ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF DIETARY PLANTS AGAINST GROUP A ß-HAEMOLYTIC STREPTOCOCCI

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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF DIETARY PLANTS AGAINST GROUP A ß-HAEMOLYTIC STREPTOCOCCI

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ABSTRACT

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF DIETARY PLANTS AGAINST GROUP A ß-HAEMOLYTIC STREPTOCOCCI

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Fresh produces, fruit juices and herbal teas used in our regular diet may have importance in the protective treatment of some infectious diseases. In this study, dietary produces were investigated for their antioxidant activities and antimicrobial activities against group A ß-haemolytic streptoccoci. *Streptococcus pyogenes*, a member of the group A ß-haemolytic streptococci, is a very dangerous pathogen, which may cause diseases such as tonsillopharyngitis, meningitis, rheumatic arthritis.

Fruits and vegetables; onion, radish, carrot, plum, fruit juices; orange, peach, pomegranate, grape and teas; sage, anise, rosehip, chamomile were chosen as samples of regular daily diets. Dry extracts were obtained either by lyophilizing or fractionating in ethyl acetate. Antioxidant activities of extracts were examined by total phenolic content determination, and 2,2-diphenyl-1-picrylhydrazyl radical scavenging

(DPPH) methods. Antimicrobial activities of extracts were studied by disk diffusion test, minimum inhibitory and bactericidal concentration methods.

Sage, plum, onion and radish displayed high radical scavenging activity with EC_{50} values of 0.043, 0.049, 0.148 and 0.414 mg/mL, respectively. Plum, sage, onion and radish were found high in total phenolic contents with µg gallic acid equivalent of 50.506, 48.299, 44.427 and 13.135 in mg extract, respectively. High antimicrobial activities were obtained by onion, radish, anise, carrot and peach extracts as tested by disk diffusion method with respective 20, 16, 16, 14 and 14 millimeters clear growth inhibition zones. Carrot, onion and radish extracts were found as effective bacteriostatic and bactericidal agents with minimum inhibitory and bactericidal respective concentrations of 0.008, 0.125, 0.250 mg/mL and 0.06, 0.5, 1 mg/mL.

Keywords: Group A ß-haemolytic streptococci, plant extraction, protective treatment, antioxidant, antimicrobial activity

ÖZ

A GRUBU ß-HEMOLİTİK STREPTOKOKLARA KARŞI BİTKİSEL BESİNLERİN ANTİOKSİDAN VE ANTİMİKROBİYAL ETKİNLİKLERİ

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Günlük beslenmede tüketilen meyve, sebze, meyve suları ve bitkisel çayların bazı enfeksiyon hastalıklarından korunmada önemi olduğu düşünülmektedir. Bu çalışmada, besin öğeleri A Grubu ß-Hemolitik Streptokoklara karşı antioksidan ve antimikrobiyal aktiviteleri bakımından araştırılmıştır. A Grubu ß-Hemolitik Streptokoklardan *Streptococcus pyogenes;* tonsillofarenjit, kızıl, menenjit, romatoid artrit gibi hastalılara sebep olabilen çok tehlikeli bir patojendir.

Günlük diyette kullanılan soğan, turp, havuç ve erik meyve sebzeleri; portakal, şeftali, nar ve üzüm suları; adaçayı, anason, kuşburnu ve papatya çayları örnek olarak seçilmiştir. Kuru özütler liyofilize edilerek ya da etil asetat içinde fraksiyonlama işlemiyle elde edilmiştir. Özütlerin antioksidan aktiviteleri toplam fenolik madde tayini ve 2,2-difenil-1-pikrilhidrazil radikal sönümleme metoduyla bulunmuştur. Özütlerin

antimikrobiyal aktiviteleri de disk difüzyon testi, minimum inhibe edici ve bakterisidal konsantrasyon tayin metodlarıyla çalışılmıştır.

Adaçayı, erik, soğan ve turp sırasıyla 0.043, 0.049, 0.148 ve 0.414 mg/mL EC_{50} değerleriyle yüksek radikal yakalama aktivitesi göstermişlerdir. Erik, adaçayı ve turp 1 mg özütte sırasıyla 50.506, 48.299, 44.427 ve 13.135 µg gallik asite eşdeğer fenolik madde içermektedir. Disk difüzyon yöntemiyle yüksek antimikrobiyal aktivitesi gösterenler sırasıyla 20, 16, 16, 14 ve 14 millimetrelik inhibisyon alanlarıyla soğan, turp, anason, havuç şeftali özütleridir. Havuç, soğan ve turp özütleri sırasıyla 0.008, 0.125, 0.250 mg/mL konsantrasyonlarda etkili bakteriyostatik ve 0.06, 0.5, 1 mg/mL konsantrasyonlarda etkili bakterisidal ajanlar olarak tespit edilmiştir.

Anahtar Kelimeler: A grubu beta hemolitik streptokok, bitki özüt eldesi, koruyucu hekimlik, antioksidan, antimikrobiyal aktivite

To my family

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LIST OF ABBREVATIONS

mg	Milligram
mL	Milliliter
µL	Microliter
µm	Micrometer
mm	Milimeter
nm	Nanometer
GAS	Group A ß-haemolytic streptococci
ABT	Acute bacterial tonsillopharyngitis
RA	Rheumatoid arthritis
CLSI	Clinical Laboratory Standards Institute
HPLC	High pressure liquid chromatography
DPPH	2,2-diphenyl-1-picrylhydrazyl
TP	Total Phenolics
GAE	Gallic acid equivalent
CFU	Colony forming unit
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
UV	Ultra violet visible spectroscopy
PBS	Phosphate buffer saline

CHAPTER 1

INTRODUCTION

1.1 Group A ß-haemolytic streptococci

"Streptococcus pyogenes (group A streptococcus) is an important species of gram-positive extracellular bacterial pathogens" (Cunningham, 2000). There are more than fifty classified species those which belong to the genus *Streptococcus.* Streptococci generally cause infection and/or colonize as part of the normal flora of a broad range of hosts which can be humans and domesticated animals. *Streptococcus pyogenes* causes "strep" throat and *Streptococcus pneumoniae* a foremost important cause of pneumonia are among the two major human pathogens both in children and adults (Bessen, 2009; Gonzalez-Rey, 2003).

Scientific classification:

Kingdom : Eubacteria
Phylum : Firmicutes
Class : Bacilli
Order : Lactobacillales
Family : Streptococcaceae
Genus : *Streptococcus*
Species : *Streptococcus pyogenes* (Rosenbach, 1884)

Group A ß-haemolytic streptococci (GAS) can easily be spread through air by droplets of saliva or nasal secretions by close contact.

Emergence of streptococcal pharyngitis in crowded institutional places, the military, schools and families is most likely (Hayes, 2001).

Streptococcus pyogenes can be the reason for a simple pharyngitis and skin infection as well as to life-threatening invasive illness including pneumonia, bacteraemia, necrotizing fasciitis, streptococcal toxic shock syndrome (TSS), and nonsuppurative sequelae such as acute rheumatic fever and glomerulonephritis (WHO, 2007).

Bessen has reported in 2009 that "*S. pyogenes* is responsible for a minimally estimated 616 million cases of throat infection (pharyngitis, tonsillitis) worldwide per year, and 111 million cases of skin infection (primarily non-bullous impetigo) in children of less developed countries" (Bessen, 2009).

Group A ß-haemolytic streptococci, mostly occurs in under-developed countries where it is harder to gather disease data. In order to understand the world-wide importance of GAS diseases, more information is necessary. Only reliable data is available for the rheumatic heart disease among GAS diseases all over the world (Carapetis, 2005).

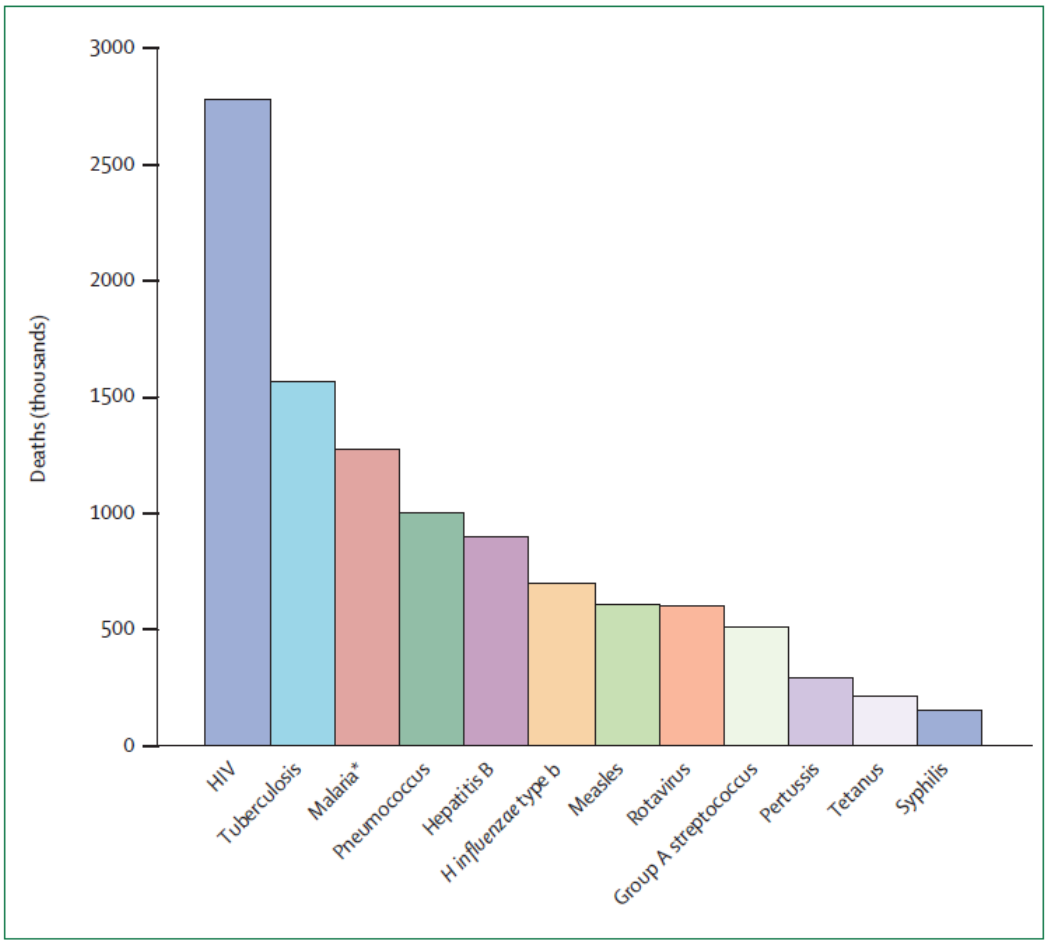

Figure 1.1 Estimated global mortality from individual pathogens in 2002.

Estimated 2004 World Health Report found in fact sheets on the World Health Organization (WHO) website (http://www.who.int) (Carapetis, 2005).

1.2 Diseases caused by Group A ß-haemolytic streptococci

1.2.1 Tonsillopharyngitis

According to the WHO number one childhood illness continues to be *Streptococcal pharyngitis* throughout the world (WHO, 2007).

Streptococcus pyogenes is the primary reason for acute bacterial tonsillopharyngitis (ABT), accounts for 30-40% of all acute tonsillopharyngitis which are mainly viral (Lilja, 1999; Gehanno, 2003).

1.2.2 Streptococcal toxic shock syndrome

Streptococcal toxic shock syndrome is one of the serious diseases caused by *Streptococcus pyogenes* (Honore, 1999; Ferry, 2009).

Hypotension and shock, multiple organ failure, systemic toxicity, severe pain, rapid necrosis of subcutaneous tissues and skin, and gangrene are among the traits of invasive streptococcal infections (Cunningham, 2000).

1.2.3 Rheumatic fever

Rheumatic fever is one of the serious consequences group A streptococcal pharyngitis. The disease appears as an inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea), skin (erythema marginatum), and/or subcutaneous nodules (Cunningham, 2000).

The occurrence of rheumatic fever has declined in industrialized countries since the 1950s and now has an annual incidence of around 1 out of 200 000 children of school age, however, in developing countries, number of cases increase up to 400 (WHO, 2007).

1.2.4 Rheumatoid arthritis

Rheumatoid arthritis (RA) manifests itself as chronic inflammation of the joints, usually continues as joint damage and disability. The pathogenesis of RA is still unknown, as a result of this, antirheumatic therapies are focused on nonspecific suppression of disease activity (Van Gestel, 1996).

1.2.5 Rheumatic heart disease

Rheumatic heart disease (RHD), is known as carditis developed by recurrent rheumatoid arthritis with progressive and permanent valvular lesions, affects more than one-third of children in developing countries. However, RHD occurrence decreased with use of penicillin and better living standards in the developed countries (Marijon, 2009).

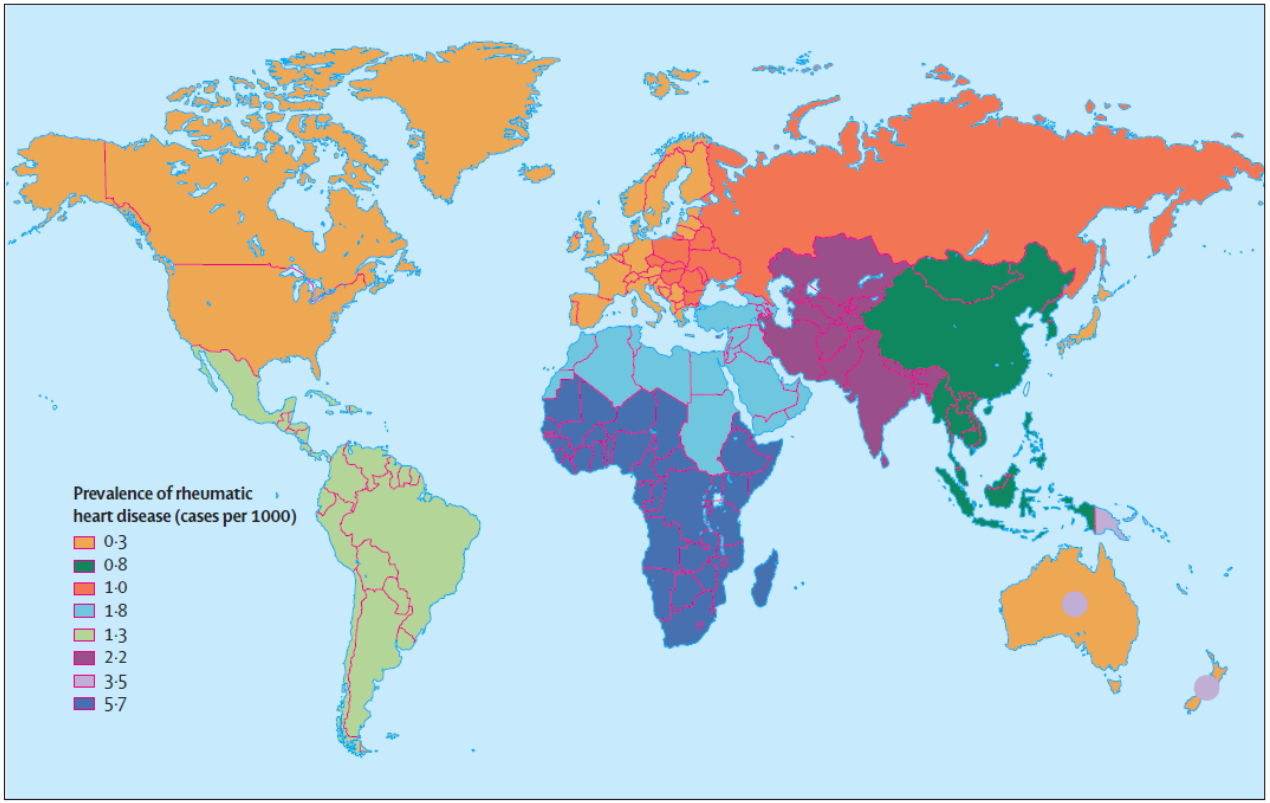

Figure 1.2 Prevalence of rheumatic heart disease in children aged 5–14 years. The circles within Australia and New Zealand represent indigenous populations (and also Pacific Islanders in New Zealand) (Carapetis, 2005).

1.2.6 Meningitis

Before the discovery of antibiotics, bacterial meningitis was considered as a lethal illness. Even today, bacterial meningitis continues to be one of the serious cause of childhood death, and with one third of the survivors having either a transient or permanent deafness, or other neurological abnormalities, in spite of an increased access to refined intensive care establishments, and various antimicrobials including active β-lactams (Sáez-Llorens, 2003; Theodoridou, 2007).

1.3 Antimicrobials against Group A ß-haemolytic streptococci

Penicillin is one of the most favored low-cost antibiotics against GAS infections, since its discovery in 1943 (Nir-Paz, 2006).

A group of antibiotics, called ß-lactams, mimics a section of the bacterial cell wall structure and causing bacteria to burst open and die. There are four subgroups of ß-lactams: penicillins, cephalosporins, carbapenems, and monbactams. Penicillins include the original drug, and various derivatives as ampicillin, amoxicillin, and methicillin. Cephalothin, cefoxitin, ceftazidime, and cefipime are in the subgroup of cephalosporins. Carbapenems include thienamycin and imipenem, and monobactams contains aztreonam (Guilfoile, 2006).

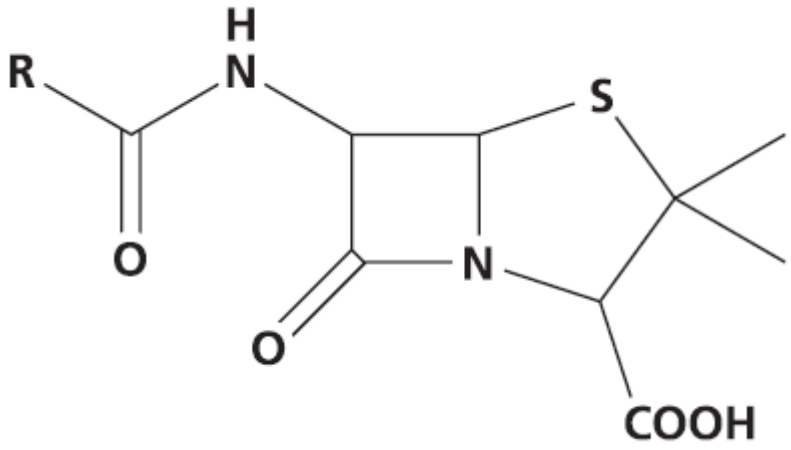

Figure 1.3 Penicillin, a ß-lactam antibiotic excerpted from (Guilfoile, 2006).

In this era, the biggest problem stems from the vast misuse of antibiotics, results in bacterial resistance as given in Table 1.1 (Guilfoile, 2006; Johnson, 2000; Gehanno, 2003).

Table 1.1 Date when an antibiotic became available and when resistance was first reported (Guilfoile, 2006).

	INTRODUCED	**RESISTANCE OCCURRED**
Sulfonamides	1930s	1940s
Penicillin	1943	1946
Streptomycin	1943	1948
Bacitracin	1945	1953
Chloramphenicol	1947	1959
Cephalosporin	1960s	late 1960s
Neomycin	1949	1950
Tetracycline	1948	1953
Erythromycin	1952	1988
Vancomycin	1956	1988
Kanamycin	1957	1966
Methicillin	1960	1961
Ampicillin	1961	1973
Gentamicin	1963	1969
Carbenicillin	1964	1974
Clindamycin	1969	1970
Amoxicillin	1972	1975
Piperacillin	1980	1981
Augmentin	1984	1984
Aztreonam	1984	1985
Imipenem	1985	1985
Ciprofloxacin	1987	1987
Quinupristin-Dalfopristin	1999	2000
Linezolid	2000	2002

1.4 Botanical information about the plant species

1.4.1 Onion

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Monocots
Order : Asparagales
Family : Liliaceae
Genus : *Allium*
Species : *A. cepa*

Binomial name

Allium cepa L.

