Phytochemical Analysis and Biochemical Analysis of *Washingtonia filifera* Fruits and Seeds

Hatice Uluçınar

Submitted to the
Institute of Graduate Studies and Research
in partial fulfilment of the requirements for the degree of

Master of Science
in
Chemistry

Eastern Mediterranean University
July 2017
Gazimağusa, North Cyprus

Approval of the Institute of Graduate Studies and Research

Prof. Dr. Mustafa Tümer
Director

I certify that this thesis satisfies the requirements as a thesis for the degree of Master of Science in Chemistry.

Prof. Dr. İzzet Sakallı
Chair, Department of Physics and Chemistry

We certify that we have read this thesis and that in our opinion it is fully adequate in scope and quality as a thesis for the degree of Master of Science in Chemistry.

Asst. Prof. Dr. Mehmet İlktaç
Co-Supervisor

Assoc. Prof. Dr. Mustafa Gazi
Supervisor

Examining Committee

1.Prof. Dr. Müberra Koşar

2.Prof. Dr. Osman Yılmaz

3.Assoc. Prof. Mustafa Gazi

ABSTRACT

The investigation of antibacterial activities of natural plants is a potential guide for treating infectious diseases. Many years from now on, roots, fruits, seeds or leaves of natural plants have been used for medicinal agents. Therefore, natural plants have a wide range of pharmacological and biological activities such as antimicrobial, diuretics and anti-inflammatory functions. The main benefit of using natural sources is to decrease the side effects of synthetic drugs which are used for treatments.

The aim of this research is to investigate antibacterial activities and antioxidant activities of *Washingtonia filifera* (California Desert Palm) seeds and fruits. The antibacterial activity of seeds and fruits were tested against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) pathogenic bacteria. The antioxidant activity was tested with $ABTS^{+\bullet}$ radical scavenging activity assay and $DPPH^{\bullet}$ scavenging activity assay. The *Washingtonia filifera* was chosen as an investigation plant because there is no research or investigation yet about *W. filifera*'s antibacterial activities, antioxidant activities and its medicinal effects. The other reason is that *W. filifera* is found in most of the cities or villages in Cyprus. So, it is the plant that can be found easily and if there is any antibacterial activity or antioxidant activity, the plant can be used as a natural source for treatment of some diseases. Thus, this result may lead to design a new drug with using *W. filifera* seeds and fruits.

Keywords: *Washingtonia filifera*, antibacterial activity, antioxidant activity medicinal plants.

ÖZ

Doğal bitkilerin anti-bakteriyel aktivitelerinin incelenmesi, bulaşıcı hastalıkların tedavisinde potansiyel bir yol gösterici olmaktadır. Geçmişte de, doğal bitkilerin kökleri, meyveleri, tohumları ve yaprakları tıbbi olarak kullanılmaktaydı. Bu nedenle, doğal bitkiler geniş bir farmakolojik ve biyolojik aktiviteye sahiptirler, örneğin; anti-mikrobik, diyüretik ve iltihap giderici fonksiyonlar. Doğal kaynakları kullanmaktaki temel fayda, tedavilerde kullanılan sentetik ilaçların yan etkilerini azaltmaktır.

Bu tezin amacı, California çöl palmiyesi olarak da bilinen *Washingtonia filifera* tohum ve meyvelerinin anti-bakteriyel ve antioksidan aktivitelerinin araştırılmasıdır. Tohum ve meyvelerin anti-bakteriyel aktivitesi Gram-pozitif (*Staphylococcus aureus*) ve Gram-negatif (*Escherichia coli*) patojenik bakterilere karşı test edilmiştir. Antioksidan aktivite, $ABTS^{+\bullet}$ radikal süpürgeme aktivite ve $DPPH^{\bullet}$ süpürgeme aktivite deneyleriyle test edilmiştir. *Washingtonia filifera* araştırma bitkisi olarak seçildi çünkü *W. filifera*'nın anti-bakteriyel, antioksidan ve tıbbi etkileriyle ilgili henüz bir araştırma ya da inceleme bulunmamaktadır. Bir diğer neden ise, *W. filifera* Kıbrıs'taki çoğu şehir veya köylerde bulunmaktadır. Bu yüzden, bitki kolayca bulunabilmektedir. Eğer anti-bakteriyel ya da antioksidan aktivite aktivitesi var ise bu bitki bazı hastalıkların tedavisinde doğal bir kaynak olarak kullanılabilir. Böylece, bu sonuç *W. filifera*'nın tohum ve meyveleri kullanılarak yeni bir ilaç tasarlamaya yol açabilir.

Anahtar kelimeler: *Washingtonia filifera*, anti-bakteriyel aktivite, antioksidan aktivite, tıbbi bitkiler.

DEDICATION

I dedicate my thesis to beloved people who have meant and continue to mean so much to me, my family and my fiancé. They have supported me throughout the process. I will always appreciate for all the things that they have done for me. I am really blessed for having them in my life.

ACKNOWLEDGMENT

First of all, I would like to thank to my supervisor Asst. Prof. Dr. Mustafa Gazi for his assistance, motivation, patience and support in my research. His guidance helped me to understand my research topic very clearly. This thesis would not have been possible to complete without his inspiration.

I would like to extend my gratitude to Mehmet İlktaç and Prof. Dr. Müberra Koşar for their help, support and encouragement in my research.

I am blessed and so grateful for my family for their endless love, care, trust and support throughout my life. I would like to also thank to my fiancé for supporting me always.

ABBREVATIONS

ABTS+●	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic /acid) radical
BHT	Butylated hydroxytoluene
DNA	Deoxyribonucleic acid
DPPH$^{\bullet}$	1,1-Diphenyl-2-picrylhydrazyl radical
E. coli	*Escherichia coli*
H1A	Hexane extract of immature seed
H1B	Hexane extract of mature fruit
H1C	Hexane extract of mature seed
H2A	Ethyl acetate extract of immature seed
H2B	Ethyl acetate extract of mature fruit
H2C	Ethyl acetate extract of mature seed
H3A	70% Methanol extract of immature seed
H3B	70% Methanol extract of mature fruit
H3C	70% Methanol extract of mature seed
n.d.	not detected
S. aureus	*Staphylococcus aureus*
W. filifera	*Washingtonia filifera*
WHO	World Health Organization
W. robusta	*Washingtonia robusta*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

1.1 General information about medicinal plants

Plants that possess beneficial pharmacological effects and therapeutic characteristics on living organisms especially humans, are determined as medicinal plants [1]. The medicinal plants have been used for treatment of illnesses and have been played important role in the treatment of diseases on humans [2]. Plants are the possible source of antimicrobial agents [3]. In developing countries, about 60 to 90% of population use drugs which are derived from plants for most of the diseases [4]. So, plants are widely used in many countries as a medicine for therapeutic purposes and especially, as herbal medicine for the treatment of infectious diseases [2], [5]. The herbal medicine which is also called as phytomedicine or botanical medicine is increasingly becoming popular as an alternative to synthetic medicines. Herbal medicine cause less side effects than synthetic drugs and generally cheaper than most of the synthetic drugs [6].

1.2 Secondary Metabolites and their Importance

The bioactive compounds which are responsible for many functions in plants are synthesized by primary or secondary metabolism (Fig.1) in living organisms [9]. There are two types of metabolites that a plant cell produces; primary metabolites which are responsible for metabolism and growth, such as proteins, carbohydrates, vitamins, and lipids [11]. The primary metabolites have important roles in the survival of the plant. For example, primary metabolites have a key role in the

function of photosynthesis and respiration [11]. The other metabolite is secondary metabolites which are considered as an end product of primary metabolism but not in metabolic activity. The secondary metabolites act as defence chemicals [11]. The secondary metabolites have phytochemicals which protect plants against pests and microbial infections [8]. Phytochemicals possess therapeutic properties therefore they are considered as a drug or medicine [8]. These bioactive chemicals include phenols, alkaloids, steroids, tannins and essential oils etc. The absence of these chemicals does not cause any bad influence in the plants [11]. Likewise, absence of secondary metabolites does not result in immediate death [11]. On the other hand, the plants have to cope with many challenges, for example their own pollination, coexistence of pathogens and herbivores in their environment and fluctuations in the supply of nutrients that they require to synthesize their food [13]. Therefore plants have evolved secondary biochemical pathways to synthesize secondary metabolite chemicals. So that they are able to response to pathogen attacks, herbivore induced damage or nutrient deprivation [11]. The secondary metabolites are limited to occurrence and some kinds may not be found in particular taxonomic group of family, species or genus [11].

1.3 Phytochemicals in plants

There are considerably amount of evidences that some plants have many healthful properties. The plants have a wide range of pharmacological and biological activities such as, antibacterial, anti-fungal and anti-inflammatory properties [7]. These medicinal plants naturally synthesize some organic compounds, such as carbohydrates, flavonoids, saponins, tannins, alkaloids, steroids, resins, terpenes, glycosides, volatile oils etc. [1]. It is generally assumed that these active substances which have preventive and protective effects are the minerals, vitamins and

phytochemicals [7]. Phyto means “plant” in Greek language [8]. The phytochemicals are present in many plants, seeds, fruits and leaves, thus considered as important substances for humans and animals as well [7]. They provide certain physiological functions on the human body [9].

