbil

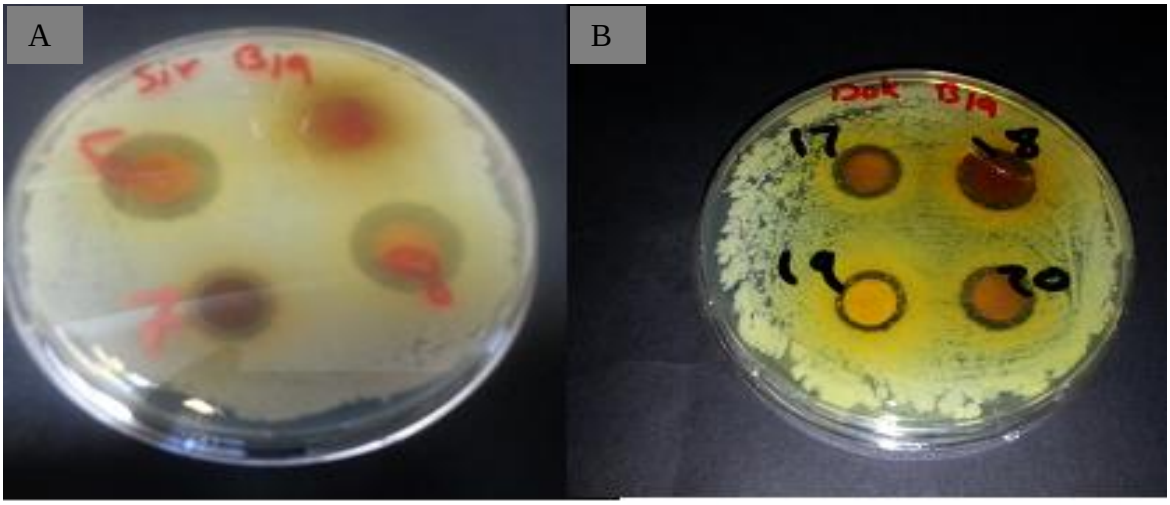

Figure A14. Effect of *Glycyrrhiza glabra* L. against *Pseudomonas aeruginosa*
Note : A: liquorice from Şırnak; B: liquorice from Sulaymaniyah

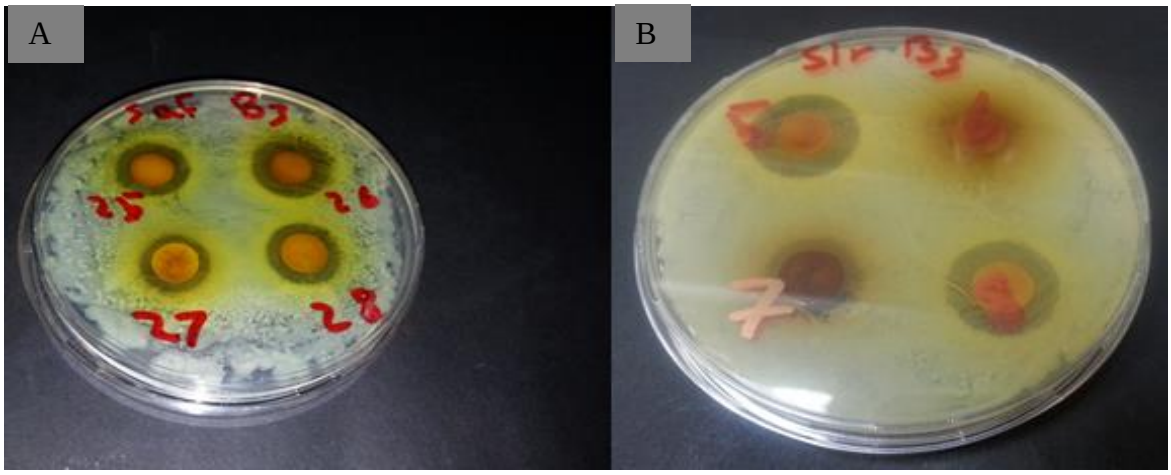

Figure A15. Effect of *Glycyrrhiza glabra* L. against *Enterococcus faecium*
Note : A: liquorice from Erbil; B: liquorice from Şırnak