1.4.2 Radish

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Rosids
Order : Brassicales
Family : Brassicaceae
Genus : *Raphanus*
Species : *R. sativus*

Binomial name

Raphanus sativus L.

1.4.3 Carrot

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Apiales
Family : Apiaceae
Genus : *Daucus*
Species : *D. carota*

Binomial name

Daucus carota L.

1.4.4 Plum

Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Rosales
Family : Rosaceae
Genus : *Prunus*
Species : *P. domestica*

Binomial name

Prunus domestica L.

1.4.5 Sage

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Lamiales
Family : Lamiaceae
Genus : *Salvia*
Species : *S. fruticosa*

Binomial name

Salvia fruticosa Mill

1.4.6 Anise

Scientific classification

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Apiales
Family : Apiaceae
Genus : *Pimpinella*
Species : *P. anisum*

Binomial name

Pimpinella anisum L.

1.4.7 Rosehip

Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Rosales
Family : Rosaceae
Genus : *Rosa*
Species : *R. canina*

Binomial name

Rosa canina L.

1.4.8 Chamomile

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Asterids
Order : Asterales
Family : Asteraceae
Genus : *Anthemis*
Species : *A. arvensis*

Binomial name

Anthemis arvensis L.

1.4.9 Orange

Scientific classification:

Kingdom : Plantae
(unranked) : Angiosperms
(unranked) : Eudicots
(unranked) : Rosids
Order : Sapindales
Family : Rutaceae
Genus : *Citrus*
Species : *C. sinensis*

Binomial name

Citrus sinensis L.

1.4.10 Peach

Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Rosales
Family : Rosaceae
Genus : *Prunus*
Species : *P. persica*

Binomial name

Prunus persica L.

1.4.11 Pomegranate

Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Myrtales
Family : Lythraceae
Genus : *Punica*
Species : *P. granatum*

Binomial name

Punica granatum L.

1.4.12 Grape

Scientific classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Vitales
Family : Vitaceae
Genus : *Vitis*
Species : *V. vinifera*

Binomial name

Vitis vinifera L.

1.5 Plants as the origin of medicinal agents

Plants are used worldwide for the treatment of diseases. There are more than 20,000 species of plants used in traditional medicines would remain as prospective medicine reservoirs. Moreover, bioactive components of medicinal plants may lead to new drug discoveries (Amor, 2009; Gupta, 2008).

Albeit the increased progress in pharmacology and synthetic organic chemistry, still the use of natural products stay unaffected in the therapy of bacterial diseases (Gupta, 2008).

The plant originated natural antibiotics may have a significant clinical value in cure of resistant microbial strains since they may have bacteriostatic activity by different mechanisms than those currently used artificial antibiotic compounds (Barbour, 2004; Benli, 2008).

1.6 Importance of antioxidant compounds

Antioxidant substances are synthetic or natural compounds prevent or delay the deterioration of systems by scavenging free radicals produced by oxidation reactions. In biological systems, antioxidants are enzymes or other organic substances, such as vitamin E or β-carotene, which are capable of reducing the harmful effects of oxidation (Huang, 2005).

There is an increasing interest towards the antioxidant phenolics found in dietary sources. Thus, many fruit juices as grapes and berry juices have received attention due to their antioxidant activity. Phenolic compounds may produce their beneficial effects by scavenging free radicals (Gil, 2000).

1.7 Methods for the determination of antimicrobial activities

1.7.1 Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) is stated as the lowest concentration of an antibiotic that will inhibit the growth of the organism being tested. The MIC is helpful for determining the concentration necessary for the antimicrobial agent to inhibit the growth of a pathogen. A complete guide to define standards of this protocol can be found in the Clinical Laboratory Standards Institute (CLSI) guidelines document M7-A7 (Schwalbe, 2007).

Agar dilution and broth dilution are the most commonly used techniques to determine the minimal inhibitory concentration (MIC) of antimicrobial agents that inhibit the growth (bacteriostatic activity) of bacteria (Wiegand, 2008).

1.7.2 Determination of minimum bactericidal concentration

The minimum concentration of an antimicrobial of interest eradicating the majority (99.9 %) of a bacterial inoculum is described as the minimum bactericidal concentration (MBC). In some infections (i.e., evclocaditis), it is necessary to kill the microorganism rather than inhibiting its growth. The test performed to determine the ability of antimicrobial to kill the bacteria is referred as MBC test. In routine laboratory studies, the micro agar dilution method is used to determine MBC as an adaptation of the agar dilution method with small volumes in 96 micro-well plate (Schwalbe, 2007).

1.7.3 Kirby-Bauer Disk Diffusion Method

The Kirby-Bauer disk diffusion method is one of the earliest methods for testing antimicrobial effects on bacteria by measuring the diameter of bacteria cleared zone in agar plates. In the earlier studies, the bacteria of interest was used to be smeared perpendicular to a well made on agar plates, and antibiotic solutions would be applied to the well by diffusing out to inhibit the growth of bacteria. Later, antibiotic solutions in agar wells or cylinders were substituted by the paper discs soaked in antibiotics. As soon as an antibiotic disk comes in contact with an inoculated agar surface, an immediate race between antibiotic and bacteria begins. Antibiotic molecules diffuse out from the disk into the agar, creating a dynamically changing gradient of antibiotic concentrations, while the test organism starts to divide and grow. The zone edge is formed at the concentration of antibiotic that is just able to inhibit the organism reaches an undefeatable mass of cell (Schwalbe, 2007).

1.8 Aim of the study

This study was designed to investigate the antioxidant and antimicrobial activities of the extracts prepared from a variety of fresh produces and herbal teas daily consumed in our diets. Antimicrobial activities of the extracts were studied against the group A ß-haemolytic streptoccoci. *Streptococcus pyogenes*, a member of the group A ß-haemolytic streptococci, is a very dangerous pathogen, known to cause tonsillopharyngitis, scarlet fever, meningitis, and rheumatic arthritis. Many commercial antimicrobial agents are available to struggle against these pathogens, nevertheless the expenses of treatments and the resistance acquisition of bacteria, force researchers to explore new alternates. In conclusion, the investigation of fresh produces and herbal teas for

antimicrobial activities would reveal a totally new era of “foods for medicine” against pathogens.

CHAPTER 2

MATERIAL AND METHODS

2.1 MATERIALS

2.1.1 Chemicals

Agar plate medium for identifying *Streptococcus pyogenes* including %5 sheep blood agar were purchased from Salubris. Luria Bertani agar, Luria Bertani broth, Mueller Hinton agar, Mueller Hinton broth and brain heart infusion broth for preparing medium used in antimicrobial assays were purchased from Merck (Darmstadt, Germany).

Streptococcus pyogenes used for bacterial culturing were obtained from American Type Culture Collection, UK.

Antimicrobial susceptibility test disks (6 mm diameter) used in disk diffusion test were bought from Oxoid (Hants, UK).

Antimicrobial disk standards clarithromycin (15 μg), clindamycin (2 μg), erythromycin (15 μg), ciprofloxacin (5 μg), penicillin (10 μg) and azithromycin (15 μg) used in disk diffusion test were purchased from Bioanalyse, Ankara, TÜRKİYE.

Penicillin G potassium salt, cell culture tested grade in powder form were obtained from Sigma Chemical Company, (St.Louis, MO, USA).

HPLC grade methanol, acetone, ethanol, ethyl acetate, acetonitrile and hexane used in solvent effect experiment were purchased from Merck (Darmstadt, Germany). Cell culture grade dimethylsulfoxide was purchased from AppliChem. Milli-Q system (Millipore, Bedford, MA, USA) was used to generate ultrapure water. Distilled water used to prepare extracts was got via Milli-pore walled system.

Disposable syringe filter (pore size: 0.22 µm and 0.45 µm Diameter: 33mm) is purchased from Millipore Corporation (Bedford, MA USA).

2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate (Na_2CO_3), were purchased from Sigma Chemical Company, (St.Louis, MO, USA). Folin Ciocalteu's phenol reagent was purchased from Merck (Darmstadt, Germany).

Rosehips from *Rosa canina* fruits were dried and ground to prepare rosehip infusion tea.

Arifoğlu brand (İstanbul, TÜRKİYE) anise (*Pimpinella anisum*) herbal tea, Lipton (Unilever Ltd., İstanbul, TÜRKİYE) sage (*Salvia fruticosa*) herbal tea, Doğadan brand (Ankara, TÜRKİYE) chamomile (*Anthemis arvensis*) herbal tea in the form of 20 packages of tea bags were bought from local markets in Ankara, TÜRKİYE.

Onion (*Allium cepa*), radish (*Raphanus sativus*), carrot (*Daucus carota*), plum (*Prunus domestica*), orange (*Citrus sinensis*), peach (*Prunus persica*), pomegranate (*Punica granatum*) and grape (*Vitis vinifera*) were bought freshly from local market.

2.1.2 Apparatus

Class II Safety Cabinet used for creating a sterile environment for microbiological work was purchased from ESCO, Thailand.

All the spectroscopic data were obtained in Cary 50 Bio UV-VIS spectrophotometer (Varian, USA). Other instruments used for the experiments are listed as followings:

- Bandelin Sonorex (ultrasonic bath)
- SpectraMax 340PC384 (96 micro-well plate reader)
- Optic Ivymen System (incubator)
- Rotary evaporator (Heidolph Laborota 4000)
- Lyophilizator (Heto-Holten Model Maxi-Dry Lyo)
- Blender: Waring model 32BL80 (New Hartford, CT, USA)
- Nuve FN-50 incubator
- Philips Cucina HR1840 Food Processor

2.2 METHODS

2.2.1 Preparation of samples

Selected fresh fruits, vegetables, and commercial herbal teas were bought from local markets. All fresh fruits and vegetables were immediately washed, dried and kept at 4 °C in dark until use. Onion, radish, carrot and plums were classified as fruits and vegetables, and freshly squeezed pomegranate, peach, orange and grapes were grouped as the fruit juices. Commercially available rosehip dried fruits and tea brands including anise, sage, and chamomile in the form of sealed envelope tea bags were bought from local markets and stored at room temperature in dark until use.

Figure 2.1 In order to investigate fruits and vegetables; onion, radish, carrot and plums, fruit juices; pomegranate, peach, orange and grape were bought fresh from market, washed, dried and kept in 4 °C in dark. Brands containing anise, sage, rosehip and chamomile.

2.2.2 Preparation of plant extracts

2.2.2.1 Preparation of fruit and vegetable extracts

Using food processor (Philips HR1840), 497.1 g of peeled onions, 308.1 g of radishes, 332.7 g of carrots and 422.4 g of plums were blended in small portions for three minutes. Each fruit and vegetable blend was collected and mixed with purest available ethyl acetate solvent in ratio of 1:6 (w/v). Then, the mixtures were incubated at rocking-incubator for 24 hours at 37 °C at 180 rpm. The ethyl acetate phases were collected using separatory funnel, then water phase was fractionated with ethyl acetate once more. After collection of the ethyl acetate phases, solvents were evaporated by rotary evaporator at 40 °C.

2.2.2.2 Preparation of fruit juice extracts

1665.5 g of arils of pomegranates, 655 g of peach, 1241.8 g of orange and 474.9 g of grapes were blended separately using a commercial food processor which helps to separate juice from the pulp. Juices were filtered through filter paper utilizing glass funnels in the cold-room at 4 °C kept in dark. Final volumes given in Table 3.2 were lyophilized to dryness and refrigerated (4 °C) until used in further experiments.

2.2.2.3 Preparation of tea extracts

Selections of commercial tea-bags, containing sage, anise and chamomile, were cut open and contents were weighted to obtain 40 g of each. Rosehip fruits were washed, dried and ground. Infusion was performed at two steps. Fourty grams of each tea sample was let to infuse in 480 mL boiled distilled water for about one hour standing at room temperature. Then, to increase the yield, infused tea solutions were incubated at 37 °C for 24 hours using rocking-incubator at 180 rpm. After incubation, the tea infusion solutions were filtered through filter paper and filtrates were lyophilized to dryness. The lyophilized tea extracts were kept at 4 °C in dark until used.

2.2.3 UV-VIS absorption spectra from plant extracts

Twelve extracts from the fresh produces and herbal teas were dissolved in 99.5 % methanol and their UV-VIS absorption spectra were scanned using wavelength range between 200 to 800 nm by Cary 50 Bio UV-VIS spectrophotometer (Varian, USA).

2.2.4 Preparation of Microbial Strain

The microbial strain group A ß-haemolytic streptococci, *Streptococcus pyogenes* ATCC strain, which was used in microbiological studies was bought from Refik Saydam Hygiene Center. We performed our experiments with the control strain.

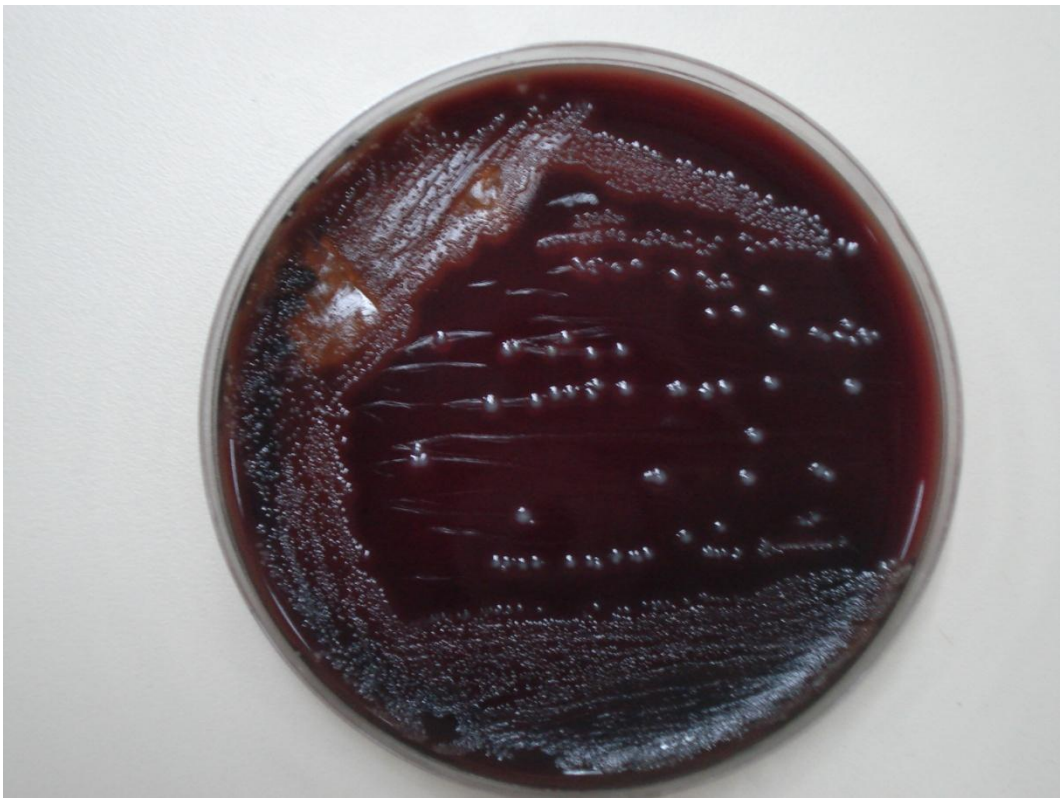

Figure 2.2 The microbial strain group A ß-haemolytic streptococci, *Streptococcus pyogenes* ATCC strain, which was used in microbiological studies was bought from Refik Saydam Hygiene Center and transferred to *Streptococci* medium incubated at 37 °C for 24 hours.

The group A ß-haemolytic streptococci, *Streptococcus pyogenes* pellet were suspended in 1mL of brain heart infusion broth and aseptically spread onto *Streptococci* agar medium and incubated at 37 °C for 24 hours. Those plates were stored at 4 °C.

In order to prepare -80 °C stocks for long term storage, three to five morphologically similar colonies isolated from 4 °C agar plates were aseptically transferred and suspended in 1 mL Mueller Hinton broth. Then 100 µL of the suspension were inoculated to 100 mL Mueller Hinton and brain heart infusion broths. They were incubated at 37 °C at 180 rpm until they reach a final OD_{600} of 0.6 when the cells were at mid log phase. Then, 100 µL of bacterial suspension was mixed with filter sterilized pre chilled 25 % 4,9 mL glycerol. Final mixture was carefully mixed with several inversions and then 250 µL of the bacterial solution were delivered to pre chilled centrifuge tubes and put in -80 °C freezer for long term storage.

2.2.5 Bacterial growth curve

Bacteria were inoculated to 100 mL Luria Bertani broth to reach a final OD_{600} of 0.03 and were incubated at 37 °C at 185 rpm for 24 hours. The absorbance measurements were performed at the given hours: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24. In addition to absorbance measurements, to achieve colony counts at 4, 8, 12, 16, 20 and 24, at least four proper dilutions of the sample were performed with phosphate buffer saline (PBS) and were spread to agar plates and incubated at 37 °C, until visible colony growth occurs, approximately for 12-16 hours.

2.2.6 Minimum inhibitory concentration of solvents

In a 96 micro-well plate, ten different concentrations of eight solvents (methanol, dimethylsulfoxide, acetone, ethanol, ethyl acetate, acetonitrile, hexane and ultrapure water) were prepared by two fold dilutions of the highest concentration containing first well. When working with 96 micro well plates, all solution transfers were carried out by using Thermo Scientific Finnpipettes (12 multi-channel 5-50 µL or 50-300 µL). All wells, excluding the sterility controls, were inoculated with *Streptococcus pyogenes.* Final composition of the wells was arranged to

be 85 µL of broth, 10 µL of 99.5 % solvent and 5 µL of inoculum containing approximately $5x10^5$ cells. Wells of 11^{th} horizontal lane, containing only 100 µL of Mueller-Hinton broth, served as sterility control. Growth controls were prepared in 12^{th} horizontal lane wells and contained 95 µL of Mueller-Hinton broth and 5 µL of inoculum. The lowest concentration of solvent in which no visible growth observed was labeled as minimum inhibitory concentration. All experiments were performed in triplicates.

2.2.7 Minimum inhibitory concentration determination by micro broth dilution method

A modified version of National Committee for Clinical Laboratory Standards protocol for micro broth dilution was used to determine minimum inhibitory concentrations (Wiegand, 2008). Stock solutions of each sample were prepared according to the data given in Table 2.1. In a 96 micro-well plate, twelve different concentrations of the samples were prepared by two fold dilutions of the previous well each time, starting with the highest concentration first well. All wells, excluding the sterility control ones, were inoculated with *Streptococcus pyogenes.* In each set of experiment using side to side two 96 micro-well plates, twelve concentrations from twelve different extracts were assayed. Final composition of the wells was arranged to contain 85 µL of Mueller-Hinton broth, 10 µL of extract solution and 5 µL of inoculum containing approximately $5x10^5$ cells. In each experiment, 13^{th},14^{th}, 15^{th} and 16^{th} horizontal lanes were set as antibiotic control, antibiotic sterility control, growth control and sterility control, respectively. In 13^{th}, antibiotic control lane, penicillin concentration was set to 0.20 µg/mL and inoculated with bacteria. In 14^{th} antibiotic sterility control lane, antibiotic was added but not inoculated. In 15^{th} growth control lane, only 95 µL of Mueller-Hinton broth was mixed with 5 µL of inoculum without addition of any antimicrobial solutions. Sixteenth sterility control lane contained only 100 µL of Mueller-Hinton broth. Minimum inhibitory concentration was

defined as the lowest concentration where no bacterial growth was observed as monitored at 600 nm using SpectraMax 340PC384 (96 micro-well plate reader). All experiments were performed in triplicates.