The phytochemicals which are bioactive non-nutrient compounds in fruit, seeds, vegetables or different plant parts are related to reduce the risk of main chronic diseases [10]. It is estimated that, there are more than 8,000 phenolics, 12,000 alkaloids and 25,000 terpenoids but there are still very large number of unknown phytochemicals [11]. According to recent evidences, the benefits of phytochemicals may be greater than anticipated because oxidative stress induced by free radicals which may be the reason of chronic diseases can be controlled by antioxidants [12]. So, to understand how phytochemicals can affect health of human, the most important phytochemicals must be investigated in detail.

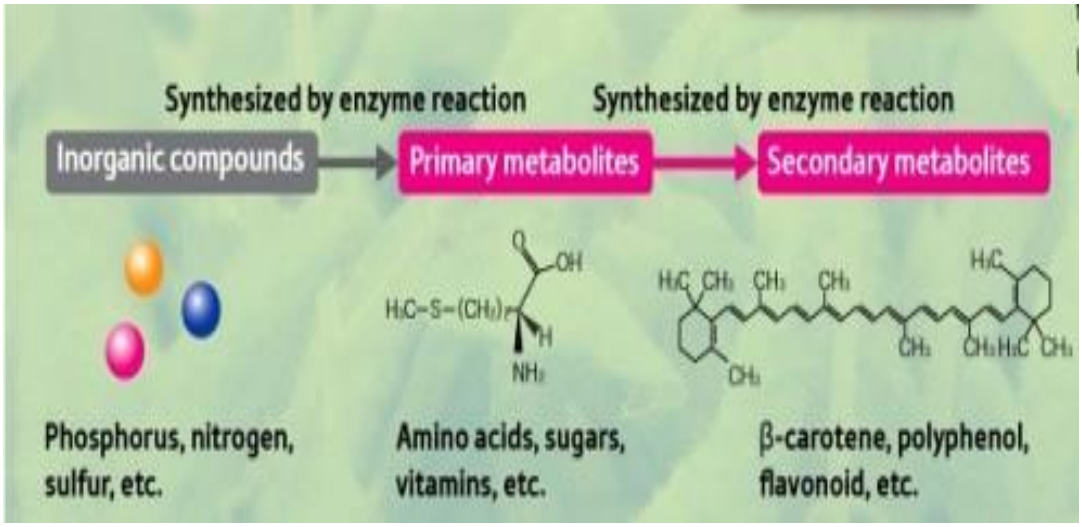


Figure 1:Flowchart of metabolites [11].

Secondary Metabolites

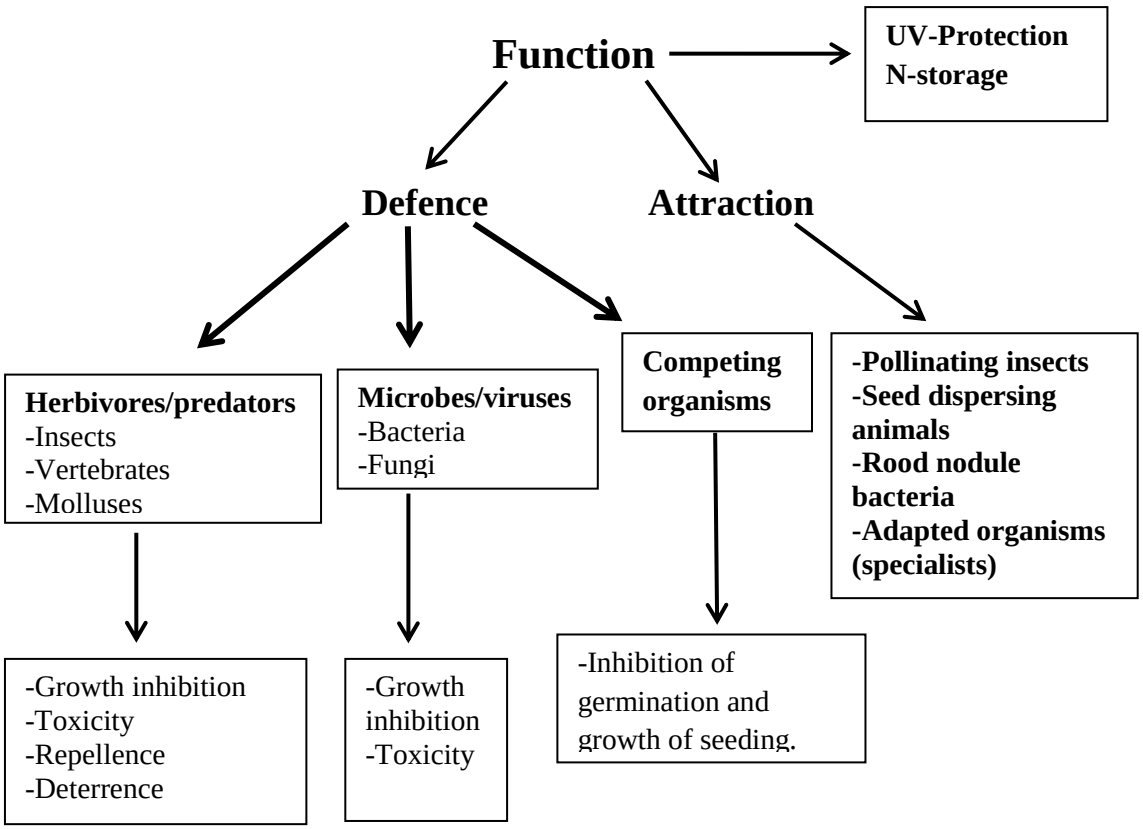
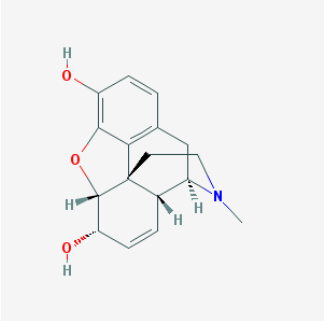
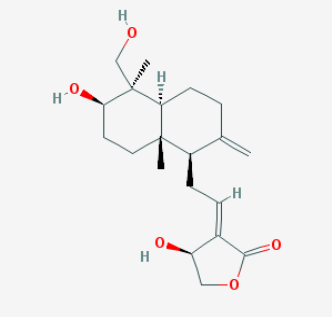
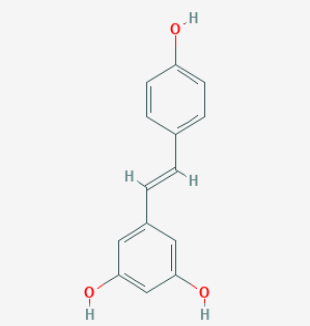
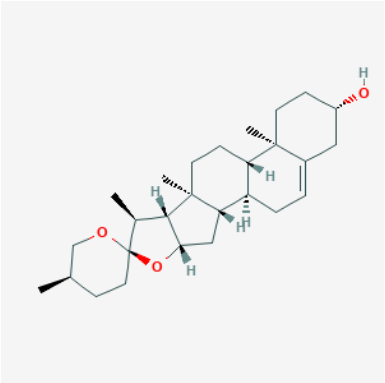
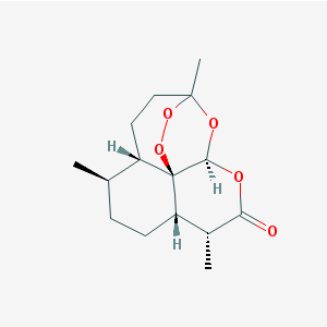
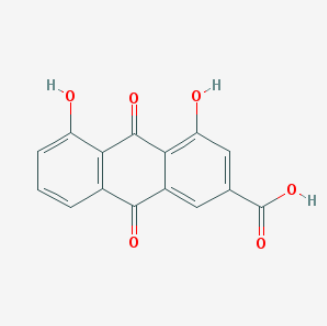


Figure 2:The functions of Secondary metabolites [14].

Table 1:The classification of main phytochemicals [8].