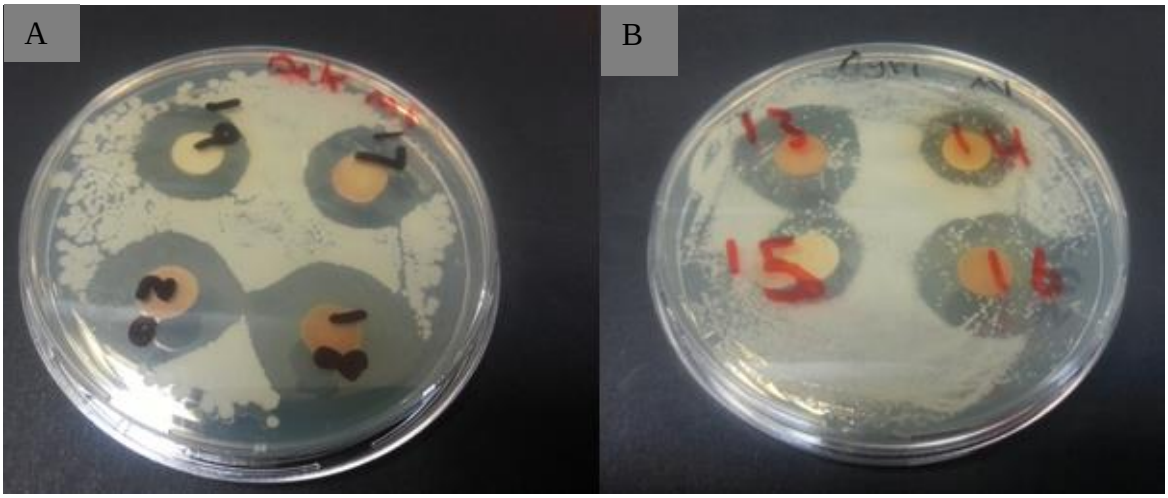

Figure A 16. Effect of *Glycyrrhiza glabra* L. against *Candida albicans*
Note : A: liquorice from Sulaymaniyah; B: liquorice from Ağrı

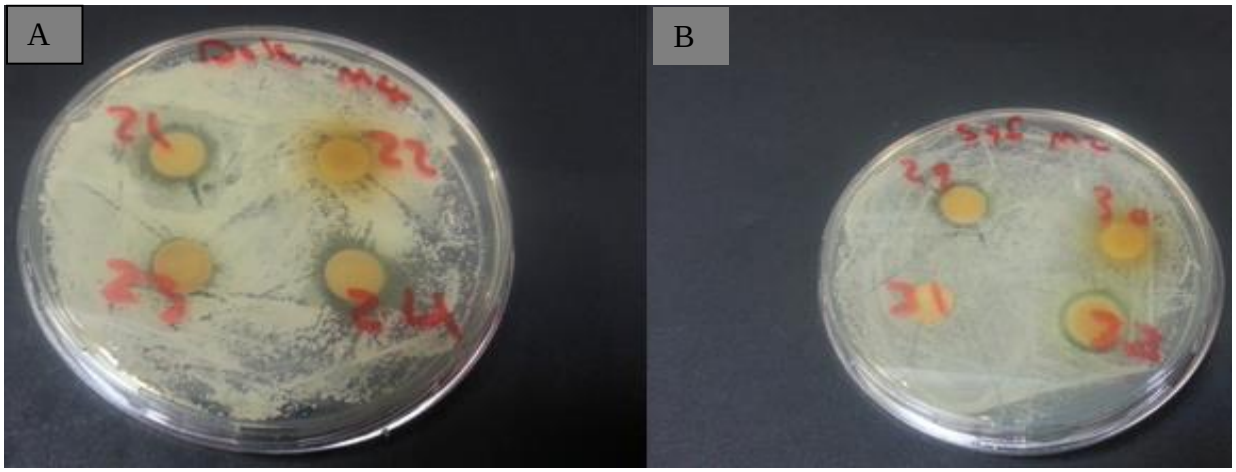

Figure A17. Effect of *Glycyrrhiza glabra* L. against A: *Yarrowia lipolytica*; B: *Candida utilis*
Note : A: liquorice from Sulaymaniyah; B: liquorice from Erbil