Table 2.1 Stock solutions (mg/mL) prepared in methanol for subsequent dilutions for determination of minimum inhibitory concentration experiments.

	Sample	Stock conc. (mg/mL)
1	**Onion**	80
2	**Radish**	80
3	**Carrot**	80
4	**Plum**	80
5	**Sage**	320
6	**Anise**	320
7	**Rosehip**	320
8	**Chamomile**	320
9	**Orange**	320
10	**Peach**	320
11	**Pomegranate**	320
12	**Grape**	320

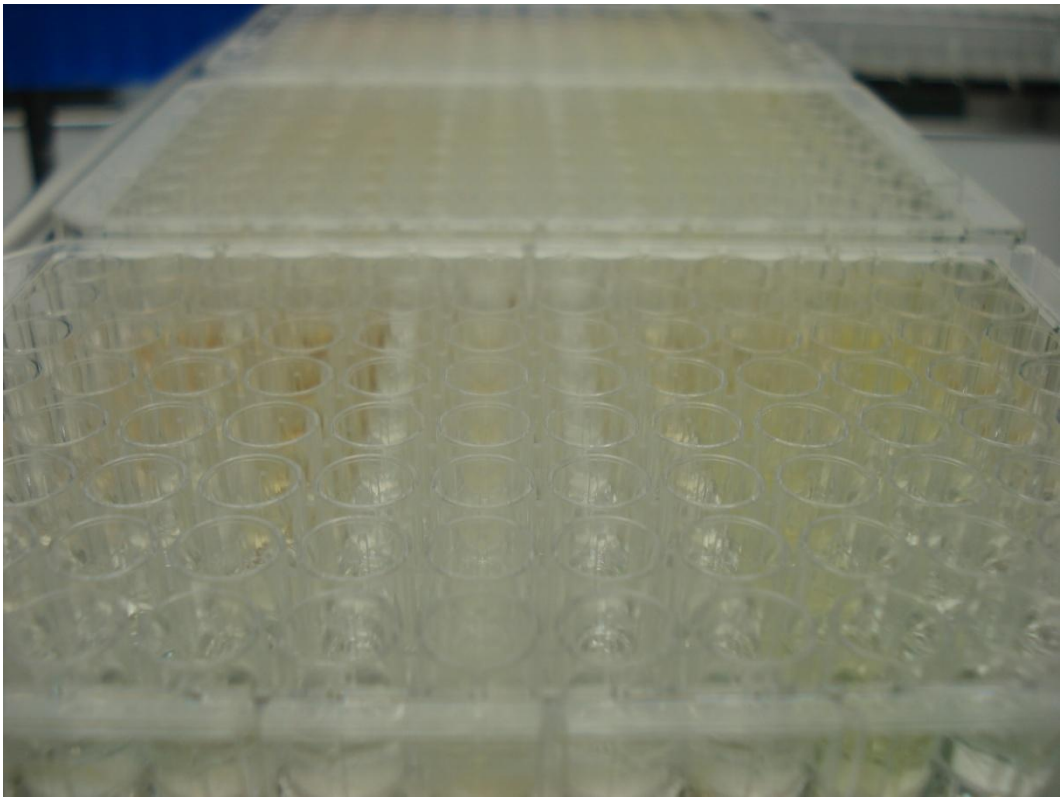

Figure 2.3 Three 96-microwell plate. Minimum inhibitory concentration experiment. All experiments were done in triplicates

2.2.8 Minimum bactericidal concentration determination by micro agar dilution method

A modified version of National Committee for Clinical Laboratory Standards protocol for micro agar dilution was used to determine minimum bactericidal concentration (Wiegand, 2008). After micro broth dilution experiment, a 10 µL of solution was transferred using Thermo Scientific Finnpipettes (12 multichannel 5-50 µL) from each well to another 96 micro-well plates which contained 200 µL of Mueller-Hinton agar in each well. Minimum bactericidal concentration was determined by the lowest concentration of extract where no visible bacterial growth was observed on the agar surface in the wells of 96-well micro plate. All experiments were performed in triplicates.

2.2.9 Kirby-Bauer Disk Diffusion Method

Approximately 25 mL of autoclaved Muller Hinton Agar was distributed into sterile 100 mm petri plates and allowed to solidify. Cultures from overnight grown brain heart infusion broth were diluted to achieve 10^8 colony forming unit per mL (cfu.mL^{-1}). One hundred micro liters of bacteria suspension was inoculated by spreading evenly on agar surface. All plates contained approximately 10^7 colony forming units (cfus). After 5 minutes of incubation, sterile 6 mm paper disks were set on agar surface carefully. Twenty µL of 40 mg/mL concentrations of 12 extracts were applied on filter disks. Agar petri plates containing disks were incubated at 37 °C for approximately 16 hours. After incubation, plates were observed for inhibition zones, and the diameters were measured in millimeters. Six antimicrobial disk standards containing clarithromycin, clindamycin, erythromycin, ciprofloxacin, penicillin and azithromycin were used as positive controls. An empty disk with 20 µL of water was used as the negative control. Each plate contained four disks and each experiment was repeated twice.

2.2.10 Evaluation of antioxidant activity

2.2.10.1 Free radical scavenging activity by DPPH method

A 0.05 mg/mL of DPPH ethanol solution which absorbs at 517 nm, produces nearly 1.3 unit of absorbance. Dry extracts were dissolved in ethanol and 0.1 mL of extract solutions was added to 1.4 mL of DPPH solution. With this process a series of extract solutions with varying concentrations have been prepared. Then the absorbance at 517 nm was recorded after 15 min of incubation for carrot, sage, anise, chamomile, orange, peach, pomegranate, grape and 30 min of incubation for onion, radish, plum and rosehip extract solutions at room temperature. Absorption of blank sample containing the same amount of ethanol and

DPPH solution was prepared and measured. Quercetin was used as the reference compound in the radical scavenging studies. These experiments were carried out in triplicates. Radical scavenging effect of extracts was calculated as:

$$DPPH\ radical\ scavenging\ (\%) = [(A_0 - A_1)] / A_0 \times 100$$

After incubation time; A_0 is the absorbance of the control with ethanol and A_1 is the absorbance of the sample in the presence of the extracts dissolved in ethanol.

50 % effective concentration (EC_{50}) values were calculated after constructing the percent *radical scavenging* versus log (extract concentration) plots.

2.2.10.2 Determination of total phenolic contents of extracts

Total concentration of phenolic compounds in extracts was determined according to method of Singleton and Rossi (1963) with some modifications. A 0.1 ml of each extract solution was mixed with 2 ml aqueous solution of 2 % Na_2CO_3 and vortexed vigorously. Blank solution for control was prepared by replacing extract solution with 0.1 mL of ethanol. The same procedure was also applied to obtain a standard curve by using gallic acid concentrations in the range of 0.05-0.3 mg/mL. After 3 minutes incubation time, a 0.1 mL of 50 % Folin–Ciocalteu's phenol reagent was added and each mixture was vortexed again and waited for a 30 minutes of incubation time at room temperature before the measurements of absorbance. At the end of the 30 minutes of incubation time, absorbance of each mixture was monitored at 750 nm and blanks were subtracted. Results were recorded as micrograms of total phenolics (TP) contained in milligrams of extract as the gallic acid equivalents (GAE).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Extraction of fresh produces and herbal teas

In this section, data obtained from extraction of 12 different samples of selected fresh produces and herbal teas were presented.

3.1.1 Extraction of fresh produces

Four samples of fresh produces onion, radish, carrot and plum were selected to extract and study their antioxidant and antimicrobial activities. A 497.1 grams of blended fresh onions was immediately mixed with ethyl acetate for extraction. Resultant dry extract weight was 2.90 g.

Blended weight of 308.1 g of radishes, 332.7 g of carrots and 422.4 g of plums extracted in ethyl acetate as explained in section 2.2.2.1, respective dry extracts in weights of 0.58 g, 0.80 g and 0.9 g were obtained after evaporation. Extraction yields (%,w/w) are calculated and presented in Table 3.1.

Table 3.1 Ethyl acetate extraction data of selected fruits and vegetables.

Fruit and vegetables	Onion	Radish	Carrot	Plum
Total weight (g)	497.1	308.1	332.7	422.4
Total extract (g)	2.90	0.50	0.80	0.90
Extract yield (%, w/w)	0.58	0.16	0.24	0.21

Onion extraction has yielded the highest percentage with a value of 0.58 % (w/w).

3.1.2 Extraction fruit juices

In order to investigate antimicrobial activities of fruit juices, we have selected four different species: Pomegranate, peach, orange and grape.

1665.5 grams of arils of pomegranate weighted and pulp and juice were separated. Subsequently, filtered 407 mL of pomegranate juice was immediately lyophilized and resultant dry extract was 59.2 grams.

Blend weights of fresh fleshes of 655 g of peaches, 1241.8 g of oranges and 474.9 g of grapes were processed in the food processor. Respective volumes of 452 mL, 524 mL and 371 mL of filtered fruit juices were lyophilized to dryness.

In Table 3.2 blend weights, volumes of juices, dry extract weights and percent extract yields were presented.

Table 3.2 Extraction data of selected fruit juices.

Juices	**Pomegranate**	**Peach**	**Orange**	**Grape**
Total weight (g)	1665.5	655.00	1241.8	474.9
Juice mL	407.00	452.00	524.00	371.00
Total Extract (g)	59.20	52.00	51.60	42.40
Yield (%, w/v)	14.55	11.50	9.85	11.43

Pomegranate displayed the highest yield of extraction with a value of 14.55 %(w/v).

3.1.3 Extraction of herbal tea infusions

Herbal tea samples were selected as sage, anise, rosehip and chamomile to prepare extracts by infusion method as described in section 2.2.2.3.

40 grams of each tea samples were extracted in 480 mL of boiled water and after filtration, each herbal tea infusions were lyophilized to dryness, providing 23.7 g, 6.2 g, 22.2 g and 8 g of dry extracts from chamomile, sage, rosehip and anise, respectively.

Table 3.3 shows the total weight (g), infusion volume (mL), dry extract weight (g) and yields in (%, w/w).

Table 3.3 Extraction data of selected herbal tea infusions.

Teas	**Chamomile**	**Sage**	**Rosehip**	**Anise**
Total weight (g)	40	40	40	40
Infusion volume (mL)	480	480	480	480
Extract (g)	23.70	6.20	22.20	8.00
Yield (%, w/w)	59.25	15.50	55.50	20.00

Among the herbal tea infusion extractions, chamomile has displayed highest percent yield (w/w) with a value of 59.25.

3.2 UV-VIS absorption spectra from plant extracts

UV-VIS absorption of twelve extracts dissolved in methanol was scanned up to 800 nm and resultant plots were displayed in Appendix A.

3.3 Bacterial growth curves

In antimicrobial experiments, bacteria count may affect the determination of the antimicrobial concentration. For instance, production of a compound by bacteria that would inhibit or reduce the activity of an antimicrobial agent could cause reduction of antimicrobial activity directly related with the bacteria count. Therefore, calibrating inoculum density according to the standards is very important. In antimicrobial experiments using equation of colony count versus optical density for the bacteria of interest, one could easily approximate the number of colony forming units for related optical density. This information was useful for preparing 4 °C, -80 °C stock solutions, for determination of MIC or MBC.

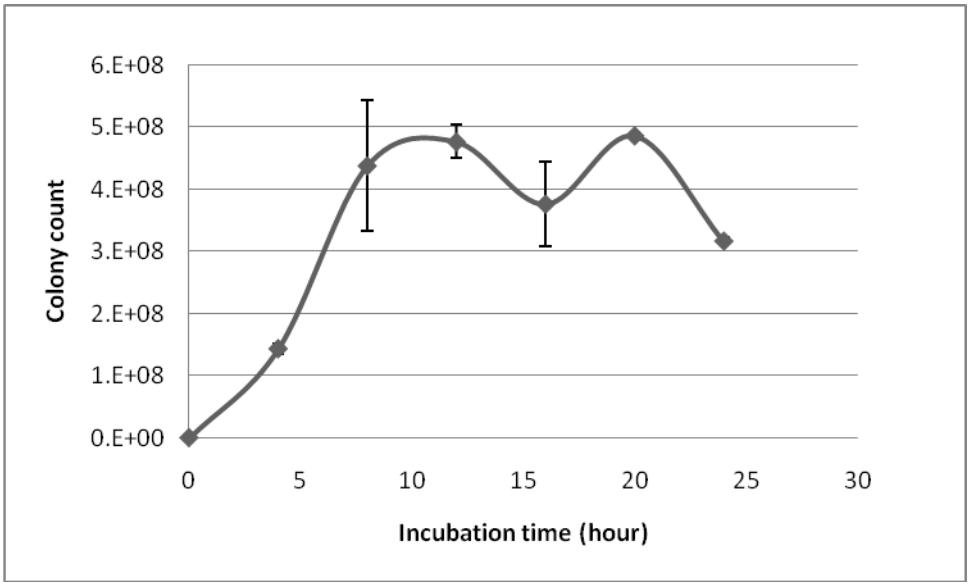

Figure 3.1 Plot of direct colony counts at different time intervals starting with a final OD_{600} of 0.03 as colony count versus incubation time.

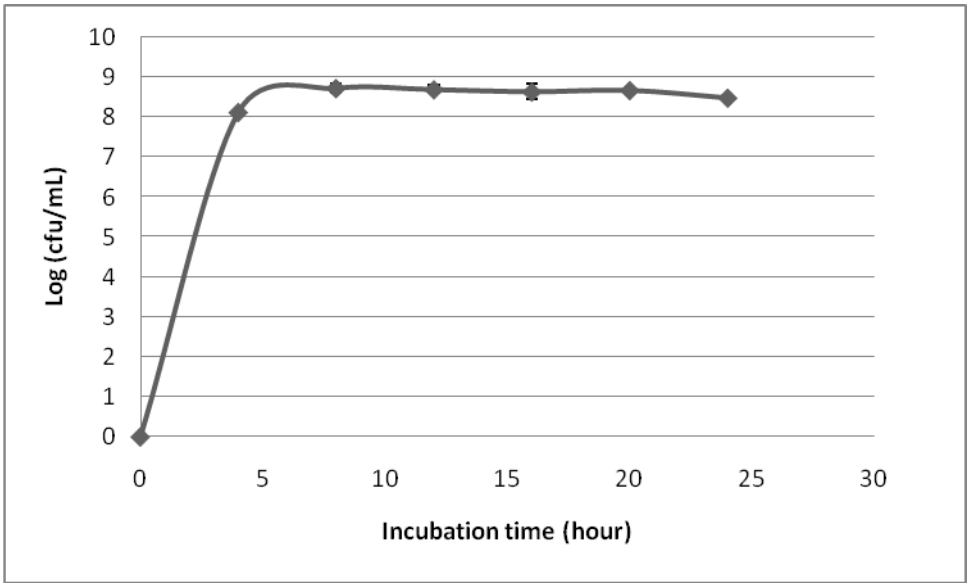

Figure 3.2 Base logarithmic representation of colony forming bacteria count per mL at different time intervals starting with a final OD_{600} of 0.03 as log (CFU/mL) versus incubation time.

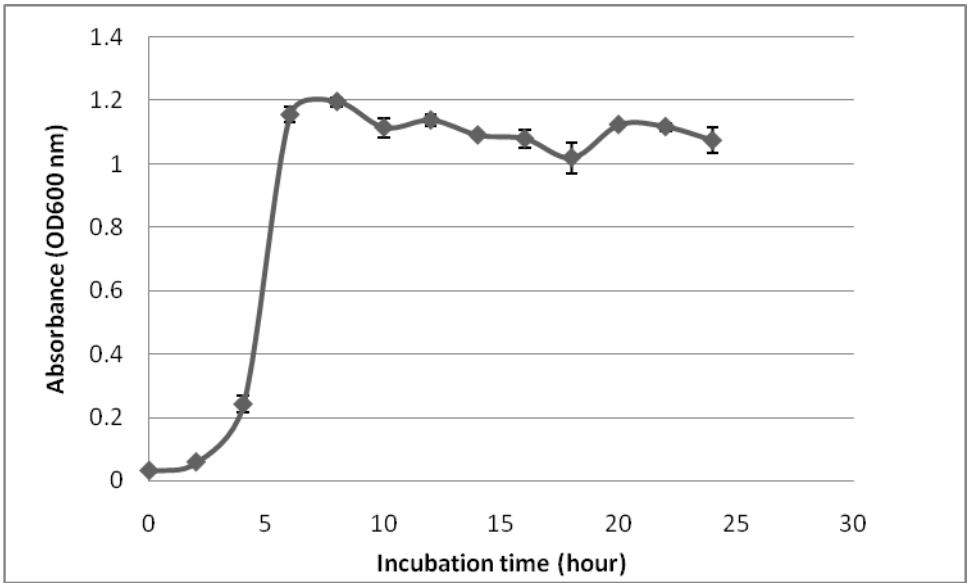

Figure 3.3 Plot of OD_{600} values at different time intervals starting with a final OD_{600} of 0.03 as OD_{600} versus incubation time.

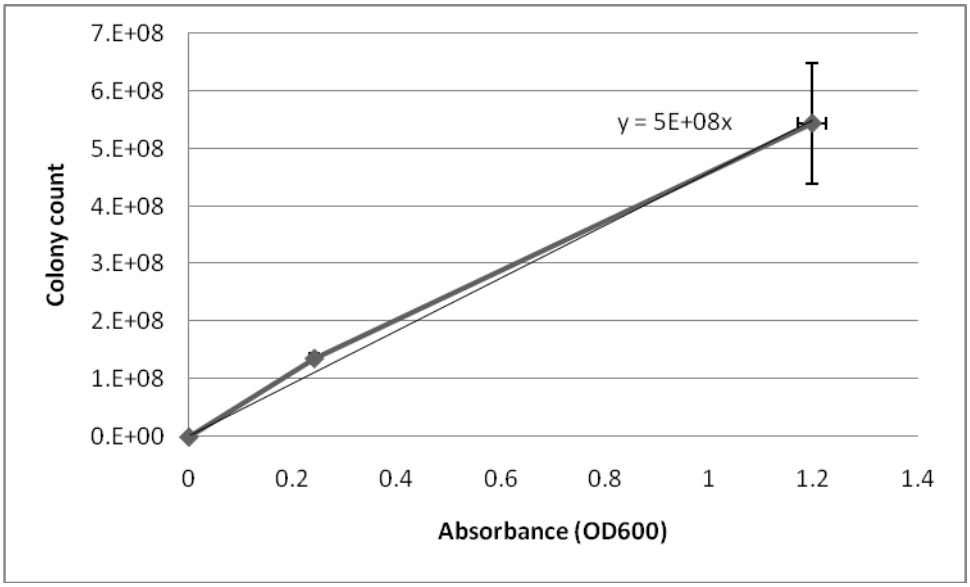

Figure 3.4 As a result of the OD_{600} and CFU/mL versus time experiments; we plotted the colony count vs OD_{600}. By using the equation, we can approximate the colony forming unit per mL count for a measured OD_{600} value. As a result, 1 OD_{600} is approximately $5x10^8$ CFU/mL

In Figure 3.4, bacterial colony count plotted against optical density monitored at 600 nm. The trendline equation from the plot was obtained as $y = 5x10^8x$. This equation was crucial to approximate the colony forming unit per mL for any value of OD at 600 nm.