S. No.	Phytochemicals	Chemical Structure	Examples
1.	Alkaloids	Nitrogen atom in heterocyclic rings  Morphine [15]	Caffeine, Morphine, Berberin, codeine
2.	Glycosides	Derived from carbohydrates and non-carbohydrates molecules  Andrographolide [16]	Amygdalin, gentiopicrin and rograpolide, polgalin, Cinnamyl acetate
3.	Polyphenols (Flavonoids, Phenolic Tannins)	Aromatic aliphatic ring containing phenols  Resveratrol [17]	Quercetin, quercitrin, caffeic acid, flavones, rutin, naringin, hesperidin and chlorogenic, tannic acid, gallic acid and ellagic acid

4.	Saponins	Sugar attached to triterpene or steroid aglycone  Diosgenin [18]	Diosgenin and hecogenin
5.	Terpenes (Steroids)	Long unsaturated aliphatic chains (isoprene units)  Artemisinin [19]	Artemisinin, lycopene, lutein
6.	Anthraquinones	Derivatives of phenolic and glycosidic compounds  Rhein [20]	Rhein, salinos poramide and luteolin

1.3.1 Phenolic Compounds

Plants contain a large variety of **phenol** groups. Phenol groups are the compounds that have one or more hydroxyl groups that are attached directly to the aromatic ring (Fig.3 and Table 2) [21]. These substances are named as phenolic compounds or phenolics [13]. Plant phenolics are chemically heterogeneous, some of them are soluble in organic solvents, some of them are water soluble glycosides and carboxylic acids, and others are insoluble polymers [13]. Due to their chemical diversity, phenolic compounds have a variety of important roles in the plant. Most of the phenolic compounds serve as defence mechanism against pathogens and herbivores [13]. The other phenolics have functions in mechanical support, in attraction of pollinators and fruit dispersers etc. [13]. Phenolics, differ from simple, low molecular weight, single aromatic ringed compounds to large and complex tannins and also derived polyphenols. They can be classified due to arrangement and number of carbon atoms [11]. Phenolics have a diverse and very large group of chemical substances. These substances are classified into groups due to the number of carbons in the molecule which is designed by Harborne and Simmonds in 1964 (Table 1.) [21].

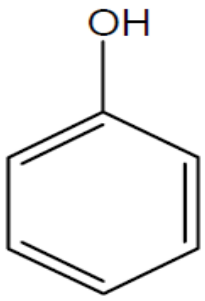


Figure 3:Phenol structure, a hydroxyl group is directly attached to the aromatic ring (benzene) [21].

Table 2: Classification of the phenolic compounds [21].

Structure	Class
C_6	Simple phenolics
$C_6 – C_1$	Phenolic acids and related compounds Acetophenones and phenylacetic acids
$C_6 – C_2$	Cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols
$C_6 – C_3$	Coumarins, isocoumarins, and chromones
$C_6 – C_3$	
C_{15}	Flavans
C_{15}	Flavones
C_{15}	Flavanones
C_{15}	Flavanonols
C_{15}	Anthocyanidins
C_{15}	Anthocyanins
C_{30}	Biflavonyls
C_6-C_1-C_6, C_6-C_2-C_6	Benzophenones, xanthones, stilbenes
C_6,C_{10},C_{14}	Quinones
C_{18}	Betacyanins
Lignans	Dimers or oligomers
Lignin	Polymers
Tannins	Oligomers or polymers

1.3.1.1 Flavonoids

Flavonoids (Fig.4) are one of the main groups of polyphenolic compounds and contain fifteen carbons (C_{15}), with two aromatic rings which are connected by a three carbon bridge [11]. Flavonoids are known as plant pigments and they are responsible for wonderful colours of flower petals [6]. They are the group of low molecular weight phenolics of phytochemicals that contain the flavonols, flavones, flavanones, flavan-3-ols, isoflavones and anthocyanins (Fig.5). The flavonoids are widely found in plants and have been reported that they have strong antioxidant activity [6]. In addition to this, they have antiviral, antibacterial properties also regulate gene expression and modulate enzymatic action [22]. They are also involved in different

processes such as pigmentation, stimulation of nitrogen fixing nodules, UV protection and disease resistance [11].

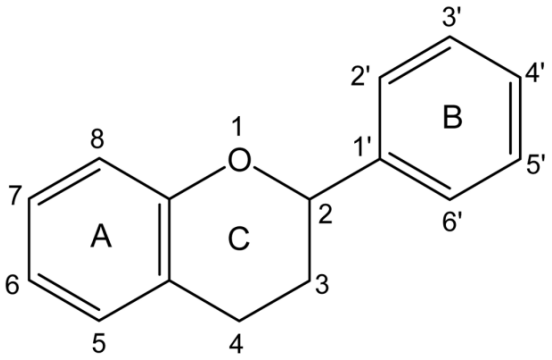


Figure 4:The main structure of Flavonoids [23].

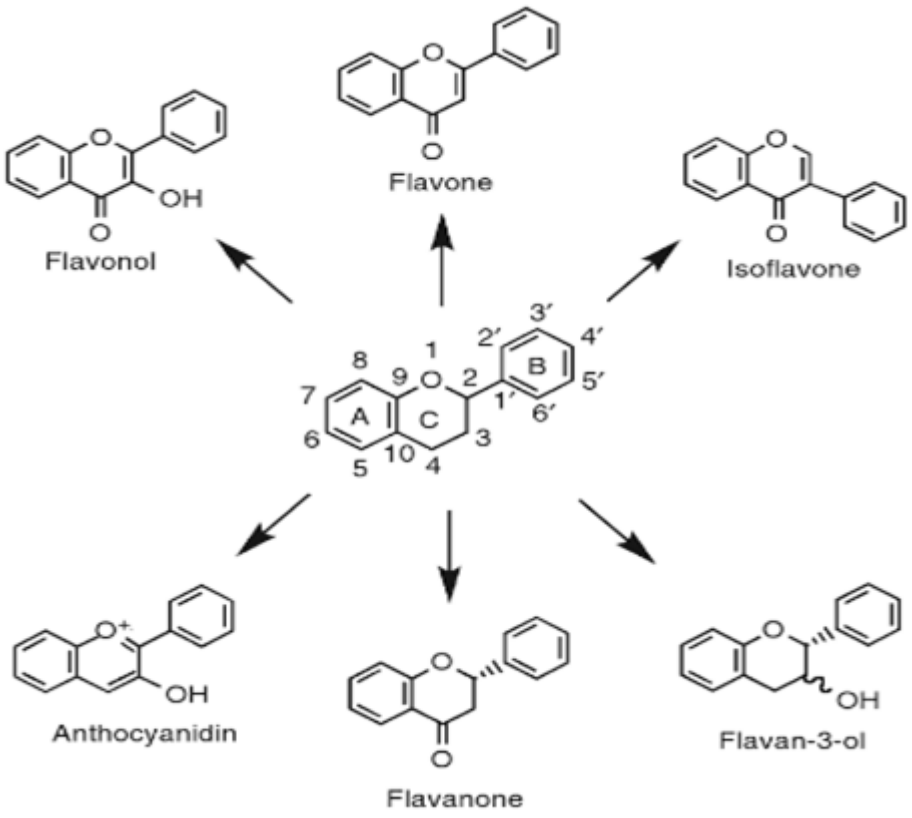
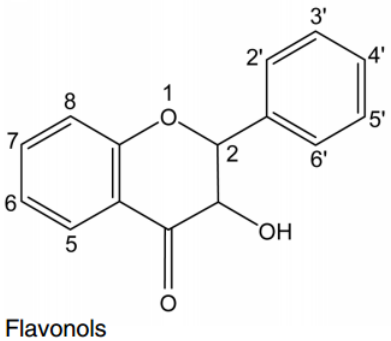


Figure 5:General Structure of main Flavonoids [24].

1.3.1.1.2 Flavonol

The flavonols are the ubiquitous flavonoids and widely present in plant foods [24]. The mostly found flavonols are quercetin, myricetin, kaempferol and isorhamnetin (Fig.6) [24]. Flavonols generally found in vegetables and fruits as glycosylated conjugate generally with the sugar [24]. These conjugated sugars are generally glucose or rhamnose but the other sugars can be involved as well such as, galactose, arabinose, glucuronic acid and xylose [25]. The presence of flavonols in the plants differs from the type of the fruit and vegetable [24]. Fruits often contain 5 to 10 different flavonol glycosides [25]. The richest sources of the flavonols are onions, broccoli, blueberries, leeks, and curly kale (Table 3.) [24].



Flavonols

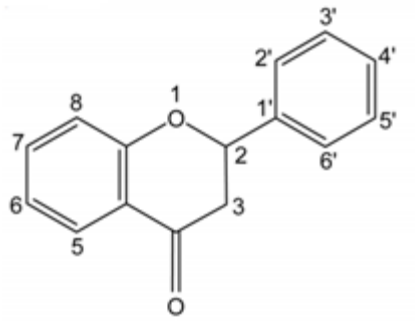
Position Compound	5	7	3′	4′	5′
Quercetin	OH	OH	OH	OH	–
Kaempferol	OH	OH	–	OH	–
Galangin	OH	OH	–	–	–
Fisetin	–	OH	OH	OH	–
Myricetin	OH	OH	OH	OH	OH

Figure 6:Flavonols and their substitution patterns [23].

1.3.1.1.3 Flavones

Flavones have a close structural relationship with flavonols [11]. On the other hand, flavones differ from flavonols by the absence of a 3-hydroxyl group [26]. Flavones have wide range substitutions including glycosylation, hydroxylation, O- and C-

alkylation and methylation [11]. The distributions of flavones are limited in vegetables and fruits when compared with flavonols [26]. Flavones can be found in plants as *O*-glycosides and also occur as *C*-glycosides [26]. The main flavones that are found in the diet are luteolin and apigenin (Fig.7) [24]. These flavones are found only in parsley, celery and in some herbs (Table 3.) [11].