Table A1. Inhibition zones (mm) of *Glycyrrhiza glabra* L. root extracts detected from Sulaymaniyah (Iraq) against micro-organisms

Micro-organisms	Conventional extraction			Microwave assisted extraction			ASE	
	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Ethanol	Methanol
B. megaterium	13.67±0.577	14.33±0.577	17.00±1.000	13.00±1.000	14.33±0.577	16.67±0.577	17.33±0.577	19.00±1.000
E. faecium	14.67±0.577	14.33±0.577	17.33±0.577	12.33±0.577	14.33±0.577	17.33±0.577	15.33±0.577	19.67±0.577
K. pneumoniae	14.33±0.577	12.67±0.577	19.33±0.577	12.67±0.577	14.00±1.000	16.33±0.577	16.00±1.000	20.00±1.000
E. coli	13.67±0.577	16.00±1.000	18.33±0.577	13.33±0.577	14.00±1.000	18.67±0.577	17.00±1.000	19.67±0.577
P. aeruginosa	13.67±0.577	14.33±0.577	17.00±1.000	12.33±0.577	13.33±0.577	17.67±0.577	16.67±0.577	17.33±0.577
S. aureus	13.33±0.577	14.33±0.577	16.67±0.577	12.33±0.577	13.00±1.000	18.33±0.577	14.67±0.577	17.67±0.577
M. luteus	16.33±0.577	18.00±1.000	18.67±1.155	13.00±1.00	16.00±1.000	19.00±1.000	18.33±0.577	20.00±1.000
B. subtilis	13.67±0.577	15.33±0.577	18.67±0.577	11.67±0.577	14.67±0.577	15.67±0.577	17.67±0.577	18.67±0.577
C. albicans	24.33±2.082	23.00±1.000	26.33±1.528	22.33±0.577	22.67±1.528	29.33±1.528	25.00±1.000	24.33±2.082
C. utilis	ND	ND	ND	ND	ND	ND	ND	ND
Y. lipolytica	12.33±1.155	ND	12.33±1.528	ND	11.33±.577	11.00±1.000	11.33±.577	13.33±0.577

Note: The values presented as mean ± SD of three replications; Mean differences were statistically significant ($P < 0.05$).
ND: Not detected.

Table A2. Inhibition zones (mm) of *Glycyrrhiza glabra* L. root extracts detected from Ağrı (Turkey) against micro-organisms

Micro-organisms	Conventional extraction			Microwave assisted extraction			ASE	
	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Ethanol	Methanol
B.megaterium	ND	12.33± 0.577	15.33±0.577	ND	12.67±0.577	12.67±0.577	12.67±0.577	15.33±0.577
E. faecium	ND	12.67±0.577	15.67±0.577	ND	14.33±0.577	13.67±0.577	13.67±0.577	15.33±0.577
K. pneumoniae	ND	12.33±0.577	13.67±0.577	ND	13.67±0.577	12.33±0.577	13.67±0.577	17.00±1.000
E. coli	ND	14.67±0.577	13.67±0.577	ND	14.67±0.577	14.33±0.577	12.67±0.577	13.33±0.577
P. aeruginosa	ND	12.67±0.577	14.67±0.577	ND	15.67±0.577	13.33±0.577	11.67±0.577	14.67±0.577
S. aureus	ND	12.67±0.577	13.33±0.577	ND	12.67±0.577	12.67±0.577	12.33±0.577	14.33±0.577
M. luteus	ND	15.67±0.577	16.00±1.000	13.33±0.577	12.33±0.577	17.33±0.577	12.33±0.577	16.67±0.577
B. subtilis	ND	12.67±0.577	13.33±0.577	ND	14.33±0.577	13.33±0.577	12.33±0.577	15.33±0.577
C. albicans	19.00±1.000	22.67±0.577	23.67±0.577	14.00±1.000	20.67±0.658	24.00±1.000	18.67±0.577	23.00±1.000
C. utilis	ND	ND	ND	ND	ND	11.33±.577	ND	11.67±.577
Y. lipolytica	ND	ND	ND	ND	ND	ND	ND	11.67±.577