3.4 Minimum inhibitory concentration of solvents

Minimum inhibitory concentrations of different solvents (methanol, dimethylsulfoxide, acetone, ethanol, ethyl acetate, acetonitrile, hexane and ultrapure water) were studied, by preparation of solvent (v/v) concentrations in Mueller-Hinton broth in a range of 0.02 to 9.95% (v/v).

As it is given in Table 3.4, it is found that only dimethylsulfoxide had MIC of 4.98% (v/v). All other solvents, when used 9.95% (v/v) and lower, presented no growth inhibitory activity.

When performing antimicrobial assays, extracts had to be dissolved in a solvent with no antimicrobial activity. Having no bacteriostatic or bactericidal activity, methanol was the solvent of choice for the preparation of appropriate concentrations in micro broth dilution assays.

Table 3.4 Result of minimum inhibitory concentration determination experiment for various solvents. For the concentrations used in the experiment none showed inhibitory activity but 9.95 and 4.98% of DMSO.

	MeOH	DMSO	Acetone	EtOH	EtOAc	Acetonitrile	Hexane	ddH2O
9.95%	+	-	+	+	+	+	+	+
4.98%	+	-	+	+	+	+	+	+
2.49%	+	+	+	+	+	+	+	+
1.24%	+	+	+	+	+	+	+	+
0.62%	+	+	+	+	+	+	+	+
0.31%	+	+	+	+	+	+	+	+
0.16%	+	+	+	+	+	+	+	+
0.08%	+	+	+	+	+	+	+	+
0.04%	+	+	+	+	+	+	+	+
0.02%	+	+	+	+	+	+	+	+
Sterility control	-	-	-	-	-	-	-	-
Growth control	+	+	+	+	+	+	+	+

3.5 Minimum inhibitory concentration of extracts

3.5.1 Minimum inhibitory concentration of fruits and vegetables

Antimicrobial activities of fruit and vegetables extracts were investigated against S*treptococcus pyogenes* using micro broth dilution method to determine minimum inhibitory concentrations of each extract as described in section 2.2.7.

Selected fresh produce extract concentrations were prepared in range of 0.004 to 8.0 mg/mL.

Considering 1.0 OD (600 nm) value was equivalent to the $5x10^8$ colony forming units per mL effect of extract concentrations on bacteria growth was monitored by measuring optical density at 600 nm as given in Figure 3.5.

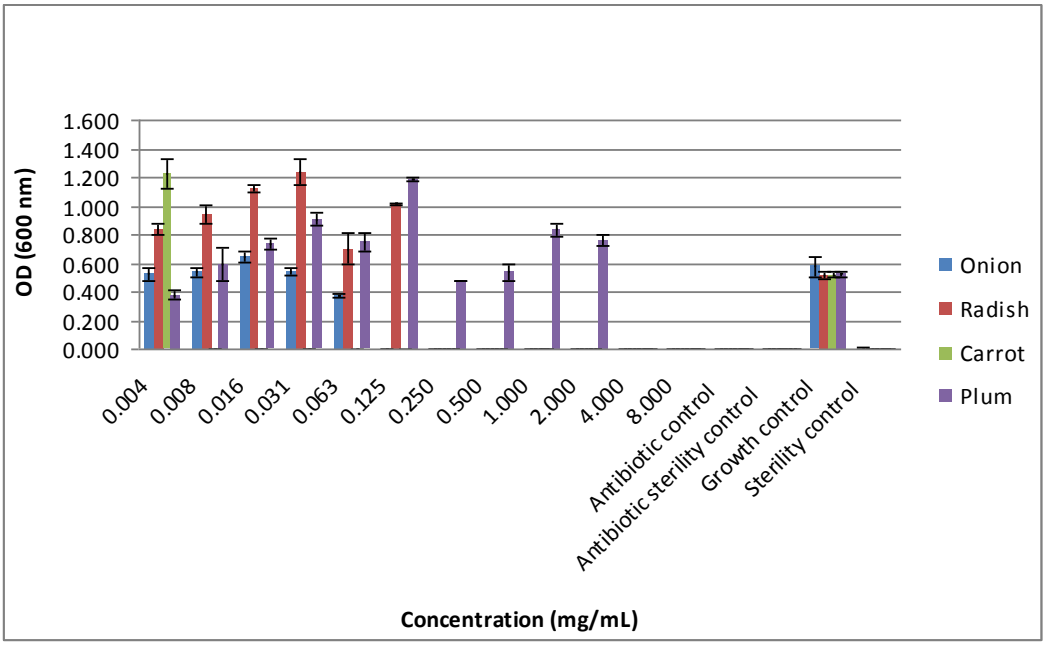

Figure 3.5 Minimum inhibitory concentration determination for various fruits and vegetables incubated for 16 hours.

Antibiotic control: Penicillin G was used as an effective reference antimicrobial compound in concentration of 0.20 μg/mL in the presence of $5x10^5$ cfus.
Antibiotic sterility control: Sterility of the antibiotic solution examined in the absence of inoculum.
Growth control: Test of normal bacterial growth in the absence of extracts and antibiotic agents.
Sterility control: Test the experiments were performed in aseptic conditions and the broth is sterile.

When OD value monitored at 600 nm equals to 0.0 absorbance, means no visible bacterial growth appeared for the given concentration of a sample. Although there may be more than one concentration values available inhibiting bacterial growth the lowest value of such concentrations would reveal the minimum concentration for inhibition of bacterial growth.

Bacterial growth was observed from 0.004 to 0.062 mg/mL concentrations of onion extracts. However, growth inhibition takes place at and above 0.125 mg/mL concentrations of onion extract. Therefore, 0.125 mg/mL was selected as MIC for onion, which is the lowest concentration where optical density was zero at 600 nm.

Carrot, onion, radish and plum with increasing minimum inhibitory concentrations were found as values of 0.008, 0.125, 0.250 and 4 mg/mL, respectively. Results are shown in Figure 3.6.

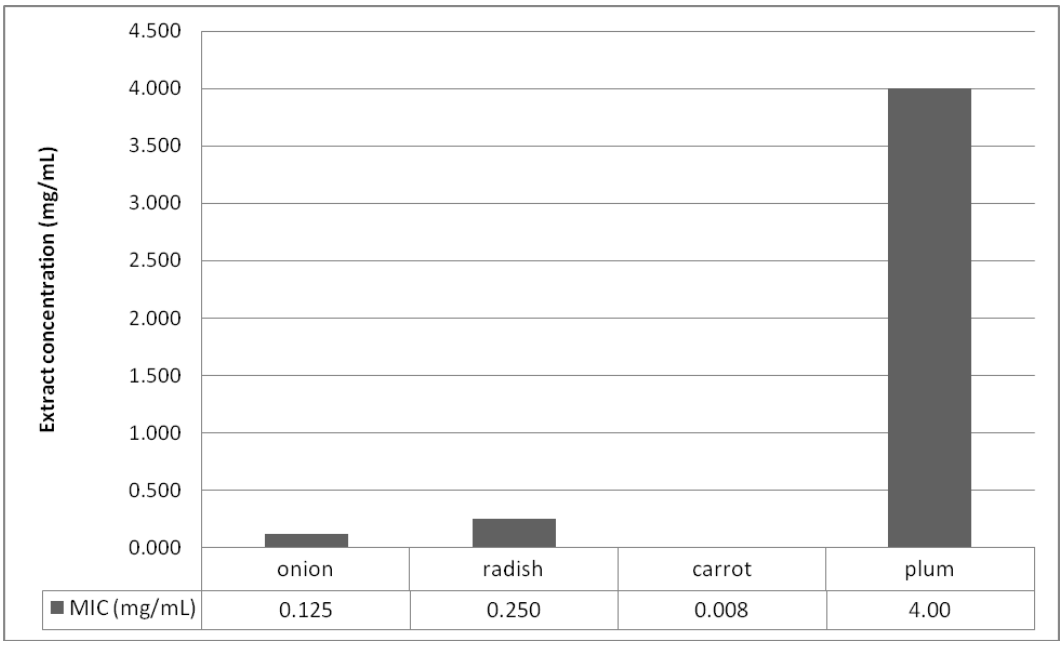

Figure 3.6 Minimum inhibitory concentrations (mg/mL) for various fruit and vegetable extracts against *Streptococcus pyogenes*.

The extract yield of carrot was found as 0.24 % (w/w), consequently 0.008 mg/mL MIC value would correspond to 1.67 mg of blended carrot in order to inhibit the growth of $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

3.5.2 Minimum inhibitory concentration of fruit juices

Fruit juice extracts were examined for their antimicrobial activities against S*treptococcus pyogenes* using micro broth dilution method. Determination of minimum inhibitory concentrations of each extract was performed as described in section 2.2.7.

Absorption at 600 nm for the bacterial growth observed for the selected fruit juice extract concentrations prepared in range of 0.016 to 32.0 mg/mL as displayed in Figure 3.7.

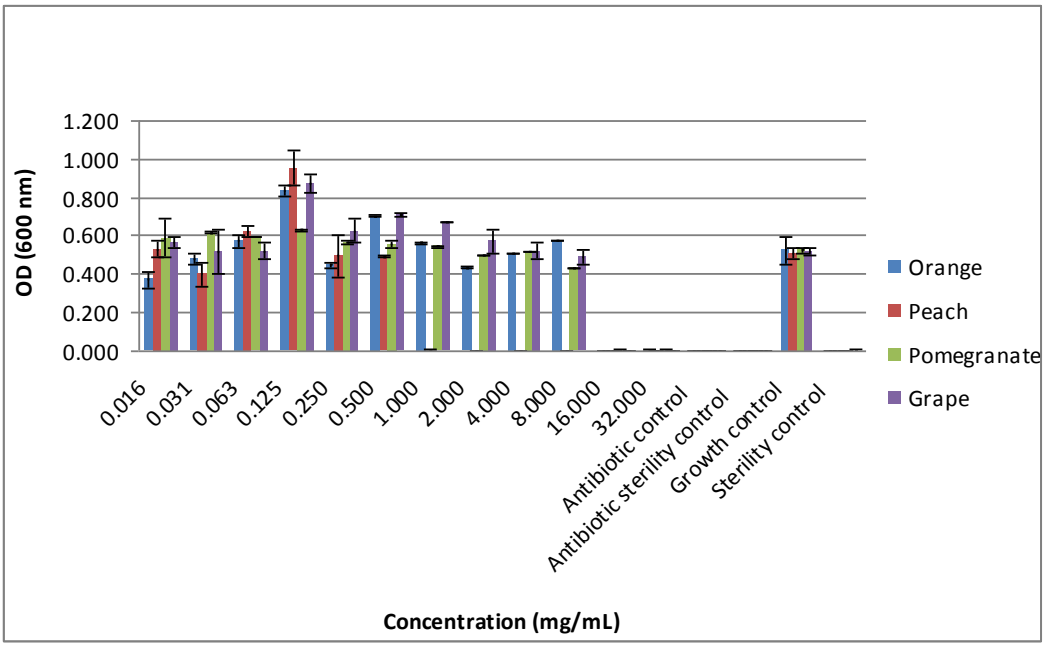

Figure 3.7 Absorbance monitored at 600 nm versus concentrations of various fruit juices extracts to determine minimum inhibitory concentrations.

Antibiotic control: Penicillin G was used as an effective reference antimicrobial compound in concentration of 0.20 μg/mL in the presence of $5x10^5$ cfus.
Antibiotic sterility control: Sterility of the antibiotic solution examined in the absence of inoculum.
Growth control: Test of normal bacterial growth in the absence of extracts and antibiotic agents.
Sterility control: Test the experiments were performed in aseptic conditions and the broth is sterile.

Minimum inhibitory concentrations results shown in Figure 3.8 for selected fruit juice extracts were found to be 1 mg/mL for peach and 16 mg/mL for orange, pomegranate and grapes.

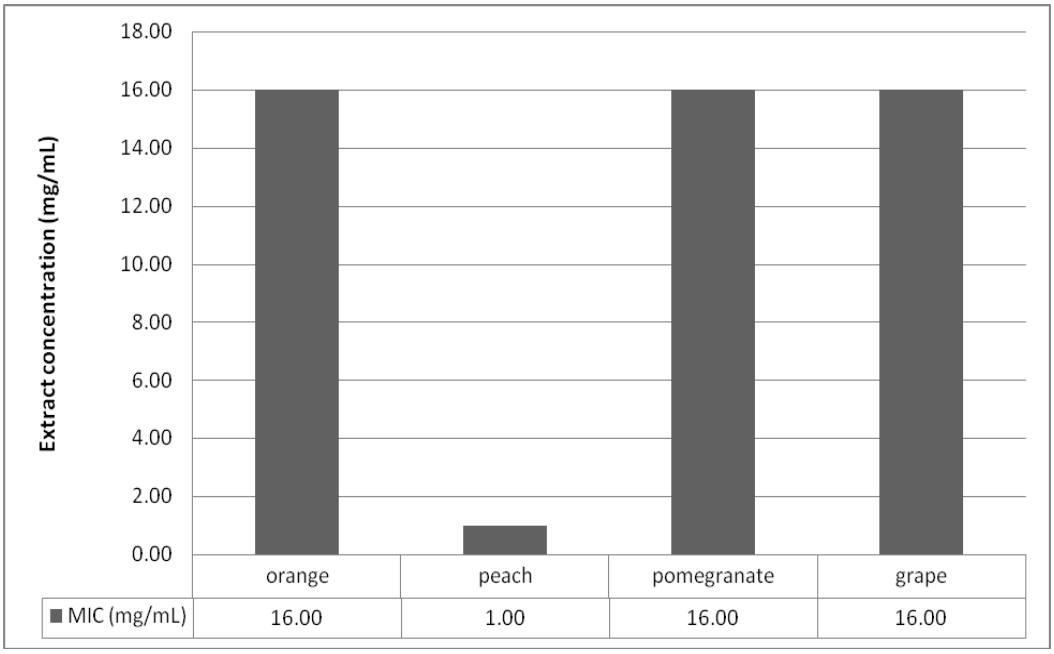

Figure 3.8 Minimum inhibitory concentrations (mg/mL) for various fruits juice extracts against *Streptococcus pyogenes*.

Peach extract was found to be the most effective with a MIC value of 1.0 mg/mL in the inhibition of bacterial growth among other orange, pomegranate and grape fruit juices.

1.0 mg/mL MIC concentration of peach juice extract could be obtained from 8.70 mL of peach juice necessary to inhibit the growth of $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

The commercial grape juice exhibited high inhibitory effect against *Listeria monocytogenes*, which have been associated with food-borne listeriosis (Rhodes, 2006).

Antimicrobial spectrum of pomegranate peel extracts (in vitro and in situ) revealed a relationship between microbial inhibition and total phenolic content of various peel extracts (Al-Zoreky, 2009).

Presence of anthocyanins in plants has revealed itself in colors of purple and red. In addition to that, anthocyanins have been reported to have antimicrobial activity. Their results further supported the association of antibacterial activity and anthocyanins (Lee, 2003).

3.5.3 Minimum inhibitory concentration of tea infusions

Antimicrobial activities of tea infusion extracts were studied against S*treptococcus pyogenes* with micro broth dilution method in the determination of minimum inhibitory concentrations of each extract as described in section 2.2.7.

Bacterial growth was monitored by absorption at 600 nm for the selected concentration range of 0.016 to 32.0 mg/mL of tea infusion extracts. Data was presented in Figure 3.9.

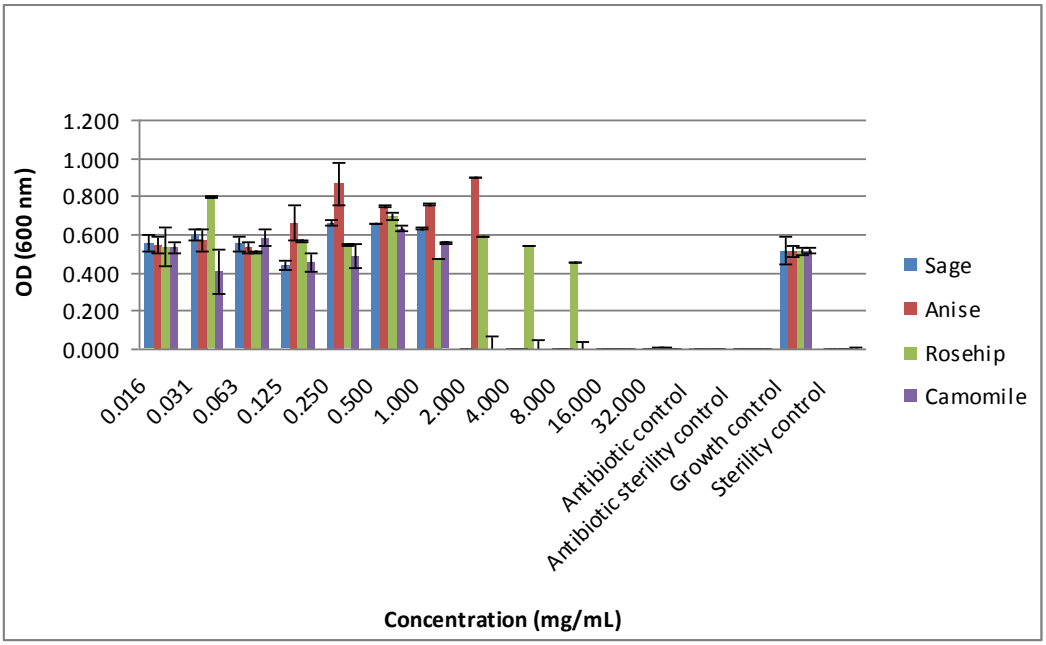

Figure 3.9 Absorbance at 600 nm versus concentrations of various fruit juice extracts to determine the effect on bacterial growth.

Antibiotic control: Penicillin G was used as an effective reference antimicrobial compound in concentration of 0.20 μg/mL in the presence of $5x10^5$ cfus.
Antibiotic sterility control: Sterility of the antibiotic solution examined in the absence of inoculum.
Growth control: Test of normal bacterial growth in the absence of extracts and antibiotic agents.
Sterility control: Test the experiments were performed in aseptic conditions and the broth is sterile.

Minimum inhibitory concentrations results shown in Figure 3.10 for selected tea infusion extracts were found to be 2 mg/mL for chamomile, 2 mg/mL for sage, 4 mg/mL for anise and 16 mg/mL for rosehip in increasing order.

Antimicrobial capacities of the essential oils from *P. Anisetum* were reported against *Streptococcus pneumoniae* by microbroth dilution method resulting the MIC value of 18 mg/mL (Tepe, 2006).

In the literature, extracts from *R. canina* fruits and R. canina hip powder containing anti-oxidative compounds especially as galactolipids, were found to present moderate potential for anti-inflammatory and anti-

nociceptive activities, and therefore mostly used in preparations to reduce the knee an hip pain in Osteoarthritis (OA) patients (Christensen, 2008).