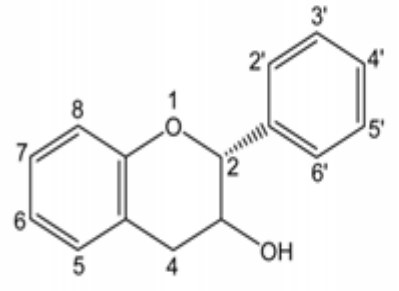
Flavones

Position Compound	5	7	3′	4′
Apigenin	OH	OH	-	OH
Luteolin	OH	OH	OH	OH
Chrysin	OH	OH	–	–

Figure 7:Flavones and their substitution patterns [23].

1.3.1.1.4 Flavan-3-ol

Flavan-3-ols are the most complex subclass of the flavonoids having both as monomers, such as (+)-catechins and its isomer (−)-epicatechin and polymeric proanthocyanidins (Fig.8) that are also known as condensed tannins [24].



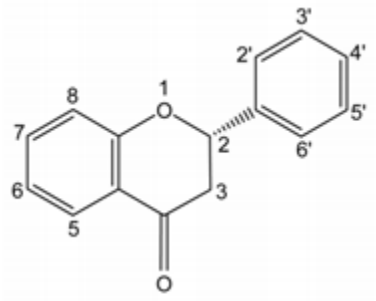
Flavan-3-ols

Position Compound	3	5	7	3′	4′	5′
(+)-Catechin	βOH	OH	OH	OH	OH	–
(−)-Epicatechin	αOH	OH	OH	OH	OH	-
(−)-Epigallocatechin	αOH	OH	OH	OH	OH	OH

Figure 8:Flavan-3-ols and their substitution patterns [23].

1.3.1.1.5 Flavanones

Flavanones are present in tomatoes and in certain aromatic plants such as mint [25]. On the other hand, they are found in high concentrations only in the citrus fruit [25]. The main flavanones (Fig.9) are hesperetin which is present in oranges, naringenin in grapefruit and eriodictyol are present in lemons (Table 3.) [27].



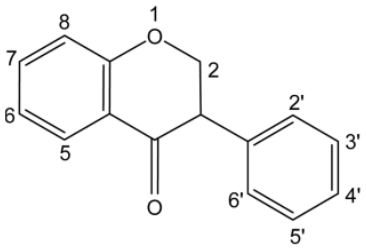
Flavanones

Position Compound	5	7	3′	4′
Naringenin	OH	OH	-	OH
Naringin	OH	*O*-Rha-Glu	–	OH
Hesperetin	OH	OH	OH	OCH_3
Hesperidin	OH	*O*-Rha-Glu	OH	OCH_3

Figure 9:Flavanones and their substitution patterns [23].

1.3.1.1.6 Isoflavones

Isoflavones have limited distribution in the plant kingdom when it is compared with other flavonoids [24]. Isoflavones are mostly present in leguminous plants such as soybeans and its processed products, soymilk and tofu (Table 3.) [25]. The most common isoflavones are genistein, genistin, and daidzein which soybeans contain them principally (Fig.10) [24]. Isoflavones have the similar structure with the estrogens [25]. Despite they are not steroids; they have hydroxyl groups in C-4 and C-7 configuration analogous to the estradiol molecule [25]. Thus, isoflavones shows pseudohormonal properties and they have the ability to find to estrogenic receptors [25].



Isoflavones

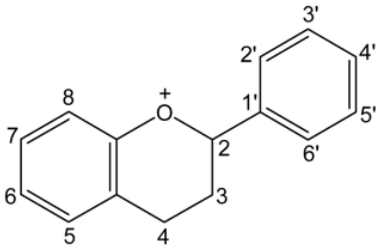
Position Compound	5	7	4′
Genistein	OH	OH	OH
Genistin	OH	*O*-Glu	OH
Daidzein	–	OH	OH
Daidzin	–	*O*-Glu	OH
Ononin	OH	*O*-Glu	CH_3

Figure 10:Isoflavones and their substitution patterns [23].

1.3.1.1.7 Anthocyanidins/Anthocyanins

Anthocyanidins are mostly present in nature as their sugar-conjugated derivatives [24]. They have the pigments that are dissolved in the epidermal tissue of fruit and flowers which are responsible for blue, purple, pink and red colours [28]. They protect the plant against excessive light to do not damage the mesophyll cells and

also have an important role to attract pollinators [24]. The most common anthocyanidins are cyanidin, peonidin, malvidin, delphinidin and pelargonidin (Fig.11) [28]. Anthocyanidins are present abundantly in berries like in cranberry, elderberry, blueberry and blackberry (Table 3.) [28].



Anthocyanidins

Position Compound	3	5	7	3′	4′	5′
Cyanidin	OH	OH	OH	OH	OH	–
Cyanin	O-Glu	OH	OH	OH	OH	–
Peonidin	OH	OH	OH	OCH_3	OH	–
Delphinidin	–	OH	OH	OH	–	OH
Pelargonidin	OH	OH	OH	–	OH	–
Malvidin	OH	OH	OH	OCH_3	OH	OCH_3

Figure 11:Anthocyanidins and their substitution patterns [23].

Table 3:Classification of some flavonoids and food sources [29].

Class	Flavonoids	Source of Dietary
Flavanol	(+)-Catechin, (-)-Epicatechin, Epigallocatechin	Tea
Flavone	Chrysin, apigenin, Rutin, and luteolin	Fruit skins, red wine, tomato skin, red pepper and buckwheat
Flavonol	Kaempferol, quercetin, myricetin, and tamarixetin	Onion, olive oil, red wine, grapefruit, and berries
Flavanone	Naringin, naringenin, hesperidin, and taxifolin	Lemons, oranges, grapefruits and citrus fruits
Isoflavone	Genistin, daidzin	Soyabean
Anthocyanidin	Cyanidin, apigenidin	Strawberry, Cherry and easberry

1.3.1.1.8 Non-flavonoids

The major non-flavonoid phenolic compounds of dietary are the C6-C1 phenolic acids, the polyphenolic C6-C2-C6 stilbenes, the C6-C3 hydroxycinammates and their conjugated derivatives [28].

1.3.1.1.8.1 Phenolic acids

Phenolic acids are generally known as hydroxybenzoates that has the principal component of Gallic acid [11]. Phenolic acids are present in the form of bound and they are components of complex structures such as hydrolysable tannins and lignins [28]. They can also present as derivatives of organic acids and sugars in the plant foods [28].

1.3.2 Terpeneoids

The terpenoids or terpenes are the largest group of secondary metabolites [13]. Terpenes are the most diverse classes of metabolites [11]. Terpenes are derived from five carbon isoprene (C_5) units [6]. Terpenes are responsible for functions in plants for development and growth, thus terpenes can be considered as primary metabolites rather than secondary metabolites [13]. Many aromatic molecules and flavour, such as menthol, linalool are formed by monoterpenes (C_{10}) with two isoprene units and sesquiterpenes (C_{15}) with three isoprene units. Other compounds are diterpenes (C_{20}), triterpenes (C_{30}) and tetraterpenes (C_{40}) have important properties [11]. Many plants contain mixtures of volatile monoterpenes and sesquiterpenes which are called as essential oils. These essential oils give odor characteristic to their foliage [13]. For instance, lemon, peppermint, sage and basil are the plants that contain essential oils [13]. These volatile terpenes are produced in order to attract specific insects for pollination or to protect plants to be used as food by certain animals [30]. Monoterpenes serve as defence against insects and other organisms [31].

Researches and studies have revealed that the terpenes possess anti-viral, anti-microbial, anti-fungal, anti-parasitic, anti-allergenic, anti-inflammatory, anti-hyperglycemic and immune modulatory properties [6].

1.3.3 Tannins

Tannins or tannic acids are water soluble polyphenols which is present in most of the plants [6]. Tannins possess a structure that they have the ability to bind and precipitate proteins [21]. Tannins are generally known as toxins. They are involved in the defence mechanism of the plant [13]. In the studies of both *in vitro* and *in vivo* have revealed that tannins serve defence against microorganisms and have antioxidant potentials [13], [6]. Tannins are present in fruits mostly in unripe fruits, leaves and bark to protect the plant against herbivores and infections [21].

1.3.4 Alkaloids

Alkaloids are another phytochemicals that are widely distributed in the plant kingdom. The alkaloids are large family and they are more than 15,000 containing nitrogen-containing secondary metabolites [13]. Alkaloids are synthesized from decarboxylation of amino acids [6]. Alkaloids are important for the well-being of the organisms. Alkaloids are responsible for defence against predators and herbivores like most of the phytochemicals [14]. Some alkaloids are antiviral, antibacterial and anti-fungal [14]. Examples of alkaloids are represented in figure 12.

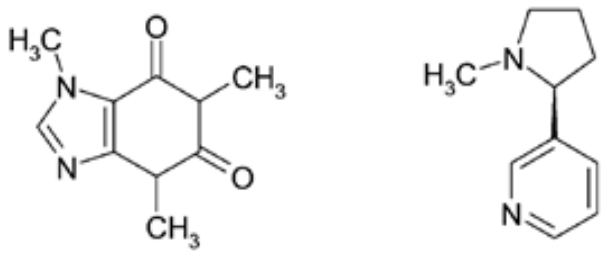


Figure 12**:**Structure of alkaloids, caffeine and nicotine [32].