Note: The values presented as mean ± SD of three replications.; Mean differences were statistically significant ($P < 0.05$).
ND: Not detected.

Table A3. Inhibition zones (mm) of *Glycyrrhiza glabra* L. root extracts detected from Erbil (Iraq) against micro-organisms

Micro-organisms	Conventional extraction			Microwave assisted extraction			ASE	
	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Ethanol	Methanol
B. megaterium	15.33±0.577	15.67±0.577	16.33±0.577	14.33±0.577	17.33±0.577	18.33+0.577	15.00±1.000	17.67±0.577
E. faecium	14.33±14.33	15.67±0.577	16.67±0.577	13.67±0.577	16.33±0.577	17.00±1.000	16.67±0.577	17.33±0.577
K. pneumoniae	11.33±0.577	15.00±1.000	15.67±0.577	15.67±0.577	15.67±0.577	16.67±0.577	14.33±0.577	17.67±0.577
E. coli	11.33±0.577	15.00±1.000	15.67±0.577	15.67±0.577	15.67±0.577	16.67±0.577	14.33±0.577	17.67±0.577
P. aeruginosa	14.67±0.577	14.67±0.577	16.33±0.577	12.33±0.577	14.67±0.577	16.67±0.577	14.33±0.577	18.00±1.000
S. aureus	14.00±1.000	13.33±0.577	18.67±1.155	13.67±0.577	16.67±0.577	15.33±0.577	14.67±0.577	18.67±1.155
M. luteus	15.33±0.577	14.67±0.577	17.33±0.577	14.33±0.577	16.33±0.577	18.67±1.155	18.33±0.577	19.33±0.577
B. subtilis	16.33±0.577	14.67±0.577	17.67±0.577	14.67±0.577	14.33±0.577	16.67±0.577	14.67±0.577	19.00±1.000
C. albicans	26.00± 1.000	23.33± 1.528	26.33±1.528	21.67 ± 1.155	23.67± 1.528	27.67 ±1.528	23.00 ± 1.000	29.00± 1.000
C. utilis	ND	ND	12.33+0.577	ND	ND	11.33±0.577	ND	13.33 ± 0 .577
Y. lipolytica	ND	ND	11.67±1.155	ND	ND	12.33±0.577	ND	15.00±1.00

Note: The values presented as mean ± SD of three replications; Mean differences were statistically significant (P< 0.05).
ND: Not detected.

Table A4. Inhibition zones (mm) of *Glycyrrhiza glabra* L. root extracts detected from Şırnak (Turkey) against micro-organisms