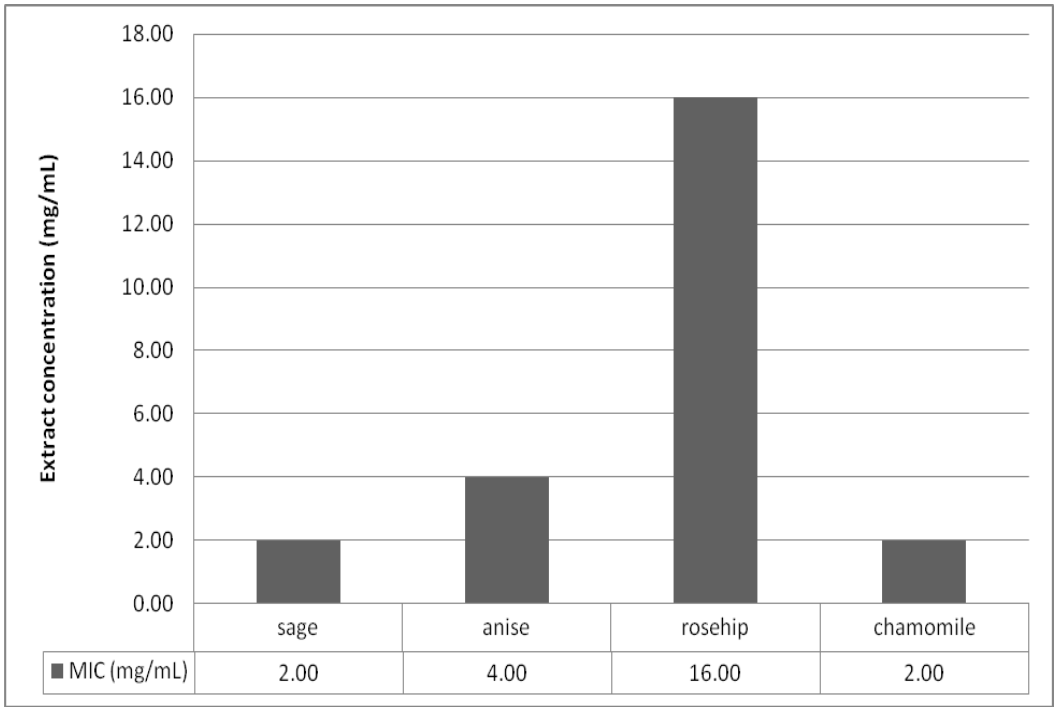

Figure 3.10 Minimum inhibitory concentrations (mg/mL) for various tea infusion extracts against *Streptococcus pyogenes*.

The extract yield of chamomile was found as 59.25 % (w/w), consequently 2 mg/mL MIC value would correspond to 0.84 mg of tea in order to inhibit the growth of $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

Chamomile has been used in traditional medicine for the treatment of inflammatory diseases. Aqueous chamomile extract in the form of tea investigated for the interference with COX-2 pathway and findings revealed effectiveness in preventing inflammation and carcinogenesis. (Srivastava, 2009)

Gülçına *et al.* has reported in 2003 about noticeable antimicrobial activity of anise (*Pimpinella anisum L.*) seed extracts (250 mg) against

gram positive and gram negative bacteria, comparing with strong antimicrobial compounds like miconazole nitrate, amoxicillin-clavulanic acid, ofloxacin, and netilmicin (Gülçına, 2003).

3.6 Minimum bactericidal concentration of extracts

3.6.1 Minimum bactericidal concentration of fruits and vegetables

Antimicrobial activities of fruit and vegetables extracts were investigated against S*treptococcus pyogenes* using micro agar dilution method to determine minimum bactericidal concentrations of each extract as described in section 2.2.8.

Minimum bactericidal concentration of fruit and vegetable extracts were determined by observing the lowest concentration where no visible growth occurred on agar surface.

Carrot, onion, radish and plum with increasing minimum bactericidal concentrations were found as values of 0.06, 0.5, 1.0 and 8 mg/mL, respectively. Results are shown in Figure 3.11.

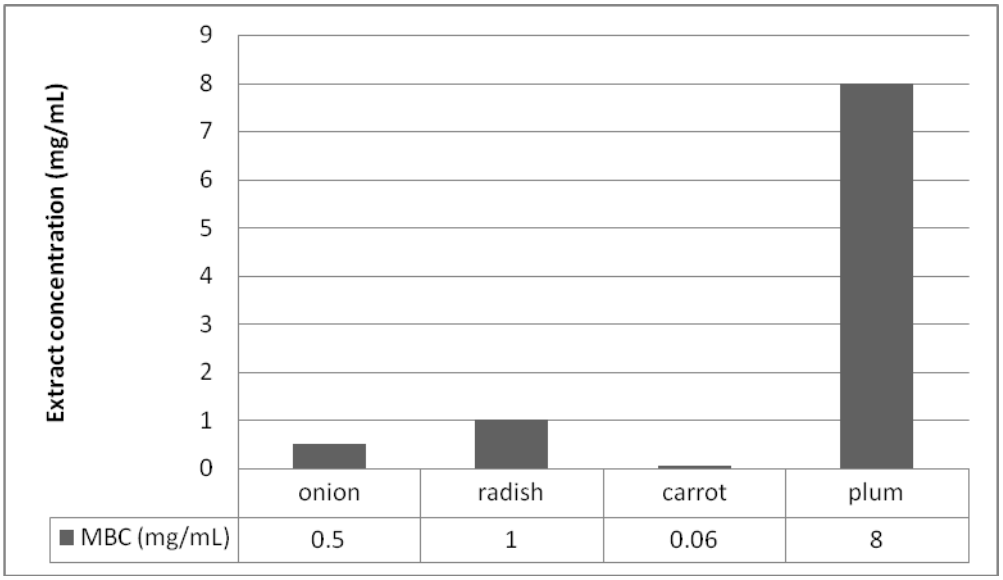

Figure 3.11 Minimum bactericidal concentrations (mg/mL) for various fruit and vegetable extracts against *Streptococcus pyogenes*.

Carrot being the most effective bactericidal next to onion among selected fruit and vegetable extracts. This also correlates with MIC data given in Figure 3.6.

The extract yield of carrot was found as 0.24 % (w/w), consequently 0.06 mg/mL MBC value would correspond to 25 mg of blended carrot in order to kill $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

3.6.2 Minimum bactericidal concentration of fruit juices

Fruit juice extracts were examined for their antimicrobial activities against S*treptococcus pyogenes* using micro agar dilution method. Determination of minimum bactericidal concentrations of each extract was performed as described in section 2.2.8.

Minimum bactericidal concentrations results shown in Figure 3.12 for selected fruit juice extracts were found to be 2 mg/mL for peach and 32 mg/mL for orange, pomegranate and grapes.

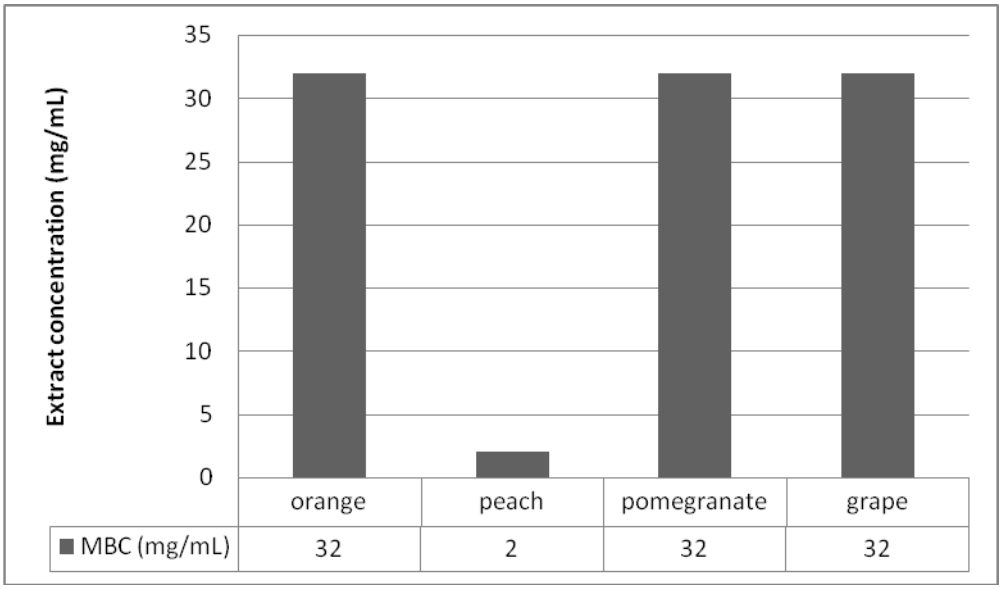

Figure 3.12 Minimum bactericidal concentrations (mg/mL) for various fruit juice extracts against *Streptococcus pyogenes*.

Peach extract was found to be the most effective with a MBC value of 2.0 mg/mL in the bactericidal effect among other orange, pomegranate and grape fruit juices.

2.0 mg/mL MBC concentration of peach juice extract could be obtained from 17.39 mL of peach juice necessary to observe the bactericidal effect on $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

3.6.3 Minimum bactericidal concentration of tea infusions

Antimicrobial activities of tea infusion extracts were studied against S*treptococcus pyogenes* with micro agar dilution method in the determination of minimum bactericidal concentrations of each extract as described in section 2.2.8.

Minimum bactericidal concentrations results shown in Figure 3.13 for selected tea infusion extracts were found to be 4.0 mg/mL for

chamomile, 16.0 mg/mL for sage, 32.0 mg/mL for anise and 32.0 mg/mL for rosehip in increasing order.

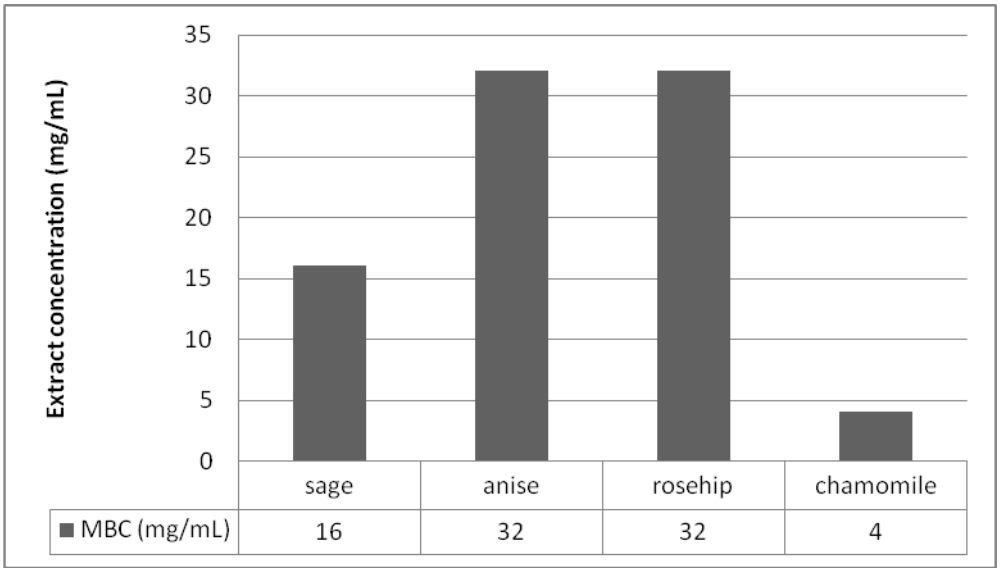

Figure 3.13 Minimum bactericidal concentrations (mg/mL) for various tea infusion extracts against *Streptococcus pyogenes*.

The extract yield of chamomile was found as 59.25 % (w/w), consequently 4.0 mg/mL MIC value would correspond to 3.36 mg of tea in order to kill $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

3.7 Antimicrobial activity of extracts by disk diffusion test

3.7.1 Antimicrobial activity of fruits and vegetables by disk diffusion test

Agar petri plates containing 6 mm disks prepared for the disk diffusion tests as described in the section 2.2.9. A 20 µL of 40 mg/mL concentrations of four selected fruit and vegetable dry extracts (800 µg) were applied on filter disks. Agar petri plates containing disks were incubated at 37 °C for approximately 16 hours. After incubation, plates

were observed for inhibition zones, and the diameters were measured in millimeters.

The growth inhibition zones measured by disk diffusion method are shown in Figure 3.14 for onion, radish, carrot and plum.

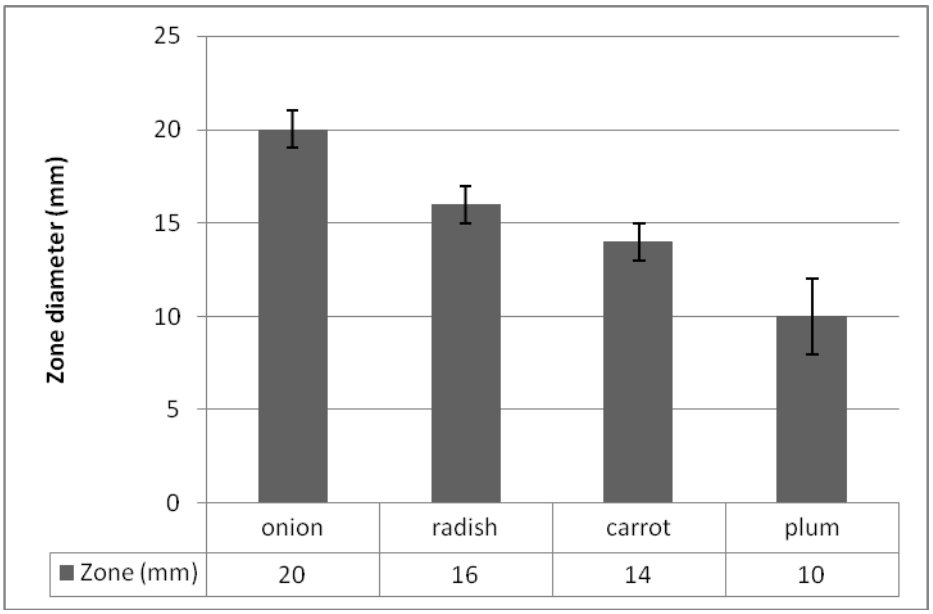

Figure 3.14 Antimicrobial activities of selected fruits and vegetable dry extracts by disk diffusion method.

Note: Diameter of inhibition zone (mm) including disk diameter of 6 mm.

Onion showed the highest antimicrobial activity when tested with disk diffusion method. Inhibition zone diameters of radish, carrot and plum were found to be 20 mm, 16 mm, 14 mm and 10 mm decreasing activity order, respectively.

3.7.2 Antimicrobial activity of fruit juices by disk diffusion test

Twenty µL of 40 mg/mL concentrations of four various fruit juices dry extracts were applied on filter disks. Therefore, each disk contained 800 µg of extract. Agar petri plates containing disks were incubated at

37 °C for approximately 16 hours. After incubation, plates were observed for inhibition zones, and the diameters were measured in millimeters.

The growth inhibition zones measured by disk diffusion method are shown in Figure 3.15 for orange, peach, pomegranate and grape.

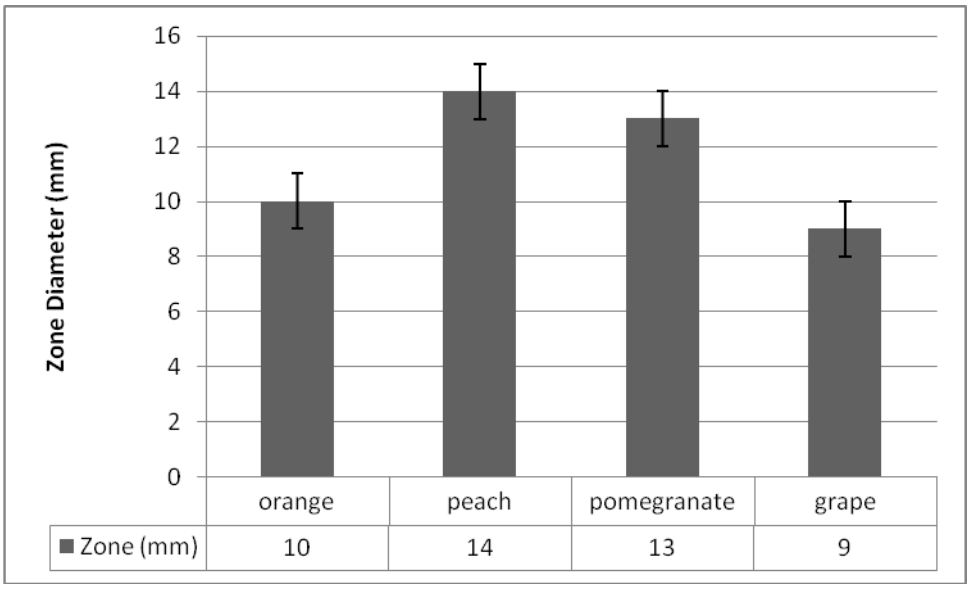

Figure 3.15 Antimicrobial activities of selected fruit juice dry extracts by disk diffusion method.

Note: Diameter of inhibition zone (mm) including disk diameter of 6 mm.

Peach showed the highest antimicrobial activity when tested with disk diffusion method with an inhibition zone value of 14 mm. Inhibition zone diameters of pomegranate, orange and grape were found to be 13 mm, 10 mm and 9 mm in decreasing activity order, respectively.

3.7.3 Antimicrobial activity of tea infusions by disk diffusion test

Agar petri plates containing disks 20 µL of 40 mg/mL concentrations of four selected tea infusion dry extracts, sage, anise, rosehip and chamomile were incubated at 37 °C for approximately 16 hours. After incubation, plates were observed for inhibition zones, and the diameters were measured in millimeters.

The growth inhibition zones measured were shown in Figure 3.16 for sage, anise, rosehip and chamomile.

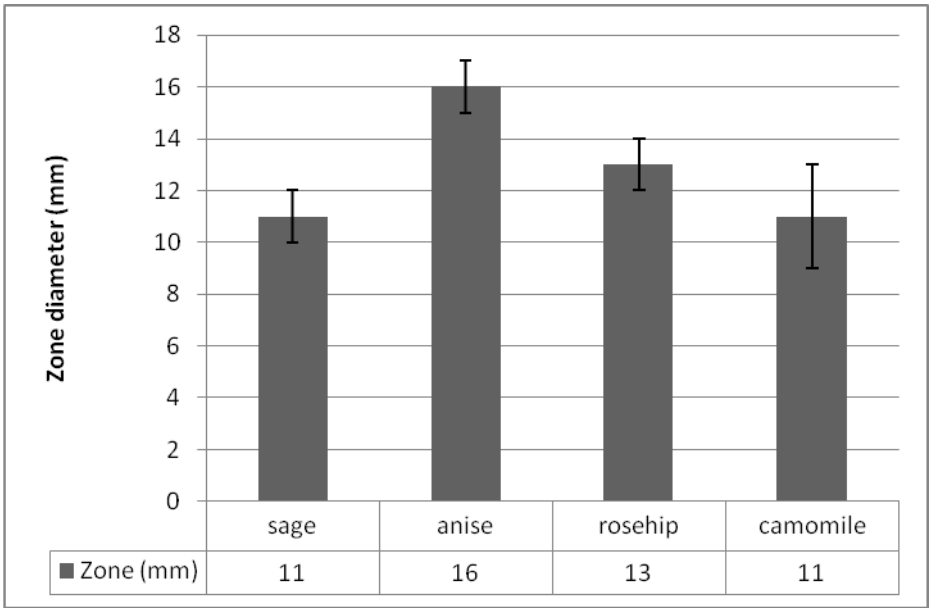

Figure 3.16 Antimicrobial activities of sage, anise, rosehip and chamomile infusion dry extracts by disk diffusion method.

Note: Diameter of inhibition zone (mm) including disk diameter of 6 mm.

Inhibition zone diameters of sage, anise, rosehip and chamomile were found to be 11 mm, 16 mm, 13 mm and 11 mm, respectively. Anise showed the highest antimicrobial activity as it can clearly be seen from Figure 3.16.