1.3.5 Glycosides

The glycosides have many categories of secondary metabolites. They bound to mono or oligosaccharide or to uronic acid [33]. The uronic acid or saccharide part is called as glycone and other past is called as aglycone [33]. The main groups of glycosides are saponins, anthraquinone glycosides, cardiac glycosides, cyanogenic glycosides and glucosinolates [33]. In addition to this, flavonoids mostly occur as glycosides.

1.3.6 Saponins

Saponins are one of the main glycosides with foaming characteristics [34]. They have a bitter taste and can be toxic to animals [13]. They are named as saponins because of their soap-like properties. The presence and combination of both water soluble (hydrophilic) sugar part and fat soluble (hydrophobic) steroid and triterpene parts cause to the foaming ability of saponins [34]. According to researches, saponins have various health effects. The investigations reveal the beneficial effects of saponins on cancer, blood cholesterol levels, immune system and bone health [33], [34]. Other studies showed that saponins have anti-fungal effects and protect the plant against pathogens [13].

1.3.7 Essential oils/volatile oils

Essential or volatile oils of the plants which can be extracted by using steam to hydro distillation methods [35]. Most of these volatile oils are belong to the group of monoterpenoids compounds. These essential oils are very important by the reason of their antioxidant, antibacterial, antifungal and most importantly anti-carcinogenic properties [35]. There are two main reasons that explain “why do plants produce essential oils?” [36]. The first reason is for protection. Plants produce and use essential oils for protection from pathogens and also, they produce essential oils for their stress response into climate changes and for to protect themselves from harsh

environmental conditions [36]. The second reason is for attraction. Plants use essential oils to attract pollinators with their fragrance [36].

1.4 Phytochemicals and their health effects

Phytochemicals, as plant bioactive components are being examined for their ability to provide health benefits toward metabolism and biochemistry [37]. Phytochemicals provide some health effects as: biochemical reaction substrates; enzymatic reaction inhibitors; cofactors of enzymatic reactions; scavengers of toxic or reactive chemicals; substances that enhance the stability or absorption of essential nutrients; inhibitors of harmful intestinal bacteria [37].

It is reported that plant based diet with more intake of vegetables and fruits can reduce the risk of the oxidative stress related diseases like cardiovascular diseases and cancer [33]. Besides, regular consumption of vegetables and fruits can reduce the risks of cataracts, Alzheimer disease, diabetes and some of the functional declines related with aging [12]. It is not easy to understand the complex role of diet in chronic diseases [33]. There are more than 25,000 bioactive compounds in plants that can modify the processes of these diseases [33].

Bioactive compounds or phytochemicals in plants are mainly antioxidants [33]. Antioxidants can eliminate many of the reactive species that causes to chronic diseases [33]. There are hundreds of antioxidants belongs to phenols and polyphenols [33]. Phenolic compounds such as flavonoids, benzoic acids, lignans, lignins etc. [33], [38]. Many of these phenolic compounds have antioxidant activities [38].

1.5 Flavonoids and their dietary intake and health effects

Flavonoids are the major antioxidants in the diet and their total dietary intake can be 1 g per day which is higher than all the other class of phytochemicals [39]. The major actions of flavonoids are antioxidant, anti-inflammatory, anticancer and antiproliferative activities and also anti-human immunodeficiency virus functions [40]. There are many *in vitro* studies on biological activities and beneficial effects of flavonoids. Currently, there are strong evidences that flavonoids support the prevention of cancers, cardiovascular diseases, and osteoporosis and also studies suggest that flavonoids have role in the prevention of diabetes mellitus and neurodegenerative diseases [39].

The recent studies showed health benefits of dietary flavonoids reduce the risk of cardiovascular death in adults [22]. In this study, both male and female subjects who take large amounts of flavonoids, compared with to those whose flavonoid intake was low [22]. The subjects who consume large amount of flavonoids showed 18% lower mortality risk of cardiovascular diseases [22]. In another study, it was demonstrated that high consumption of flavonoids (flavones and flavanols) protected people against hypertension [22].

Flavonoids show effectiveness in prevention of age related neurodegenerative diseases [22]. Their effectiveness is related with dementia, Alzheimer's and Parkinson's diseases [22]. According to some studies, it seems flavonoids can modulate neuronal functions and cognitive functions by protection of neurons, enhancement of neuron functions and regeneration [22].

It was demonstrated that using the extract of the plant gingobiloba which is rich in flavonoids, may show beneficial effects on the treatment of Alzheimer's disease and dementia [22]. The tangeretin flavonoid which belongs to the flavone subclass and present in citrus fruit showed protection in Parkinson's disease [22].

Flavonoid dietary intake plays an important role in the prevention of cancers [29]. The fruits and vegetables which contain flavonoids have been reported as cancer chemo-preventive agents [29]. Clinical studies suggest that flavonoids can prevent cancer by the interactions with genes and enzymes [22]. These substances that are found in plant foods can affect the stages of carcinogenesis, initiation, promotion a progression [22]. In the stages of initiation and promotion, flavonoids involve in: inhibition of cell proliferation, inactivation of carcinogen, reduction of oxidative stress and enhancement of Deoxyribonucleic acid (DNA) repair process [22]. In the progression stage, flavonoids can induce apoptosis which is a programmed cell death, exhibit antioxidant activity, and inhibition of angiogenesis [22]. For instance, the anticancer effects of flavonoids can be explained by the inhibition of p53. Mutations in p53 protein are the most common genetic abnormalities in cancers [29]. Flavonoids are found to downregulate the expression of p53 mutations nearly undetectable levels in the breast cancer cell lines [29].

1.6 Biological Activities:

1.6.1 Anti-bacterial Activities

Investigation of biological activities of phytochemicals have increased in recent years and researches for novel compounds have increased as well in order to understand health effects of bioactive compounds. Biological activity of the plant extracts are investigated by using *in vivo* or *in vitro* bioassay methods [6]. In the in

vivo bioassay method approach involves the use of microorganisms; on the other hand in vitro procedures involve isolated subcellular systems such as receptors and enzymes or animal and human cell cultures [6].

Plants are the source of beneficial phytochemicals which have antimicrobial activities. Antimicrobial activities of phytochemicals are investigated in *Phoenix dactylifera* which is the plant examined in the family of Palm trees.

1.6.2 Anti-oxidant activities

Phenolic compounds are mostly found in edible or non-edible plants and they are related with many biological effects, for instance anti-oxidant activity [41]. The anti-oxidant activity of plant materials is getting very important to investigate due to maintenance of health and protection from coronary heart disease and the most importantly cancer [41].

The potential sources of anti-oxidant compounds have been searched. As a result, flavonoids and phenolics, such as phenolic acids, tannins, lignans and lignin were the potential sources of anti-oxidants [41]. These compounds are important in normal growth and development and mainly in defence against injury and infection. Phenolics have anti-oxidant activities due to their redox properties, which let them to be able to act as hydrogen donators and reducing agents [41]. In addition to this, phenolics have a metal chelation potential as well [41].

1.7 New Produced Drug from Plants

Herbal plants have important roles in human life. Plants have been used as medicine, food, shelter and clothing. Herbal medicine is widely practiced and used all over the world [8]. For many years, herbal plants are used to cure allergy, colds, toothaches

and stomach-aches. Thus, there has been a change from synthetic to herbal medicines and this trend is constantly increasing which can be named as “Return to Nature” in order to prevent diseases [8]. According to World Health Organization (WHO) about 25% of the drugs are derived from plants. There are 252 drugs which are considered as basic and essential by WHO. 11% of these drugs are plant origin and significant numbers of synthetic drugs are derived from natural products [42]. Plants are beneficial nutrients which contain antioxidants, micronutrients, phytochemicals which are protective against various diseases [8]. Plants have many pharmacological roles such as, antimicrobial, antifungal, antioxidant, antiviral and anticancer etc. [8]. It has been reported that phytochemicals reduce the risk of several human diseases such as, diabetes, cardiovascular diseases, hepato-renal diseases, cancers and neurodegenerative disorders [8]. For instance, well known cancer drug Taxol® is derived from the bark of *Taxus brevifolia* which is the Pacific yew tree [43]. It is used in the treatment of breast, ovarian and lung cancer [43].

Herbal medicines and their derivatives show lesser side effects than synthetic drugs. It has been reported that herbal medicine plays important roles in the development of modern drugs [8]. Although, there are some challenges that manufactures faced with. For instance, herbal plants can easily contaminated during, growth, collection and processing. Heavy metal contamination is the most important problem [8]. Therefore, while developing a new herbal drug, the quality and quantity of bioactive compounds must be improved. Bioactive compound of herbal medicines must be clinically and scientifically evaluated. This is why the quality of herbal products is consistently questioned [8].

Chapter 2

MEDICINAL PLANTS AND HUMAN VARIOUS DISORDERS

2.1 Medicinal plants, herbs and traditional herbal medicine

All over the world, it is estimated that there are 300,000 to 500,000 number of plant species. Approximately, 250,000 plant species have been identified and classified [44]. All over the world, nearly 35,000 to 70,000 different plant species are used for medicinal purposes [45]. These plant species are named as “medicinal plants”. The term medicinal plant is used in herbalism (herbal medicine or herbology) which includes various types of plants [46]. The word “herb” was derived from the Latin word “herba” and an old French word “herbe”. Nowadays, herb refers to any part of the plant such as, leaf, flower, seed, fruit, bark, stem or root [46].