Micro-organisms	Conventional extraction			Microwave assisted extraction			ASE	
	Water	Ethanol	Methanol	Water	Ethanol	Methanol	Ethanol	Methanol
B. megaterium	ND	14.00±1.000	21.00±1.000	ND	15.33±0.577	20.33±0.577	16.33±0.577	20.00±1.000
E. faecium	ND	12.67±0.577	18.67±1.528	ND	12.33±0.577	16.67±0.577	15.33±0.577	16.33±0.577
K. pneumoniae	ND	13.67±0.577	18.33±0.577	ND	12.67±0.577	18.67±0.577	14.67±0.577	18.67±1.155
E. coli	ND	13.33±1.155	19.00±1.000	ND	12.67±0.577	17.33±1.155	13.67±0.577	17.67±0.577
P. aeruginosa	ND	13.67±1.155	19.67±0.577	ND	13.67±0.577	18.00±1.000	14.00±1.000	18.67±0.577
S. aureus	ND	14.33±0.577	19.67±1.155	ND	14.33±0.577	18.67±0.577	15.33±0.577	19.33±0.577
M. luteus	ND	13.00±1.000	22.33±1.528	ND	13.67±0.577	16.00±1.000	15.00±1.000	19.67±0.577
B. subtilis	ND	16.67±5.508	21.00±1.000	ND	16.00±1.000	20.67±0.577	15.33±1.155	20.33±0.577
C. albicans	ND	22.00±1.000	23.00±1.000	ND	25.00±1.000	25.67±1.155	21.67±1.155	23.00±1.528
C. utilis	ND	ND	ND	ND	ND	ND	ND	ND
Y. lipolytica	ND	ND	ND	ND	ND	ND	ND	ND

Note: The values presented as mean ± SD of three replications.; Mean differences were statistically significant ($P < 0.05$).
ND: Not detected

Table A5. ANOVA calculation

Methods (locations)	Solvents			Sum of Squares	DF	Mean Square	F	Sig.
Conventional extraction (Sulaymaniyah)	Water	Between Groups	(Combined)	932.970	10	93.297	123.152	0.000
		Within Groups			16.667	22	0.758	
	Ethanol	Between Groups	(Combined)	1453.879	10	145.388	319.853	0.000
		Within Groups			10.000	22	0.455	
	Methanol	Between Groups	(Combined)	5877.636	10	587.764	668.834	0.000
		Within Groups			19.333	22	0.879	
Microwave assisted extraction (Sulaymaniyah)	Water	Between Groups	(Combined)	1176.242	10	117.624	388.160	0.000
		Within Groups		6.667	22	0.303		
	Ethanol	Between Groups	(Combined)	842.061	10	84.206	115.783	0.000
		Within Groups		16.000	22	0.727		
	Methanol	Between Groups	(Combined)	1452.303	10	145.230	281.918	0.000
		Within Groups		11.333	22	0.515		
ASE (Sulaymaniyah)	Ethanol	Between Groups	(Combined)	1105.212	10	110.521	227.950	0.000
		Within Groups		10.667	22	0.485		
	Methanol	Between Groups	(Combined)	1185.394	10	118.539	139.707	0.000
		Within Groups		18.667	22	0.848		
Conventional extration (Ağrı)	Water	Between Groups	(Combined)	984.545	10	98.455	1083.000	0.000
		Within Groups		2.000	22	0.091		
	Ethanol	Between Groups	(Combined)	1269.333	10	126.933	465.422	0.000
		Within Groups		6.000	22	0.273		

Continued

	Methanol	Between Groups	(Combined)	1428.000	10	142.800	428.400	0.000
		Within Groups		7.333	22	0.333		
Microwave assisted extraction (Ağrı)	Water	Between Groups	(Combined)	894.848	10	89.485	1476.500	0.000
		Within Groups		1.333	22	0.061		
	Ethanol	Between Groups	(Combined)	1194.727	10	119.473	27.962	0.000
		Within Groups		94.000	22	4.273		
	Methanol	Between Groups	(Combined)	942.909	10	94.291	259.300	0.000
		Within Groups		8.000	22	0.364		
ASE (Ağrı)	Ethanol	Between Groups	(Combined)	978.727	10	97.873	358.867	0.000
		Within Groups		6.000	22	0.273		
	Methanol	Between Groups		286.970	10	28.697	63.133	0.000
		Within Groups		10.000	22	0.455		
Conventional extraction (Erbil)	Water	Between Groups	(Combined)	1616.848	10	161.685	410.431	0.000
		Within Groups		8.667	22	0.394		
	Ethanol	Between Groups	(Combined)	1422.182	10	142.218	276.071	0.000
		Within Groups		11.333	22	0.515		
	Methanol	Between Groups	(Combined)	430.545	10	43.055	61.774	0.000
		Within Groups		15.333	22	0.697		
Microwave assisted extraction (Erbil)	Water	Between Groups	(Combined)	1260.242	10	126.024	346.567	0.000
		Within Groups		8.000	22	0.364		
	Ethanol	Between Groups	(Combined)	1558.970	10	155.897	342.973	0.000
		Within Groups		10.000	22	0.455		
	Methanol	Between Groups	(Combined)	526.848	10	52.685	79.027	0.000
		Within Groups		14.667	22	0.667		