Antimicrobial capacities of the essential oils from *P. Anisetum* were reported against *Streptococcus pneumoniae* by disk diffusion method resulting the inhibition zone diameter as 11 mm (Tepe, 2006).

3.7.4 Antimicrobial activity of disk standards

Six antimicrobial disks containing clarithromycin, clindamycin, erythromycin, ciprofloxacin, penicillin and azithromycin were applied on filter disks. Agar petri plates containing disks were incubated in the same

conditions as tested fresh produce extracts and herbal tea infusion extracts. After incubation, plates were observed for inhibition zones, and the diameters were measured in millimeters were used as positive controls.

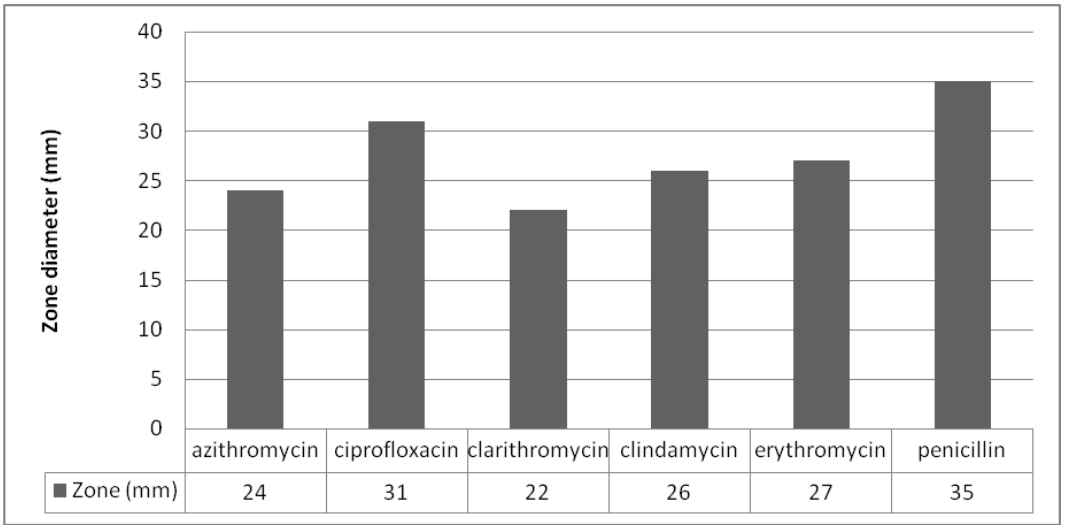

Figure 3.17 Antimicrobial activities of selected antibiotics by disk diffusion method used as reference compounds.

Note: Diameter of inhibition zone (mm) including disk diameter of 6 mm.

Inhibition zone diameters measured for disks containing penicillin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), azithromycin (15 μg) and clarithromycin (15 μg) were found to be 35 mm, 31 mm, 27 mm, 26 mm, 24 mm and 22 mm in decreasing activity order, respectively.

Onion, with an inhibition zone diameter value of 20 mm, showed highest antimicrobial activity among the tested extracts. Each disk contained 800 μg of tested material.

Highest antimicrobial activity was found to be displayed by penicillin among the other antimicrobial standard compounds with a value of 35

mm. A standard disk contained 15 μg penicillin. The activity displayed by 800 μg crude onion extract is less then that of 15 μg penicillin.

A study reported from Argentina in 1997 on the penicillin and erythronzycin susceptibility of *Streptococcus pyogenes* both by the diffusion and agar dilution methods. No penicillin-resistant streptococci were observed (MIC100 = 0.03 μg/mL) (Lopardo, 1997).

3.8 Determination of antioxidant capacities of extracts

Antioxidant capacities of the extracts from fresh fruits and vegetables regular members of daily diets were studied by using DPPH radical scavenging method and by determining the total phenolic contents in gallic acid equivalents.

3.8.1 Determination of radical scavenging capacities and total phenolic contents of fruits and vegetables

DPPH radical scavenging activity of extracts prepared in ethyl acetate from onions, radishes, carrots and plums were monitored at 517 nm. Quercetin was used as the well-known effective antioxidant standard throughout the experiments. DPPH radical scavenging activity in percentage was plotted against concentrations of extracts (mg/mL) of selected fruits and vegetables. Results are given in the Figure 3.18. On the other hand, fifty percent effective concentrations for DPPH radical scavenging activities with calculated standard deviations were presented in the Table 3.5.

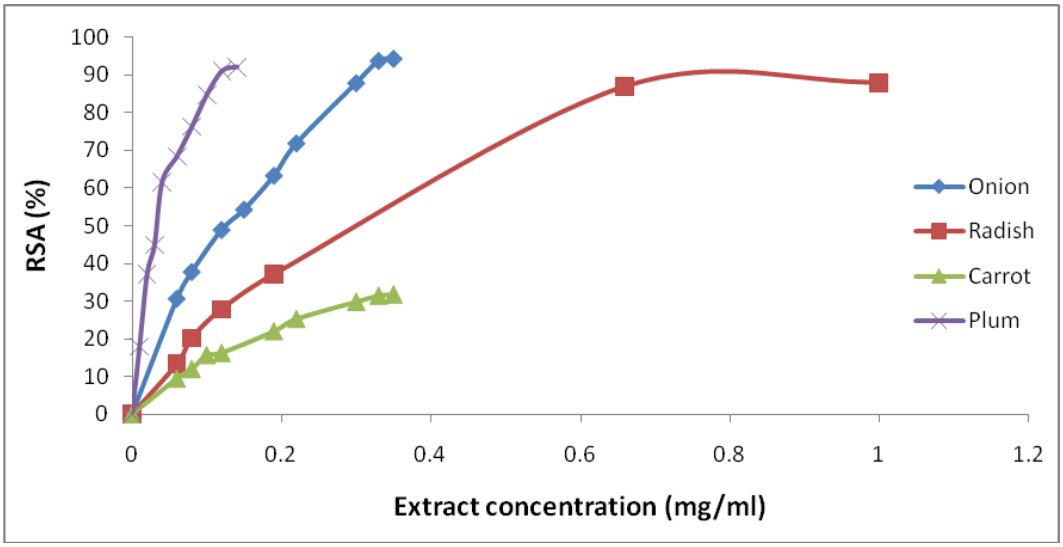

Figure 3.18 DPPH radical scavenging activity in percentile versus ethyl acetate extract concentrations (mg/mL) of selected fruits and vegetables. DPPH radical scavenging activities of onion, radish and plum were measured in 30 minutes of incubation time apart from carrot was measured in 15 minutes.

Total phenolic contents of each ethyl acetate extracts were determined by using gallic acid equivalents, the results were presented as µg phenolic equivalents of gallic acid (GAE) in mg of extract given in the Table 3.5.

Table 3.5 Fifty percent effective concentrations for DPPH radical scavenging activities and total phenolic contents of selected fruit and vegetable extracts.

	[a] DPPH RSA EC_{50} (mg/ml)	[b] TP GAE (µg/mg)
Onion	0.148 ± 0.003	44.427 ± 1.532
Radish	0.414 ± 0.008	13.135 ± 0.829
Carrot	NA	11.055 ± 0.457
Plum	0.049 ± 0.003	50.506 ± 1.439
Quercetin	0.009 ± 0.001	NA

DPPH RSA EC_{50} : Effective concentration of plant extracts for 50 % of DPPH radical scavenging activity

TP GAE : Total phenolic contents µg equivalents of gallic acid/mg of plant extract

NA: not applicable

[a] Mean of triplicate trials

[b] Mean of triplicate trials

Plum extract was found to have the highest radical scavenging activity as shown from its EC_{50} value of 0.049 mg/mL and correlates with the total phenolic content as μg gallic acid equivalent per mg extract. Even quercetin, a well known antioxidant pure compound, was only 5 times more effective than the crude ethyl acetate extract of plum.

Second best onion extract in DPPH radical scavenging activity also correlates with a high gallic acid equivalent of total phenolic content as shown in Table 3.5.

Onion spice commonly consumed in Brazil was tested for its free radical scavenging activity and found to have an EC_{50} value of 2224 ± 29 (g spice /Kg DPPH•) considered to show negligible scavenging capacity (Mariutti, 2008).

Gorinstein also reported in 2009, DPPH radical scavenging activity of white and red onion as 23.05 and 24.30 μM trolox equivalent per grams of dry weight of methanol extracts and total phenol contents with the values of 10.52 and 15.87 GAE/g dry weight, respectively (Gorinstein, 2009). Yellow onion was found to be rich in flavonols (Dimitrios, 2006).

In 2007 Arabshahi-D and his co-workers studied carrot tuber samples for their contents of ascorbic acid, α-tocopherol, b-carotene, glutathione and total phenols and results were found as 20.0 mg, 3.98 mg, 9667 μg, 129.53 mmol, and 1.68 g mg per 100 g dry weight, respectively. In the same article, antioxidant activity of carrot tuber was given as the inhibition of lineolic acid peroxidation and 1.5 mg/mL concentration of the extract inhibited 80-83% peroxidation of lineolic acid after 16 h incubation, which was found higher than the value for α-tocopherol (72%) (Arabshahi-D, 2007).

In 2006 Kima has reported total phenolic contents of fresh plums with values ranging from 174±1.5 mg GAE to 375±3.8 mg GAE per 100 g. Kima has also concluded that the distribution and composition of phenolic phytochemicals are affected by maturity, cultivars, horticultural practices, geographic origin, growing season, postharvest storage conditions and processing procedures (Kima, 2006). Plums, prunes, apples, pears, kiwi were all containing hydroxycinnamic acids, and catechins (Dimitrios, 2006).

Another group in 2009, studied the nutritional values of plums (*Prunus domestica L.*) and described the chemical characteristics of selected cultivars with the total phenolic content values ranged from 227 to 495 mg of gallic acid per 100 g of fresh mass (Rop, 2009).

Barillari *et al.* studied radish (*Raphanus sativus L.*) for kinetics of DPPH radical scavenging and also found the content of glucosinolates (GLs) that is important in healthy foods for their release of isothiocyanates (ITCs) upon myrosinase hydrolysis (Barilliari, 2008).

3.8.2 Determination of antioxidant capacities and total phenolic contents of fruit juices

Orange, peach, pomegranate and grape juice lyophilized extracts were investigated for their radical scavenging activities using DPPH method as described in section 2.2.10. Results exhibited in Figure 3.19, pomegranate extract being the most effective radical scavenger among the selected fruit juices, was almost twice as effective as of peach extract which was the second most.

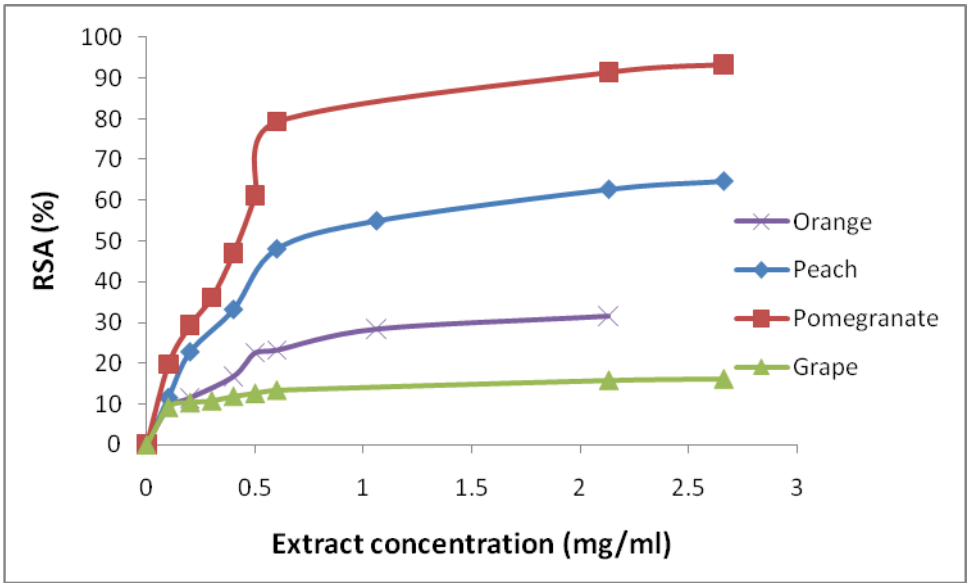

Figure 3.19 DPPH radical scavenging activity in percentile versus lyophilized extract concentrations (mg/mL) of selected fruits juices. DPPH radical scavenging activities of lyophilized fruit juice extracts were measured in 15 minutes of incubation time.

Another antioxidant capacity measurement is determination of total phenolic content of extracts by using equivalence of a phenolic standard compound. Gallic acid equivalents were studied in each of the tea infusion extracts. The amount of μg phenolics per mg of extract as gallic acid equivalents were calculated and tabulated in Table 3.6.

Table 3.6 Fifty percent effective concentrations for DPPH radical scavenging activities and total phenolic contents of lyophilized fruit juices.

	[a] DPPH RSA EC_{50} (mg/ml)	[a] TP GAE (μg/mg)
Orange	NA	1.182 ± 0.099
Peach	1.343 ± 0.095	5.196 ± 0.276
Pomegranate	0.746 ± 0.023	1.972 ± 0.112
Grape	NA	0.325 ± 0.013

DPPH RSA EC_{50} : Effective concentration of plant extracts for 50 % of DPPH radical scavenging activity

TP GAE : Total phenolic contents μg equivalents of gallic acid/mg of plant extract

NA: not applicable

[a] Mean of triplicate trials [b] Mean of triplicate trials

Although pomegranate being twice as effective as peach in DPPH radical scavenging activity, total phenolic content in gallic acid equivalence peach is almost two and half times more than that of pomegranate. Possible differences in their active radical scavenging compounds between peaches and pomegranate may explain this result.

Since 2000, phenolic contents of pomegranate juice and peels have been studied extensively by several research groups. In pomegranate juice, major compounds were found as high levels of phenolic acids (citric, malic, ascorbic acids), flavonoids, anthocyanins, punicalagins, ellagic acids, and hydrolysable tannins, delphinidin, cyanidin and pelargonidin (Li, 2006; Tezcan, 2009; Ozgen, 2008).

Amount of total phenolics in pomegranate juice, were studied recently by two Turkish research groups and Tezcan *et al.* reported their results as ranging from 2602 to 10086 mg/L mostly due to hydrolysable tannins and anthocyanins. On the other hand, Ozgen *et al.* published their work in 2008 as the amount of total phenolics found between 1245 and 2076 mg gallic acid equivalent per liter of fruit juice (Tezcan, 2009; Ozgen, 2008).

Citrus fruits, are highly consumed in Mediterranean diets, contain L-Ascorbic acid, carotenoids, flavonoids, caffeic, p-coumaric, ferulic and sinapic acids and other polyphenolic compounds. These compounds' possible beneficial effects are due to their antioxidant activity, which is related to the development of atherosclerosis and cancer, and to anti-inflammatory and antimicrobial activity (Tripoli, 2007; Polydera, 2005; Klimczak, 2007).

The content of total polyphenols in fresh orange juices, calculated simply as the sum of total hydroxycinnamic acids and flavanones determined by HPLC, was found to be ranging from 202.776.8 mg/L to

226.776.4 mg/L. The concentration of total polyphenols determined by Folin–Ciocalteu method was higher than the concentration obtained by HPLC method- ranging from 634.6 to 684.2 mg of caffeic acid equivalents per liter. In the DPPH assay, the scavenging activity was given in % ranging from 47.5 to 49.2 of 0.1 mL juice (Klimczak, 2007). Citrus fruits were studied by another research group found rich in flavanones, flavonols, phenolic acids (Dimitrios, 2006).

In 2002, Versari *et al.* reported the composition of peach juices having significant differences among cultivars in terms of glucose, fructose, sorbitol, citric and malic acids, pH, L*, a*, catechin, isoquercetin, caffeic and chlorogenic acid content (Versari,2002).

3.8.3 Determination of antioxidant capacities and total phenolic contents of herbal tea infusions

Selected herbal tea infusions were extracted for 24 hours, and dried by lyophilizing. Each sample was tested for their DPPH radical scavenging capacities by keeping the incubation time at 15 minutes for sage, anise and chamomile, for rosehip at 30 minutes. Radical scavenging activities of each tea extract in percentile were plotted against extract concentrations given in Figure 3.20.

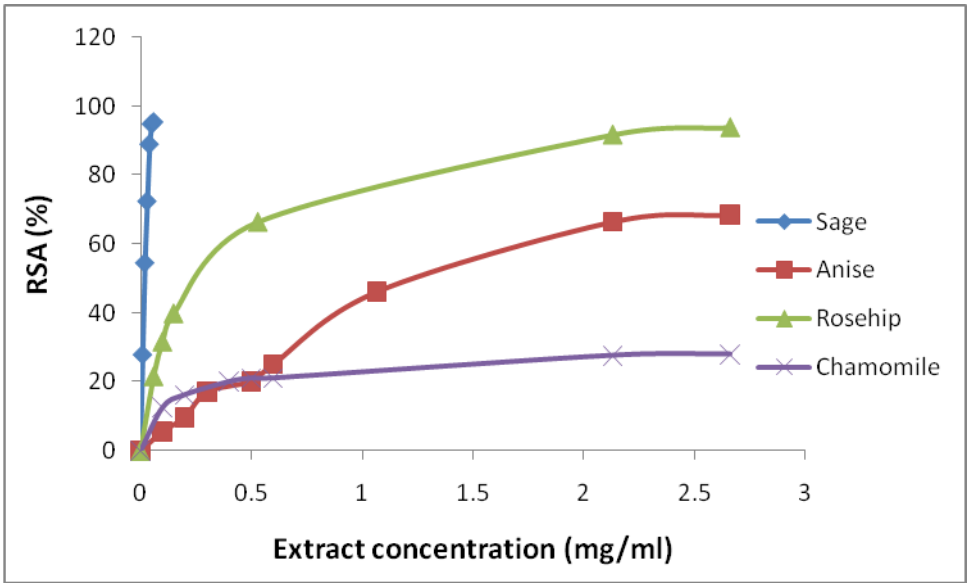

Figure 3.20 DPPH radical scavenging activity versus extract concentrations (mg/mL) of selected teas. DPPH radical scavenging activities of lyophilized tea extracts.

Fifty percent effective concentrations of DPPH radical scavenging activities of selected lyophilized tea infusion extracts were compared for their total phenolic contents in Table 3.7.

Table 3.7 Fifty percent effective concentrations for DPPH radical scavenging activities and total phenolic contents of lyophilized tea infusions.

	[a] DPPH RSA EC_{50} (mg/ml)	[a] TP GAE (µg/mg)
Sage	0.043 ± 0.001	48.299 ± 1.498
Anise	1.598 ± 0.017	3.773 ± 0.152
Rosehip	0.823 ± 0.019	7.157 ± 0.385
Chamomile	NA	2.990 ± 0.127

DPPH RSA EC_{50} : Effective concentration of plant extracts for 50 % of DPPH radical scavenging activity

TP GAE : Total phenolic contents µg equivalents of gallic acid/mg of plant extract

NA: not applicable

[a] Mean of triplicate trials

[b] Mean of triplicate trials

Sage and rosehip were found most effective in radical scavenging with EC_{50} values of 0.043 and 0.823 mg/mL, respectively. Amount of phenolics given as gallic acid equivalences were also found high for sage and rosehip teas with values of μg gallic acid equivalence of 48.299 and 7.157 per mg of extract. This result correlates with their high radical scavenging activities.