Medicinal plants have been traditionally used for the treatment of many diseases. Traditional systems of medicine continue to be widely used because of the high cost of treatments, inadequate supply of drugs, side effects of synthetic drugs and resistance to currently used drugs for infectious diseases [46]. World Health Organization (WHO) is estimated that 80% of people trust herbal medicines. According to WHO, nearly 21,000 plant species are estimated that have the potential to be used as medicinal plants [46].

2.1.1 Oxidative Stress Related Diseases

The bioactive compounds such as polyphenolics, terpenoids, tocopherols and etc. are assumed as alternative therapeutic treatment agents for various human disorders [47]. Free radicals are molecules that include an unpaired electron [48]. Most of the radicals are unstable and very reactive [48]. Radicals can accept or donate an electron from other molecules, thus they act like reductants or oxidants [48]. If free radicals overcome the ability of body to regulate them, a condition which is known as "oxidative stress" occurs. In this condition, oxidative stress damages proteins, lipids, cells, DNA and nucleic acids, then leads to cellular death, apoptosis [47].

Many degenerative diseases including skin diseases, ageing, stomach disorders, asthma, urinary tract infections, wounds, inflammatory diseases, kidney problems, malarial fevers, cardiovascular diseases, immune deficiency, sensory and nervous system dysfunctions, cancer and many psychiatric disorders such as panic disorder, major depression, obsessive-compulsive disorder, autism, Parkinson's disease and Alzheimer's disease are all associated with oxidative stress [47]. Antioxidants have the ability in coping this oxidative stress. Therefore, there are many researches to find nontoxic and effective natural products with antioxidative activity [48].

Medicinal plants can be found easily in nature and this is the biggest advantage. The most important fact about medicinal plants is that, the use of herbal medicine has no restrictions of any sexes and any age groups [46].

2.1.2 Infectious Diseases

Human kind has long been battled with diseases that can spread rapidly among the population by resulting serious damaging effects. The microbes are the main reason of this type of diseases which are called as "Infectious Diseases". The invasion and

multiplication of microorganisms such as viruses, bacteria and parasites which are not ordinarily present in the body, is the best way to explain infection [3]. The most important reason to study about microorganisms is to understand the disease that they cause and to find the ways to control them and prevent infections [64]. An infection may cause symptoms and it can appear clinically but on the other hand an infection may not cause any symptoms and can be subclinical [3]. In a different manner, an infection may remain localized or it may spread to other part of the body such as to the lymphatic vessels or to the blood and become systemic [3].

According to World Health Organization (WHO), infectious diseases are a significant cause of mortality and morbidity worldwide [65]. Infectious diseases cause 20% of deaths in the America and 50% of all deaths in tropical countries [65]. These types of diseases are caused by microorganisms which are pathogenic such as viruses, bacteria, fungi or parasites. Microorganisms are found in everywhere for example; in water, in air and in soil. A person can be infected by drinking, eating, touching or even by breathing.

2.1.2.1 Bacterial Infections

Infections that are caused to disease by bacteria or pathogenic bacteria are termed as "Bacterial Infections". On the other hand, bacteria can be beneficial, such as gut bacteria that help digestion of food [66]. Bacteria have a simple structure even so some of them are responsible for serious infections. They are prokaryotic organisms which mean that they are simple unicellular organisms without nuclear membrane, mitochondria, endoplasmic reticulum or Golgi bodies [64]. The bacterial cell wall is a complex structure. It consists of a gram-positive cell wall having thin

peptidoglycan layer and gram-negative cell wall having thin peptidoglycan layer and an overlaying outer membrane [64].

2.1.2.2 Bacterial Classification

The classification of bacteria is divided into two main groups; Gram-positive and Gram-negative bacteria. The distinction between both groups is based on the Gram stain due to their structure of the cell wall [67]. A Gram-positive bacterium has a prokaryotic cell, thick and multi-layered cell wall which consists of peptidoglycan but Gram-positive bacteria do not have outer membrane [67]. The gram-negative bacterium has thin cell wall; their peptidoglycan layer is thin (Fig.13). However, Gram-negative bacteria have outer membrane with higher lipid and lipoprotein content than Gram-positive bacteria (Fig.13) [68],[87].

The Gram-positive bacterium which is used as test organism is *Staphylococcus aureus*. This is the bacterium that causes staphylococcal food poisoning which is the form of gastroenteritis [69]. *S. aureus* is mostly found in the air, water, soil and also found on the skin and in the nose of humans [70]. *S. aureus* is a Gram-positive bacterium with non-spore forming spherical bacterium which is from *Staphylococcus* genus. The growth and survival of *S. aureus* depends on conditions of the environment like temperature, pH, presence of food and oxygen. The suitable temperature range of *S. aureus* for growth is between 7-48°C.

The Gram-negative bacterium as a test organism is *Escherichia coli*. This is the bacterium that causes intestinal disease in human [69]. It causes diarrhea with blood and severe cases can cause some kidney problems [69].

The method that shows the difference between Gram positive and Gram negative bacteria is discovered by Danish scientist Hans Christian Gram [87]. The method differentiates these two types of bacteria according to their differences in cell walls [87]. In this method, a bacterium that retains the crystal violet dye is the bacterium which has thick layer of peptidoglycan and coloured as purple is called Gram-positive bacteria [87]. On the other hand, Gram-negative bacterium does not retain the violet dye and coloured as pink [87].

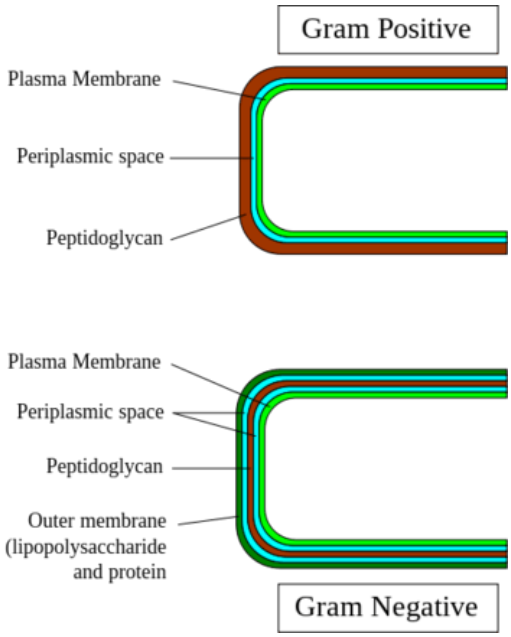


Figure 13: Differences between Gram-positive and Gram-negative bacteria [87].

Medicinal plants and their products have been used as prevention and treatment of infectious diseases for many years. According to the World Health Organization (WHO), medicinal plants are good source of many drugs [69]. WHO estimated 80% of people worldwide choose plant based medicines as a primary healthcare [70]. The global interest to traditional medicines is increasing due to their fewer side effects, tolerance or simply traditional medicines are safer for some chronic diseases [69].

2.2 Medicinal Plants in Cyprus

The flora of Cyprus is rich as the flora of the other areas in the Mediterranean region. The number of factors, such as geographic location, geological structure, climatic conditions and the surrounding sea are the main factors that Cyprus has a rich flora [49]. The number of plants taxa is recorded about 1900 species until now in Cyprus. There are 52 species of trees, 131 species of shrubs, 88 of subshrubs and 1637 species of herbs are found in Cyprus [49].

Initiatives about aromatic and medicinal plants in Cyprus began in 1991. The project which was called “Development of Aromatic and Medicinal plants in Cyprus” was set up during the year, 1991 [50]. The project involves the identification of aromatic plants, such as Sage, Oregano, Mint, Rosemary, Thyme, Melissa, Lavender, Tarragon, Sideritis, Dictamus etc. The main intention of the project is to define where those plants can grow best in Cyprus and which areas are economically available [50].

There have not any research found about palm trees even the distribution of palm trees are very high in all around the Cyprus.

2.3 The Plant: *Washingtonia filifera*

The family Arecaceae which is known as Palmae is a large group that possess approximately 2,500 species and 183 genera [51]. These species of Arecaceae found in tropical, subtropical and equatorial areas of the world [52]. The Arecaceae family include many plants which are economically very important for humans. Especially, many foodstuffs are made from *Cocos nucifera* which is a coconut palm. The date palm, *Phoenix dactylifera* has a great economic importance as it yields a huge

amount of fruits [7]. Other species are important for the production of vegetable fibres such as, Sabal, Trachycarpus, Chamaerops, Borassus, etc. On the other hand, many species are used in milder climate regions to provide gardens, avenues and parks. The most widely used ones are *Phoenix dactylifera, Phoenix canariensis, Washingtonia robusta*, *Washingtonia filifera,* etc. [7].