Continued

ASE (Erbil)	Ethanol	Between Groups	(Combined)	1520.182	10	152.018	334.440	0.000
		Within Groups		10.000	22	0.455		
	Methanol	BetweenGroups	(Combined)	459.576	10	45.958	68.936	0.000
		Within Groups		14.667	22	0.667		
Conventional extraction (Şırnak)	Methanol	Between Groups	(Combined)	2086.545	10	208.655	215.175	0.000
		Within Groups		21.333	22	0.970		
	Ethanol	Between Groups	(Combined)	989.394	10	98.939	5.718	0.000
		Within Groups		380.667	22	17.303		
Microwave assisted extraction (Şırnak)	Ethanol	Between Groups	(Combined)	1484.667	10	148.467	376.877	0.000
		Within Groups		8.667	22	0.394		
	Methanol	Between Groups	(Combined)	1994.970	10	199.497	346.495	0.000
		Within Groups		12.667	22	0.576		
ASE (Şırnak)	Ethanol	Between Groups		1345.576	10	134.558	201.836	0.000
		Within Groups		14.667	22	0.667		
	Methanol	Between Groups	(Combined)	2243.636	10	224.364	370.200	0.000
		Within Groups		13.333	22	0.606		

Note: Df: Degree of freedom; F: F-value; Sig: significant.

Table A6. ANOVA calculation between locations(Conventional extraction - water)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4837.356	3	1612.452	50.637	0.000
Within Groups	4075.939	128	31.843		
Total	8913.295	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(I) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	12.93939*	0.000	10.1906	15.6882
	Erbil	.06061	0.965	-2.6882	2.8094
	Ağrı	11.21212*	0.000	8.4633	13.9609
Ağrı	Şırnak	1.72727	0.216	-1.0215	4.4761
	Erbil	-11.15152*	0.000	-13.9003	-8.4027
	Sulaymaniyah	-11.21212*	0.000	-13.9609	-8.4633
Erbil	Şırnak	12.87879*	0.000	10.1300	15.6276
	Sulaymaniyah	-.06061	0.965	-2.8094	2.6882
	Ağrı	11.15152*	0.000	8.4027	13.9003
Şırnak	Erbil	-12.87879*	0.000	-15.6276	-10.1300
	Sulaymaniyah	-12.93939*	0.000	-15.6882	-10.1906
	Ağrı	-1.72727	0.216	-4.4761	1.0215

*The mean difference is significant at the 0.05 level.

Table A7. ANOVA calculation between locations(microwave assisted extraction - water)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3711.295	3	1237.098	47.306	.000
Within Groups	3347.333	128	26.151		
Total	7058.629	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	11.18182*	.000	8.6908	13.6728
	Erbil	-.96970	.443	-3.4607	1.5213
	Ağrı	8.72727*	.000	6.2363	11.2183
Ağrı	Şırnak	2.45455	.053	-.0365	4.9456
	Erbil	-9.69697*	.000	-12.1880	-7.2060
	Sulaymaniyah	-8.72727*	.000	-11.2183	-6.2363
Erbil	Şırnak	12.15152*	.000	9.6605	14.6425
	Sulaymaniyah	.96970	.443	-1.5213	3.4607
	Ağrı	9.69697*	.000	7.2060	12.1880
Şırnak	Erbil	-12.15152*	.000	-14.6425	-9.6605
	Sulaymaniyah	-11.18182*	.000	-13.6728	-8.6908
	Ağrı	-2.45455	.053	-4.9456	.0365

*The mean difference is significant at the 0.05 level.