All the methods that have been studied such as DPPH, TP, MIC, MBC and disk diffusion test and their results for all tested fresh produce, fruit juice and herbal tea infusion extracts were tabulated in Table 3.8.

Table 3.8 Comparison of DPPH EC_{50} (mg/mL), TP GAE (µg/mg), MIC, MBC (mg/mL) and Disk diffusion test (mm) results for all tested fresh produce, fruit juice and herbal tea infusion dried extracts.

	[a] DPPH EC_{50} (mg/ml)	[a] TP GAE (µg/mg)	MIC (mg/mL)	MBC (mg/mL)	Disk diffusion (mm)
Onion	0.148 ± 0.003	44.427 ± 1.532	0.125	0.5	20
Radish	0.414 ± 0.008	13.135 ± 0.829	0.250	1	16
Carrot	NA	11.055 ± 0.457	0.008	0.06	14
Plum	0.049 ± 0.003	50.506 ± 1.439	4	8	10
Sage	0.023 ± 0.001	48.299 ± 1.498	2	16	11
Anise	1.598 ± 0.017	3.773 ± 0.152	4	32	16
Rosehip	0.823 ± 0.019	7.157 ± 0.385	16	32	13
Chamomile	NA	2.990 ± 0.127	2	4	11
Orange	NA	1.182 ± 0.099	16	32	10
Peach	1.343 ± 0.095	5.196 ± 0.276	1	2	14
pomegranate	0.746 ± 0.023	1.972 ± 0.112	16	32	13
Grape	NA	0.325 ± 0.013	16	32	9

DPPH RSA EC_{50} : Effective concentration of plant extracts for 50 % of DPPH radical scavenging activity
TP GAE : Total phenolic contents µg equivalents of gallic acid/mg of plant extract
Diameter of inhibition zone (mm) including disk diameter of 6 mm.
NA: not applicable
[a] Mean of triplicate trials [b] Mean of triplicate trials

In 2008, Yoo *et al.* reported DPPH radical scavenging activity of *Rosa rubiginosa* as 88.7 % for 100 μg/mL, and total phenolic content as 818.5 mg GAE per 100 g of extract and total flavonoid content as 400.5 mg catechin equivalents per 100 g (Yoo, 2008). Böhm *et al.* in 2003 studied the phenolic contents of rosehip as fruit of flowers (*Rosa canina L.*), and reported that β-carotene, and remarkable amounts of lycopene was found as major two constituents (Böhm, 2003).

Gülçina *et al.* have published their work on *Pimpinella anisum L.* seed extracts showing strong antioxidant activity, reducing power, DPPH radical and superoxide anion scavenging, hydrogen peroxide scavenging, and metal chelating activities when compared with different standards such as BHA, BHT, and a-tocopherol (Gülçına, 2003).

Tepe *et al.* in 2006 reported also the DPPH radical scavenging activity of *Pimpinella anisetum* as IC_{50} value of 5.62 μg/ml and found compounds of *P. anisetum* as (E)-anethole 82.8% and methyl chavicol 14.5 % (Tepe, 2006).

Miliauskasa *et al.* in 2004 reported DPPH radical % inhibition of sage (*Salvia officinalis*) in 2.5 mg/mL of ethyl acetate, acetone, and methanol extracts 91.7, 92.6, and 92.3, respectively. Total phenolic compounds in sage (*S. officinalis*) were found 22.6 mg of GAE per g of plant extract (Miliauskasa, 2004).

165 extracts from 55 *Salvia* taxa from Türkiye screened by Şenol *et al.* DPPH radical scavenging activity results shown the highest activity with *S. fruticosa* (89.23%) and *S. russelli* (86.36%) at 100 μg/mL extract prepared in dichloromethane. This notable activity might be due to a possible synergistic interaction between its terpenoids as its total phenol content which was 87.86 mg GAE per gram of extract for dichloromethane extract of *S. fruticosa*. Furthermore, *Salvia* extracts were also found to

have anticholinesterase activity besides antioxidant activity (Şenol, 2010). Sage was found to be containing carnosol, carnosic acid, lateolin, rosmanul, rosmarinic acid (Dimitrios, 2006).

In 2008 Yoo *et al.* reported that chamomile tea extract (*Chamaemelum nobilis L.*) has exhibited 91.0 % DPPH radical scavenging activity for 100 μg/mL of extract. Chamomile also showed the high antioxidant activity with a value of 960 mg/100 g of vitamin C equivalent. In the same article, total polyphenol and total flavonoid contents were also given for chamomile tea as 844.4 GAE mg/100 g and 494.5 catechin equivalents (CE) mg/100 g, respectively. Total phenolic and total flavonoids showed a high correlation with antioxidant activity (Yoo, 2008).

Srivastava *et al.* reported in 2009 HPLC analysis of aqueous chamomile extract having two flavonoid peaks corresponding to apigenin 7-O-glucoside (63.3%) and apigenin 7-O-neohespridoside (27.7%) (Srivastava, 2009).

CHAPTER 4

CONCLUSIONS

In this research, antimicrobial activities of fresh produces, fresh fruit juices and infused herbal teas consumed in daily diets against Group A ß-haemolytic streptococci were investigated. Our aim was to improve defense system, against diseases before their full progress, by consumption of food for medicine, and to minimize the intake of synthetic antibiotics. The pre-treatment that would reduce the risk, and duration of occurrence of a probable disease, is important for both society's health and national economy.

Antioxidant capacity determinations radical scavenging and total phenolic content experiments were carried out and as a result sage infusion extract was found to exhibit the highest radical scavenging activity as shown from its EC_{50} value of 0.023 mg/mL and it correlates with the total phenolic content as 48 μg gallic acid equivalent per mg extract. Plum extract exhibited the next noticeable EC_{50} value of 0.049 mg/mL in DPPH radical scavenging activity, and this result also correlates with its 50 μg gallic acid equivalent of total phenolic content. Respective decreasing order of scavenging activities was displayed by onion, radish, carrot, pomegranate and rosehip.

Minimum inhibitory concentrations of the fresh produces and juices and hot infusion teas as in dry extract forms were determined to monitor antimicrobial activity against *Streptococcus pyogenes*. We found that

carrot has the highest inhibitory effect with MIC value of 0.008 mg/mL, and then onion, radish, peach, chamomile, sage, plum, anise, rosehip, orange, pomegranate, and grape with decreasing order as displayed in the Table 3.8.

The extract yield of carrot was found as 0.24 % (w/w), consequently 0.008 mg/mL MIC value would correspond to 1.67 mg of blended carrot, similarly 1.0 mg/mL MIC concentration of peach juice extract would be obtained from 8.70 mL of peach juice and consequently the extract yield of chamomile as 59.25 % (w/w) with 2 mg/mL MIC value would correspond to 0.84 mg of tea in order to inhibit the growth of $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

Although the experimental design of the minimum bactericidal concentration shows a similarity in pattern with the design of minimum inhibitory concentration, the two experimental results don't have to be correlated. Carrot has the highest bactericidal effect with a value of 0.06 mg/mL followed by onion and radish with values of 0.5 and 1.0 mg/mL, respectively.

The extract yield of carrot was found as 0.24 % (w/w), consequently 0.06 mg/mL MBC value would correspond to 25 mg of blended carrot, and likewise, 2.0 mg/mL MBC concentration of peach juice extract would be obtained from 17.39 mL of fresh peach juice and similarly, chamomile with the extract yield of 59.25 % (w/w) and with a 4.0 mg/mL MBC value would correspond to 3.36 mg of tea in order to kill $5x10^6$ colony forming units of *Streptococcus pyogenes* in one mL.

Disk diffusion test was applied as the third method to monitor the antimicrobial activity by determining the inhibition zones in millimeters. Onion, with an inhibition zone diameter value of 20 mm, showed highest antimicrobial activity among the tested extracts. Each disk contained 800

μg of tested material. High antimicrobial activities were obtained by radish, anise, carrot and peach extracts as tested by disk diffusion method with respective 16, 16, 14 and 14 millimeters clear growth inhibition zones.

A standard disk containing 15 μg penicillin showed antimicrobial activity with a value of 35 mm. The activity displayed by 800 μg crude onion extract (20 mm) was less then that of 15 μg penicillin.

As a conclusion, foods like onion, radish, carrot, plum and sage tea can be important in protective treatments even against dangerous pathogens such as *Streptococcus pyogenes* at the beginning stage of the of the infections.

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APPENDIX A

UV-VIS absorption spectra

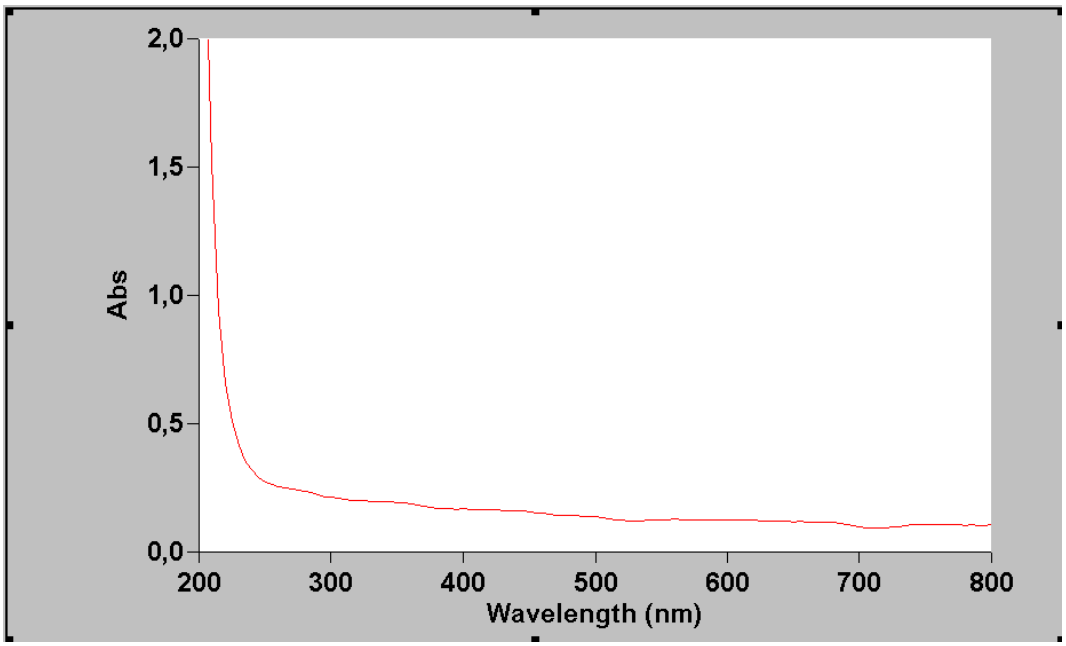

Figure A.1 UV-VIS absorption of methanol scanned using wavelength range between 200 to 800 nm.

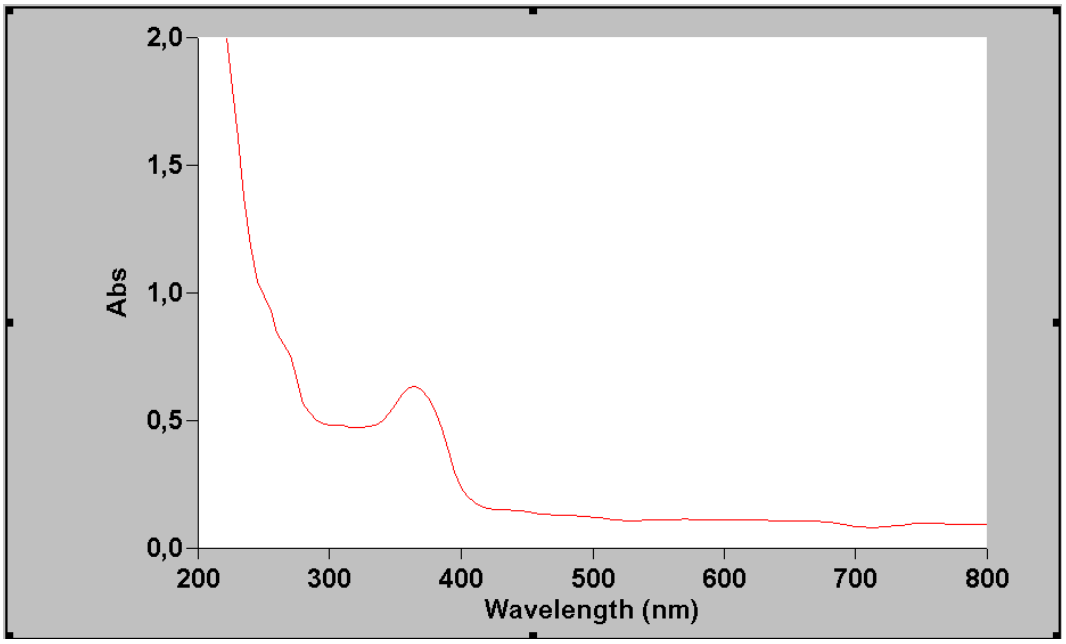

Figure A.2 UV-VIS absorption of dry onion extract scanned using wavelength range between 200 to 800 nm.

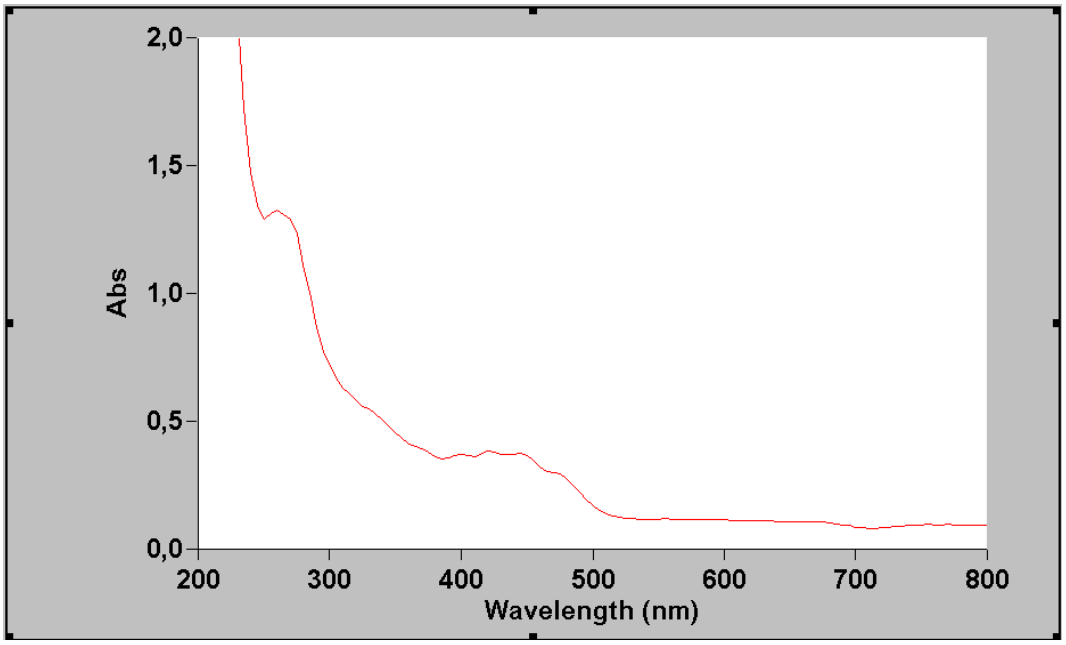

Figure A.3 UV-VIS absorption of dry radish extract scanned using wavelength range between 200 to 800 nm.

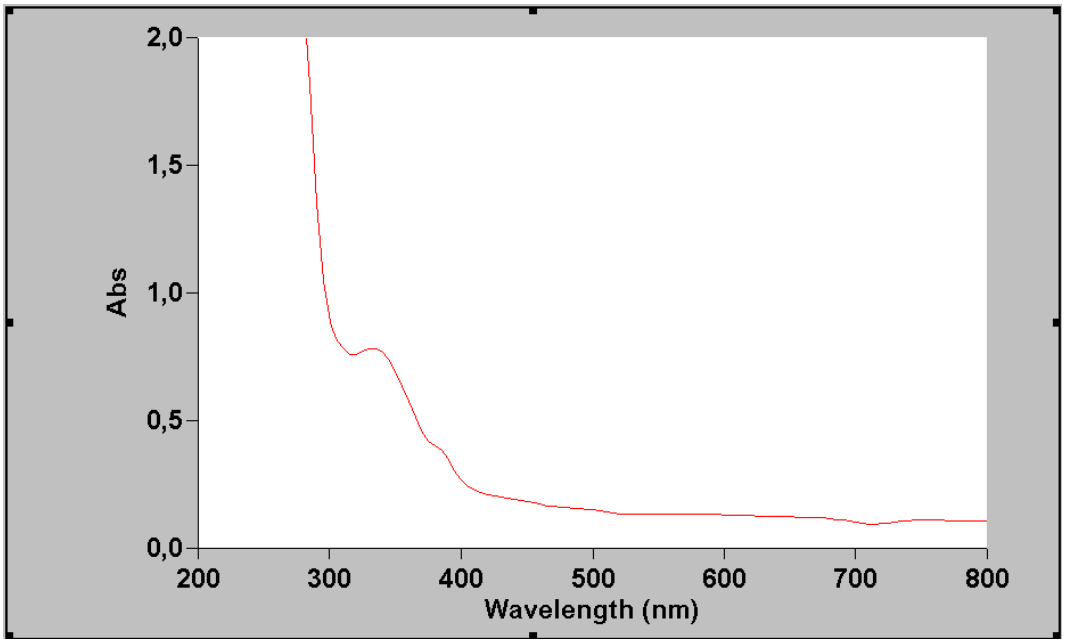

Figure A.4 UV-VIS absorption of dry carrot extract scanned using wavelength range between 200 to 800 nm.

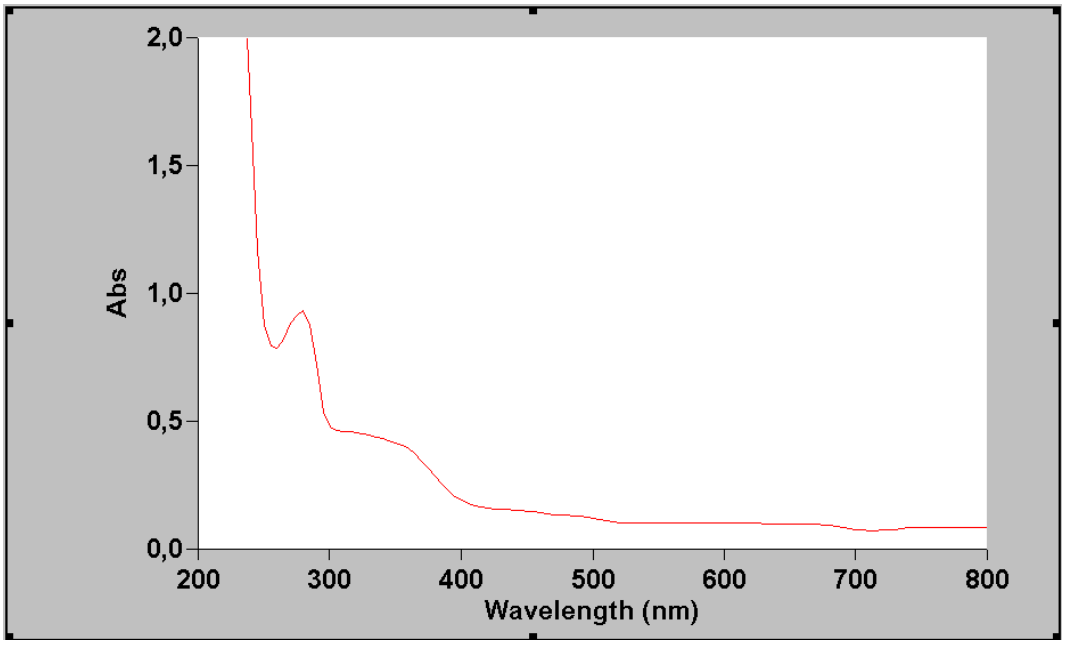

Figure A.5 UV-VIS absorption of dry plum extract scanned using wavelength range between 200 to 800 nm.