The Arecaceae family is divided into several subfamilies. These families are: a)Phytelephasieae which is characterised by flowers without a perianth, female flowers bearing 4-9 locules of multilocular ovary; b)Coryphoideae which shows floral characteristics such as berry-like fruits, pinnate, free carpels or fan-shaped leaves [52]. For example, Livistona, Sabal, Trachycarpus, Chamaerops, Phoenix and *Washingtonia* are typical kinds of Coryphoideae subfamily [52]. The third group is, c)Borassoideae which is characterised by their pan-shaped leaves, syncarpous ovary and perianth is the typical part of this subfamily. For example: Lodoicea, Hyphaene and Borassus [52]. Other subfamily is named as d)Lepidocaryoideae which is characterised by imbricate scales covered fruits and syncarpous ovary such as Calamus, Raphia and Metroxylon; e)Ceroxyloideae, characterised by pinnate leaves and syncarpous ovary such as Areca, Cocos, Arenga and Ceroxylon [52]. The last group is Nipoideae is characterised by unilocular ovary and male flowers bearing three connate stamens such as Nipa [52]. According to taxonomy of Arecacea family (Table 4.), from 217 genera, there are 2 species of Washintonia H. Wendl.(Table 5.) which are accepted [53]. These two species are *Washingtonia robusta* H. Wendl. (Washington fan palm) and second one is *Washingtonia filifera* (Linden ex André) H. Wendl. (California fan palm) [54].

Table 4:Classification of Family Arecaeae [53].

Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Liliopsida (Monocotyledons)
Subclass	Arecidae
Order	Arecales
Family	Arecaceae (Palm family)

Table 5:Classification of *Washingtonia* H. Wendl. Genus [54].

Kingdom	Plantae (Plants)
Subkingdom	Tracheobionta (Vascular plants)
Superdivision	Spermatophyta (Seed plants)
Division	Magnoliophyta (Flowering plants)
Class	Liliopsida (Monocotyledons)
Subclass	Arecidae
Order	Arecales
Family	Arecaceae (Palm family)
Genus	*Washingtonia* H. Wendl. (fan palm)
Species	-*Washingtonia robusta* H. Wendl. -*Washingtonia filifera* (Liden ex André) H. Wendl.

The plant *Washingtonia filifera* (Fig.14 and 15) belongs to the family of Arecaceae and genus of *Washingtonia* H. Wendl. is commonly known as California fan palm or desert fan palm [13]. *Washingtonia filifera* is the native palm of Western United States [14]. It is mainly distributed on the West and North edge of the Colorado Desert, Turtle Mountains, the Sonoran Desert, Southeastern Arizona, and Northern Baja, California (Fig.15) [13]. On the other hand *W. filifera* has been cultivated in Egypt, Mediterranean region and elsewhere as well (Fig.19) [55].

Desert Fan Palm (*W. filifera*) is commonly seen at 40 to 60 feet but it is able to soar up to 80 feet in height therefore its relation can be quickly recognized with single-trunked and straight street palm *W. robusta* [56]. However, *W. filifera* is better suited

at the home landscape than *W. robusta* because *W. filifera* grows slowly and it is shorter thus this allows it to be used in garden applications [56].

Figure 14:*Washingtonia filifera* in Famagusta, North Cyprus.

Figure 15:*Washingtonia filifera* in California.

Washingtonia filifera is evergreen monocot palm tree. It has large leaves. The tree is supported by a columnar trunk [57]. Trunk of the plant is covered by a mass of pendent dead leaves which are called as shag (Fig.16). The outer tissue of the trunk is thick rind [57]. The leaves of *Washingtonia filifera* is like star shaped and bigger than 92 cm (>92 cm) (Fig.17) [58]. The flowers of *Washingtonia filifera* usually forms during summer, in May and June. They are look like creamy white flowers in panicles to 3-5 m long [59]. Fruits of the plant are oval, round and they are around 13 cm size [58]. The fruits ripen during September and at the beginning they are creamy

whitefruits (Fig.23) when they ripen their colour changes to black (Fig.22) [57]. The seeds are 8 mm in size and are present inside part of the fruits (Fig.18).

Figure 16:*Washingtonia filifera* shag of dead leaves [60].

Figure 17:*Washingtonia filifera* leaves [61].

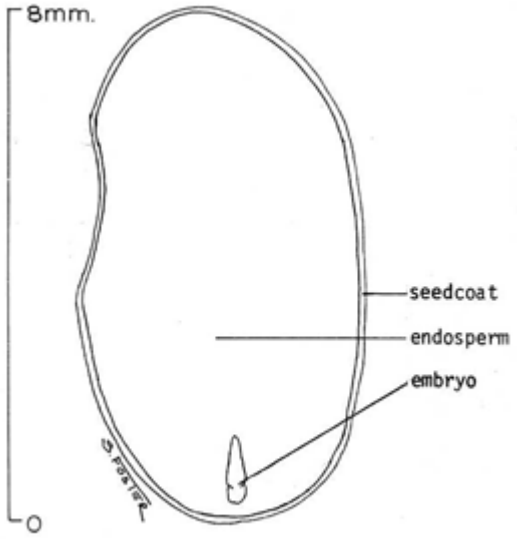


Figure 18:The parts *Washingtonia filifera* seed [56].

Figure 19:Shaded area represents potential planting area [58].

2.4 Characteristics and nutrient content of *Washingtonia filifera* seeds

The characteristics of *W. filifera* seeds from extracts were evaluated. The percentage composition of the seeds is: total carbohydrate is 77.19%, oil is 16.30%, protein content is 3.46%, and 1.37% is ash [62]. The main nutrients (mg/100g of seeds) that are found in the seeds are: calcium 187.85, potassium 67.33, magnesium 34.35 and phosphorus is 23.26 [62]. But recently, there is not any research on phytochemical analysis of *W. filifera*. Moreover, *W. filifera* is characterized by its high content of flavonoids. Mainly present flavonoids are luteolin 7-O-glucoside 4”-sulfate, luteolin 7-O-glucoside 2”-sulfate, and 8-hydroxyisoscoparin [63].

Chapter 3

Materials and Methods

3.1 Collection of plant material

Washingtonia filifera fresh fruits and leaves were collected from Famagusta, the main campus of Eastern Mediterranean University, during October to November 2016. Collected plant samples were washed with water and then washed again with distilled water. After washing process, fruits of *Washingtonia filifera* were separated from the seeds. Then both of the plant materials were dried for 5 days in the oven in 40°C. The seeds of the plant were very rigid to crush and to make powder. Because of that seeds were firstly crushed with stone mill (traditional way of Cypriots) and transferred into mortar. After that, laboratory blender was used to reduce them into powder. Fruits of the plant were reduced to powder by using laboratory blender only. Then both of them stored in airtight closed bottles for analysis.

Figure 20:Traditional Cypriot stone mill and mortar which was used for crushing the plant seeds.

Figure 21:Crushing process of the rigid plant seeds.

Figure 22:Mature plant fruits, their seeds and crushed seeds.

Figure 23:Immature plant fruits and their seeds.

3.2 Phytochemical Analysis

3.2.1 Qualitative Phytochemical Analysis

The phytochemical tests were carried out on the different parts of the plant samples to identify the presence of bioactive components and to identify them based on their abilities of their functional groups. According to phytochemical tests, functional groups will give colour changes or precipitates with specific reagents. The tests are done on the crude samples of plant [70]. Phytochemical analysis of the fruit and seed powder was done by standard methods as follows:

Test for Tannins:

The Tannins test is based on colour changes when iron (III) salts react with tannins [71]. Nearly 0.5 g of each plant sample was stirred with 10 ml of distilled water and then the mixture was filtered. Then few drops of 1% ferric chloride solution were added into 2 ml of each filtrate. A colour change occurs to green, blue-black or blue-green precipitate shows the presence of tannins [72], [73].

Test for Saponins:

One gram from each plant sample was boiled with 5 ml of distilled water and then filtered. 3ml of distilled water was added into the each filtrate and was shaken strongly for about 5 minutes. The test tube that contains mixture must be horizontal while in shaking process. The frothing with warming shows the presence of saponins [72], [74].

Test for Alkaloids:

A small amount of each plant sample was stirred with 5 ml of 1% of aqueous HCl on the water bath and then the mixture was filtered off. From the each filtrate, 1 ml was

taken and separated into 2 different test tubes. In test tube 1, few drops of Dragendorff's reagent were added and formation of orange-red precipitation is taken as positive. In test tube 2, Mayer's reagent was added and formation of buff-coloured precipitation is the evidence for the presence of alkaloids [72], [74].

Liebermann - Burchard test for steroids:

0.2 g of each plant sample was mixed with 2 ml of acetic acid. Then the solution was cooled very well in the ice bath and concentrated H_2SO_4 was further added carefully. A colour change from violet to bluish-green or to blue is the indication of the presence of a steroidal ring [72], [74].

Test for terpenoids:

A little amount from each plant sample was dissolved in ethanol and then filtered. 1 ml of acetic anhydride was added then followed by adding concentrated H_2SO_4. A colour change from pink to violet shows the presence of terpenoids [72], [74].

Borntrager's Test:

Nearly 0.2 g from each plant sample was prepared and 10 ml of benzene was added followed by shaking for a while then was filtered. 5 ml of 10% of ammonia solution was added into the each filtrate and was shaken again. The formation of red, violet or pink colour in the lower (ammoniacal) phase was the evidence of free anthraquinones in the plant sample [72], [74].