Table A7. ANOVA calculation between locations(Conventional extraction - ethanol)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62.394	3	20.798	.480	.697
Within Groups	5542.788	128	43.303		
Total	5605.182	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	1.51515	0.351	-1.6903	4.7206
	Erbil	.06061	0.970	-3.1449	3.2661
	Ağrı	1.27273	0.434	-1.9327	4.4782
Ağrı	Şırnak	.24242	0.881	-2.9630	3.4479
	Erbil	-1.21212	0.456	-4.4176	1.9933
	Sulaymaniyah	-1.27273	0.434	-4.4782	1.9327
Erbil	Şırnak	1.45455	0.371	-1.7509	4.6600
	Sulaymaniyah	-.06061	0.970	-3.2661	3.1449
	Ağrı	1.21212	0.456	-1.9933	4.4176
Şırnak	Erbil	-1.45455	0.371	-4.6600	1.7509
	Sulaymaniyah	-1.51515	0.351	-4.7206	1.6903
	Ağrı	-.24242	0.881	-3.4479	2.9630

*The mean difference is significant at the 0.05 level.

Table A8. ANOVA calculation between locations(Microwave assisted extraction with ethanol)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72.568	3	24.189	.594	.620
Within Groups	5209.091	128	40.696		
Total	5281.659	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	-.42424	0.787	-3.5317	2.6832
	Erbil	-1.78788	0.257	-4.8954	1.3196
	Ağrı	-1.51515	0.336	-4.6226	1.5923
Ağrı	Şırnak	1.36364	0.387	-1.7438	4.4711
	Sulaymaniyah	.27273	0.862	-2.8347	3.3802
	Erbil	1.78788	0.257	-1.3196	4.8954
Erbil	Şırnak	1.36364	0.387	-1.7438	4.4711
	Sulaymaniyah	.27273	0.862	-2.8347	3.3802
	Ağrı	1.78788	0.257	-1.3196	4.8954
Şırnak	Erbil	-1.36364	0.387	-4.4711	1.7438
	Sulaymaniyah	-1.09091	0.489	-4.1984	2.0166
	Ağrı	.42424	0.787	-2.6832	3.5317

*The mean difference is significant at the 0.05 level.

Table A9. ANOVA calculation between locations (ASE extraction - ethanol)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	337.939	3	112.646	2.889	0.038
Within Groups	4991.030	128	38.992		
Total	5328.970	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	2.54545	0.100	-.4963	5.5872
	Erbil	1.93939	0.209	-1.1023	4.9811
	Ağrı	4.48485*	0.004	1.4431	7.5266
Ağrı	Şırnak	-1.93939	0.209	-4.9811	1.1023
	Erbil	-2.54545	0.100	-5.5872	.4963
	Sulaymaniyah	-4.48485*	0.004	-7.5266	-1.4431
Erbil	Şırnak	.60606	0.694	-2.4357	3.6478
	Sulaymaniyah	-1.93939	0.209	-4.9811	1.1023
	Ağrı	2.54545	0.100	-.4963	5.5872
Şırnak	Erbil	-.60606	0.694	-3.6478	2.4357
	Sulaymaniyah	-2.54545	0.100	-5.5872	.4963
	Ağrı	1.93939	0.209	-1.1023	4.9811

*The mean difference is significant at the 0.05 level.