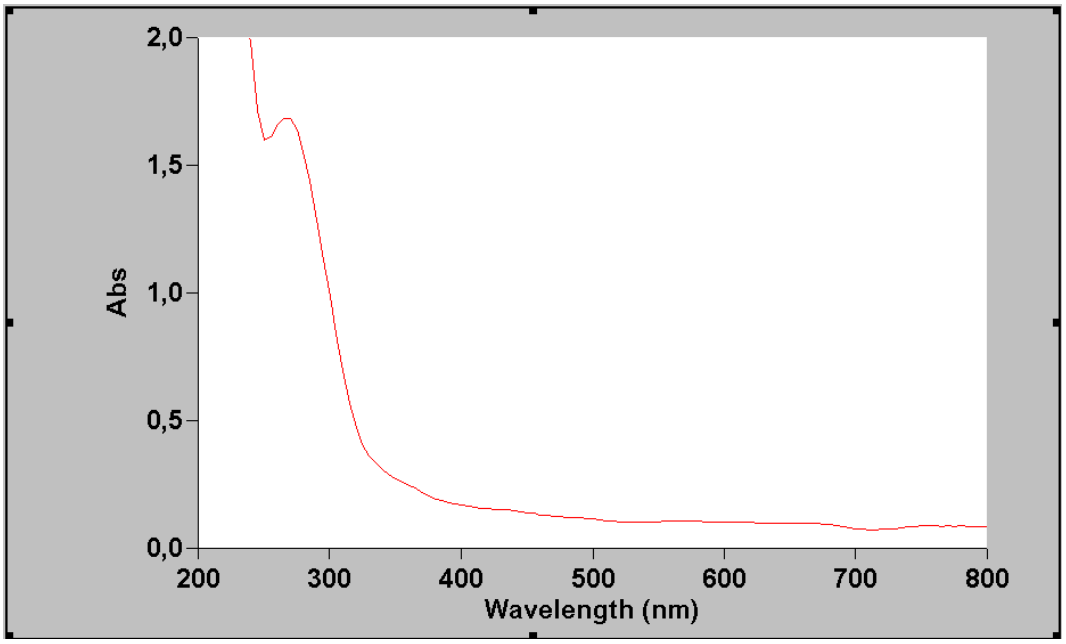

Figure A.6 UV-VIS absorption of dry pomegranate juice extract scanned using wavelength range between 200 to 800 nm.

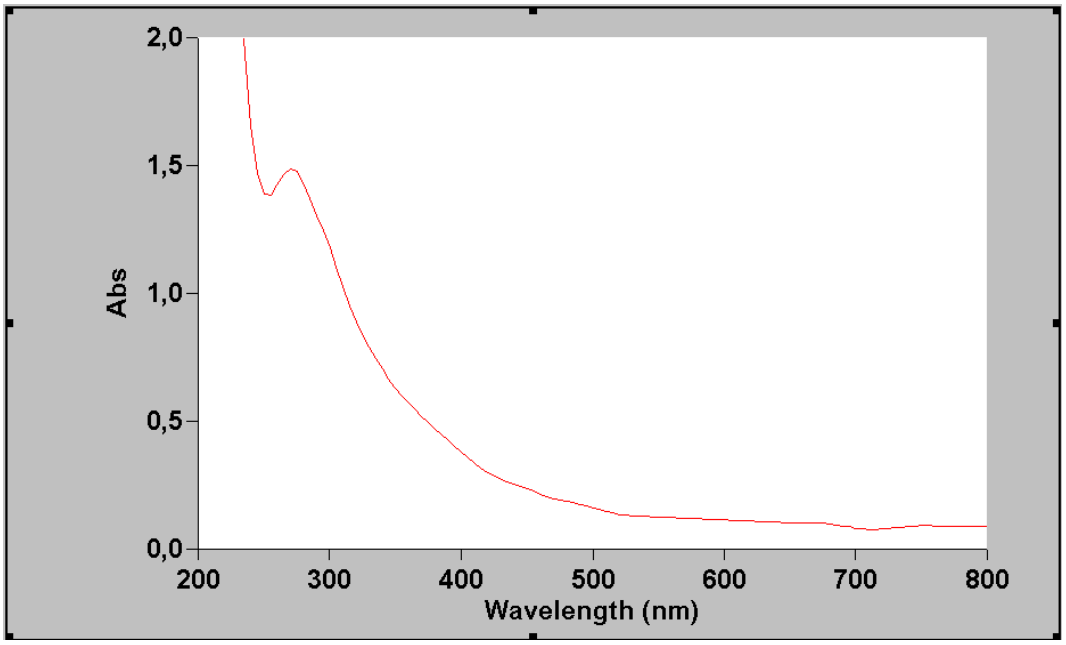

Figure A.7 UV-VIS absorption of dry peach juice extract scanned using wavelength range between 200 to 800 nm.

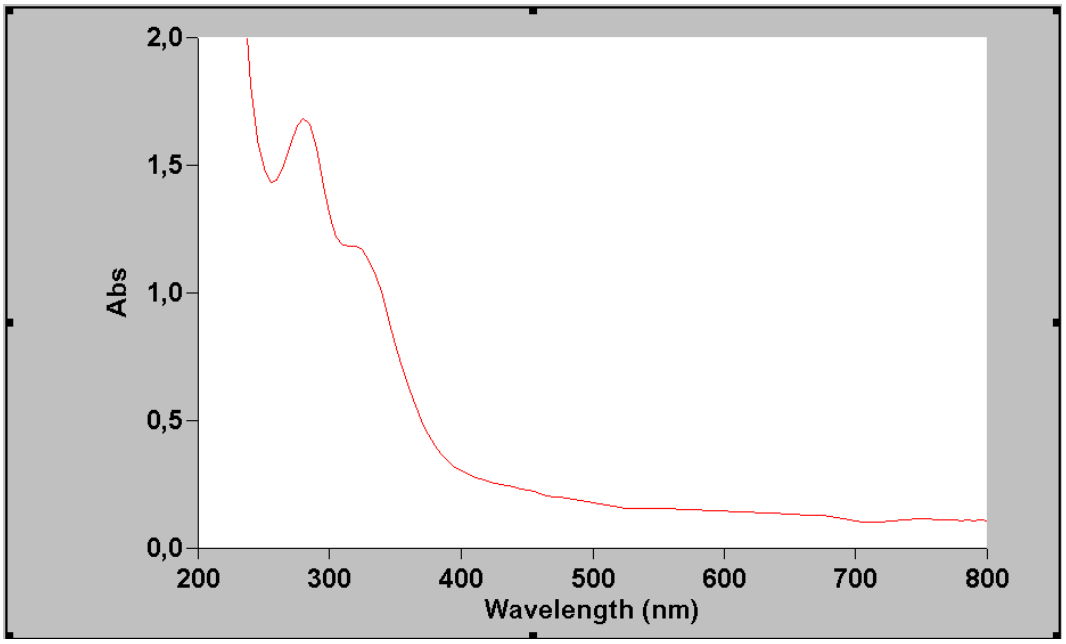

Figure A.8 UV-VIS absorption of dry orange juice extract scanned using wavelength range between 200 to 800 nm.

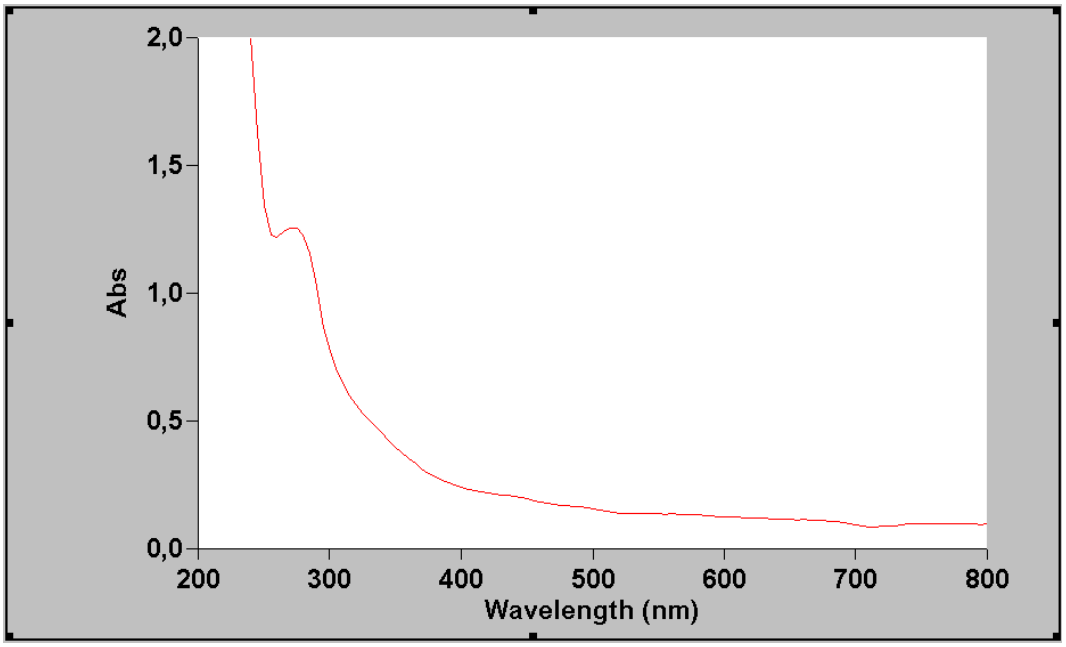

Figure A.9 UV-VIS absorption of dry grape juice extract scanned using wavelength range between 200 to 800 nm.

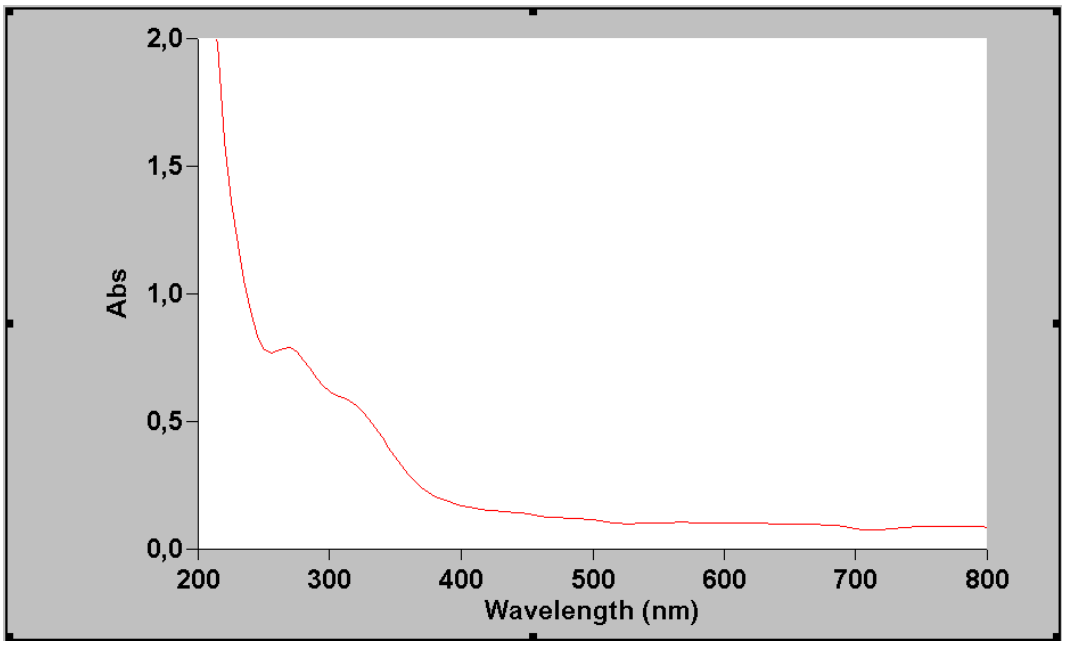

Figure A.10 UV-VIS absorption of dry chamomile tea infusion extract scanned using wavelength range between 200 to 800 nm.

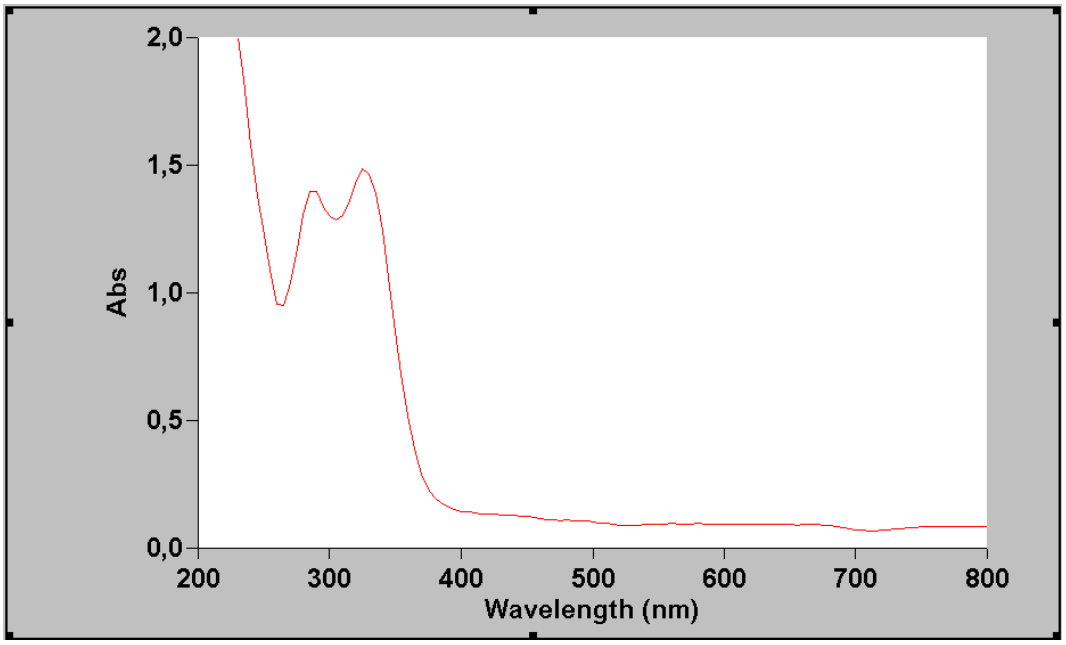

Figure A.11 UV-VIS absorption of dry sage tea infusion extract scanned using wavelength range between 200 to 800 nm.

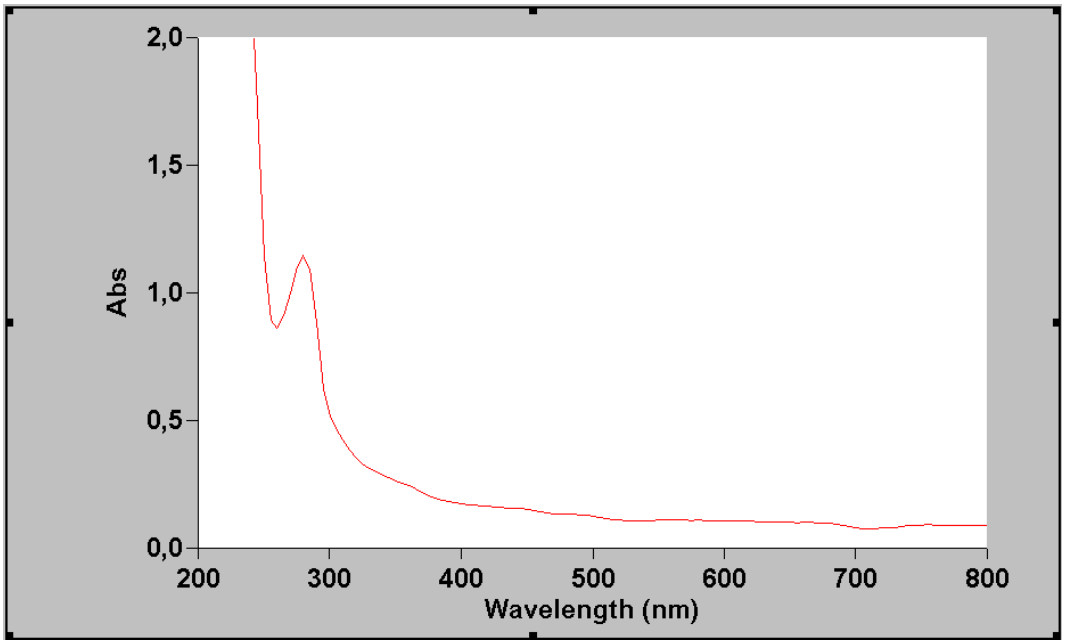

Figure A.12 UV-VIS absorption of dry rosehip tea infusion extract scanned using wavelength range between 200 to 800 nm.

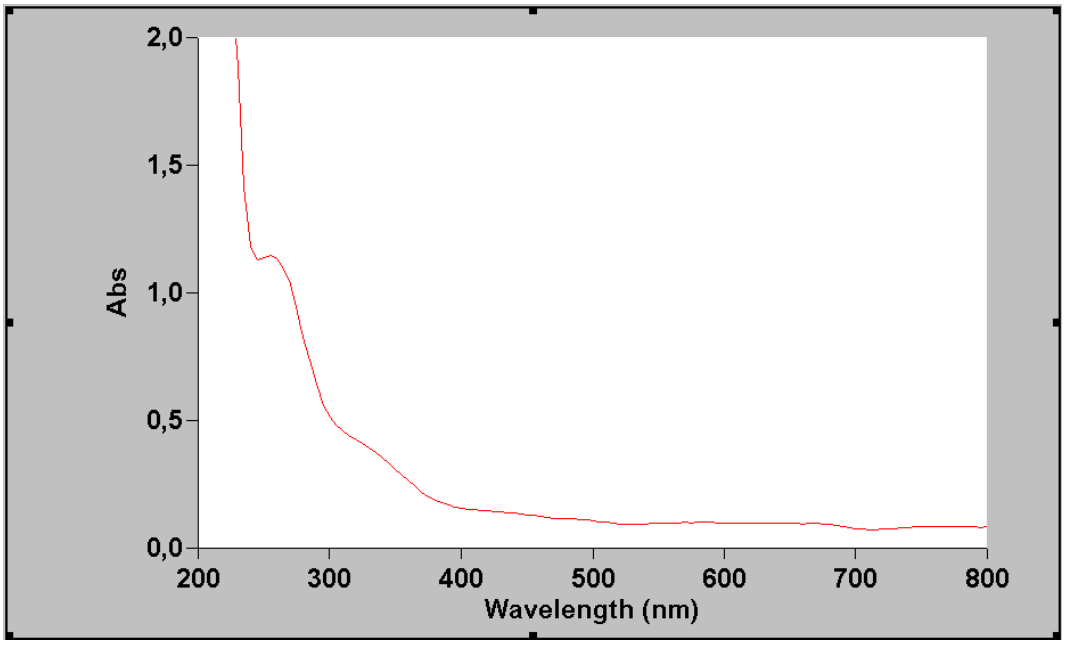

Figure A.13 UV-VIS absorption of dry anise tea infusion extract scanned using wavelength range between 200 to 800 nm.