Table A10. ANOVA calculation between locations (Conventional extraction -methanol)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1792.517	3	597.506	18.424	.000
Within Groups	4151.203	128	32.431		
Total	5943.720	131			

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	16.51515*	0.000	11.1075	21.9228
	Erbil	-.42424	0.763	-3.1983	2.3498
	Ağrı	4.82663*	0.000	2.3916	7.2616
Ağrı	Şırnak	11.68852*	0.000	6.4468	16.9303
	Erbil	-5.25087*	0.000	-7.6859	-2.8159
	Sulaymaniyah	-4.82663*	0.000	-7.2616	-2.3916
Erbil	Şırnak	16.93939*	0.000	11.5318	22.3470
	Sulaymaniyah	.42424	0.763	-2.3498	3.1983
	Ağrı	5.25087*	0.000	2.8159	7.6859
Şırnak	Erbil	-16.93939*	0.000	-22.3470	-11.5318
	Sulaymaniyah	-16.51515*	0.000	-21.9228	-11.1075
	Ağrı	-11.68852*	0.000	-16.9303	-6.4468

*The mean difference is significant at the 0.05 level.

Table A11. ANOVA calculation between locations (Microwave assisted extraction - methanol

	Sum of Squares	df	F	Sig.
Between Groups	288.545	3	2.480	.064
Within Groups	4963.697	128		
Total	5252.242	131		

Note: Df: Degree of freedom; F: F-value; Sig: significant.

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulaymaniyah	Şırnak	.72727	0.636	-2.3061	3.7607
	Erbil	-.75758	0.622	-3.7910	2.2758
	Ağrı	3.18182*	0.040	.1484	6.2152
Ağrı	Şırnak	-2.45455	0.112	-5.4879	.5789
	Erbil	-3.93939*	0.011	-6.9728	-.9060
	Sulaymaniyah	-3.18182*	0.040	-6.2152	-.1484
Erbil	Şırnak	1.48485	0.335	-1.5485	4.5182
	Sulaymaniyah	.75758	0.622	-2.2758	3.7910
	Ağrı	3.93939*	0.011	.9060	6.9728
Şırnak	Erbil	-1.48485	0.335	-4.5182	1.5485
	Sulaymaniyah	-.72727	0.636	-3.7607	2.3061
	Ağrı	2.45455	0.112	-.5789	5.4879

*The mean difference is significant at the 0.05 level.

Table A12. ANOVA calculation between locations (ASE-methanol)

	Sum ofSquares	df	MeanSquare	F	Sig.
Between Groups	182.091	3	60.697	1.836	.144
Within Groups	4232.242	128	33.064		
Total	4414.333	131			

(I) G	(J) G	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Uppe Bound
Sulaymaniyah	Şırnak	.93939	0.508	-1.8616	3.7404
	Erbil	-1.24242	0.382	-4.0434	1.5586
	Ağrı	1.93939	0.173	-.8616	4.7404
Ağrı	Şırnak	-1.00000	0.481	-3.8010	1.8010
	Erbil	-3.18182*	0.026	-5.9828	-.3808
	Sulaymaniyah	-1.93939	0.173	-4.7404	.8616
Erbil	Şırnak	2.18182	0.126	-.6192	4.9828
	Sulaymaniyah	1.24242	0.382	-1.5586	4.0434
	Ağrı	3.18182*	0.026	.3808	5.9828
Şırnak	Erbil	-2.18182	0.126	-4.9828	.6192
	Sulaymaniyah	-.93939	0.508	-3.7404	1.8616
	Ağrı	1.00000	0.481	-1.8010	3.8010

*The mean difference is significant at the 0.05 level.

CURRICULUM VITAE

Personal information

Name :	Soran Kaify Najmaldin
Country :	Iraq
Date and plave of of birth :	05/01/1990 Erbil, Iraq
Marital Status :	Single
E-mail :	sorankn@yahoo.com

Education

Degree:	B.Sc
Place of Education:	Department of biology/college of science Salaheldin University,Iraq
Date:	2011-2012

Poster

Najmaldin, S. K., ALMA, M. H., GÖÇERI, A., KARAOĞUL, E., ÖZKAN, H. G., 2015. ANTIOXIDANT PROPERTIES OF IRAQIAN AND TURKISH *Glycyrrhiza glabra* L. (LIQUORICE). 2nd Mediterranean Symposium on Medicinal and Aromatic Plants April, 22-25, Antalya - TURKEY