Shinoda's test for flavonoids:

0.5 g of each powder sample was dissolved in 5 ml of ethanol then warmed and followed by filtration. A small amount of magnesium chips were added into the

filtrate and then few drops of concentrated HCl were added. An orange, pink or red to purple colour change shows the presence of flavonoids in the plant sample [72].

Flavonoids test with Ferric chloride

0.5 g of each portion was boiled with distilled water and then filtered off. Few drops of 10% ferric chloride solution were added into each 2 ml of the filtrate. A green-blue or violet colour change indicates the presence of a hydroxyl phenolic group [72].

Molisch's test for Carbohydrates:

Molisch's reagent was used in this test. First of all, each plants sample was dissolved in distilled water and few drops of Molisch's reagent were added. Then of 1 ml of concentrated H_2SO_4 was added by the side of the test tube carefully. Then the test tube was left to stand for two minutes after that the mixture was diluted with 5 ml of distilled water. As a result, red colour formation or dull violet colour at the interphase of two layers shows the presence of carbohydrates [72], [74].

3.3 Extraction of Plant Materials

The dried *Washingtonia filifera* plant materials were powdered. Then 100g of powdered mature seeds of plant material, 100g of powdered fruit material and 40g of powdered immature seeds were extracted with three different chemicals. Firstly with hexane, secondly ethyl acetate and last extraction was done with 70% methanol.

Extraction with Hexane

Hexane (C_6H_{14}) is used as an extraction solvent for most of the plant species. Hexane is mainly used to extract edible oils from vegetables and seeds [76]. Hexane is

strongly non-polar [77]. Thus, it is used as a solvent to obtain non-polar substances from plant materials in the extraction process.

Firstly, all plant materials extracted with hexane. 500 ml of hexane was mixed with the plant materials in the conical flasks and then each flask were put to rotary shaker at 40°C for 4 hours, only for mixing by 63 rpm. Then the samples let for 1 hour to supernatant to occur after that all were filtered. Then each flask was let for dryness under the hood. Extract was stored in refrigerator for further use in each analysis [75].

Extraction with Ethyl acetate

Ethyl acetate ($C_4H_8O_2$) which is the second solvent in the extraction process is found in alcoholic beverages [78]. Ethyl acetate is a clear and colourless liquid having a fruity odour. It is used as a solvent in the manufacture of decaffeinated tea or coffee and for the manufacture of modified hop extracts [78].

Secondly, each sample was extracted with 500 ml ethyl acetate for 4 hours and again, extracts were filtered and stored. Extract was stored in refrigerator for further use in each analysis [75].

Extraction with 70% Methanol

Methanol (CH_4O) is the last solvent. Methanol is a colourless, volatile, flammable and poisonous liquid which is used in the production of acetic acid and formaldehyde and used as a solvent [79]. It is used as a solvent and in chemical synthesis methanol is used as an intermediate [79]. It is a polar solvent [77]. Thus, methanol is used as a

solvent to obtain polar substances from plant materials in the last part of the extraction process. The percentage of methanol which was used is 70%.

The third step was applied which was extracting the samples with 70% of methanol for 4 hours in rotary shaker. All extracts were stored in refrigerator for further use in each analysis [75].

When all extraction processes were done, samples stored for one day in the freezer for further usage. Next day, all the sample extracts were evaporated by using rotary evaporator in 45°C and in 47 rpm. After evaporation process all dried samples were transferred into small tubes. They are stored in the freezer for antibacterial and antioxidant activity analysis.

3.4 Total phenolic determination

The total phenols were determined by using Gallic acid equivalents which is Folin-Ciocalteu method [80]. Each extract sample was diluted as 3mg/mL and 6mg/mL with appropriate solvent. Then, 3.0 mL H_2O, 50 µL of appropriate concentration of sample were transferred into a test tube. Then 250 µL undiluted Folin-Ciocalteu reagent was added. After 1 or 2 minutes, 750 µL 20% (w/v) Na_2CO_3 were added and then 950 µL of H_2O was added to make the volume 5.0 mL in total. After 1 hour incubation at 25°C, the absorbance was measured at 760nm [80]. This process was applied to each extracts of the plant sample. All the samples were assayed in triplicate.

3.5 Antioxidant activity determination tests

3.5.1 Total antioxidant activity by 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic /acid) ($ABTS^{+\bullet}$) radical cation decolourization assay/radical scavenging activity assay

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) is a colourless dianion salt of sodium or ammonium that can form a colourful cationic radical under the oxidation of $K_2S_2O_8$ at the room temperature [81]. Then, the mixture is diluted by ethanol to give an absorbance between 0.70±0.02 which defined as the reference absorbance (A_{ref}) [81]. A_{ref} decreases to a stable value which is named as A_{detect} when $ABTS^{+\bullet}$ is mixed with an antioxidant. The percentage of an antioxidant is calculated by (1 – A_{detect}/A_{ref}) x 100 [81]. And the results from other antioxidants which reduce $ABTS^{+\bullet}$ are compared with Trolox which is expressed as the Trolox equivalent antioxidant capacity (TEAC). Trolox is assigned as reference antioxidant to interact with $ABTS^{+\bullet}$ [81]. This method performs the inhibition of the absorbance of the $ABTS^{+\bullet}$ radical cation by antioxidants [82].

The total antioxidant activity of *W. filifera* was measured by decolourization of $ABTS^{+\bullet}$ radical cation using the method. This method is applicable for both hydrophilic and lipophilic antioxidants, including flavonoids, carotenoids, and plasma antioxidants [83]. Each extract were diluted as 0.3 mg/mL and 0.6 mg/ml of solutions. The $ABTS^{+\bullet}$ radical is generated by $ABTS^{+\bullet}$ solution with ethanol and potassium persulfate ($K_2S_2O_8$). This solution was stand in dark place for 12-16 hours and then the absorbance of that solution was arranged to 734nm between 0.700-0.800 at room temperature. The radical solution was prepared according to this procedure. After that 990 µL from the radical solution was mixed with 10 µL of each extract

solution. The reaction kinetics was measured for 30 minutes with 1 minute intervals in 734nm by using by using T90+ UV/VIS spectrometer PG Instruments. All the samples were assayed in triplicate. Butylated hydroxytoluene (BHT) was as standard control.

3.5.2 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) Radical Scavenging Activity assay

DPPH$^{\bullet}$ is a stable free radical and is always used to inspect the radical scavenging properties of antioxidants [81], [82]. DPPH$^{\bullet}$ is dissolved in ethanol to result in an absorbance around 1.0 at 517 nm and the addition of antioxidants decrease the absorbance of the DPPH$^{\bullet}$ solution to a stable value [81].

The ability of the *W. filifera* plant extracts to scavenge DPPH$^{\bullet}$ radicals was determined by the method of Gyamfi et al. [84]. Tris-HCl buffer (50mM, pH 7.4) and 1mL of 0.1mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) in Methanol were mixed with 50 μL of each extracts. Extracts were diluted into appropriate concentrations with the 70% methanol. Then the mixture was incubated in dark place for 30 minutes at room temperature. The resultant absorbance was measured at 517nm by using PerkinElmer UV/VIS Spectrometer Lambda 25. The percentage inhibition was calculated by using estimated IC_{50} values. If an antioxidant shows a low value of IC_{50} that indicates a strong radical scavenger [81]. All the samples were assayed in triplicate. Butylated hydroxytoluene (BHT) was as standard control.

3.6 Determination of antibacterial activity of the plant extracts

Test organisms

Test microorganisms were: Gram positive bacteria are *Staphylococcus aureus.* Gram negative bacteria are *Escherichia coli*. All microorganisms used in the study were

obtained from Department of Microbiology, Faculty of Pharmacy, Eastern Mediterranean University, Famagusta, North Cyprus.

Preparation of Nutrient Broth Medium

25 g of nutrient broth powder dissolved in 1000 mL of distilled water. pH was arranged to 7.2±0.1 and then sterilised by using autoclaving at 10lbs pressure and 115°C for 30 minutes [85]. Then all bacteria types were transferred into nutrient broth medium in different test tubes for further use in agar well diffusion method.

Preparation of sample extract for antibacterial assay

All the plant samples were firstly dissolved in Dimethyl sulfoxide (DMSO) as 80 mg/mL. Then, diluted as 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL and 2.5 mg/mL by taking 5 mL from previous solution and adding 5 mL of sterile physiological salty water to each solution.

Agar well diffusion method

The Mueller-Hinton agar was used in this method. Agar surface was streaked by a sterile cotton swab with the reference bacterial strain that were grown in nutrient broth agar. This process was done for each plant sample extract. Agar plates were punched with a sterile glass rod of 6 mm size and 50 µL of each sample was poured with micropipette in the bores. Then all the plates were incubated at 37°C for 24h.

Chapter 4

RESULTS AND DISCUSSION

4.1 The Results of Qualitative Phytochemical Analysis

The *W. filifera* plant samples were extracted sequentially with hexane, ethyl acetate and 70% of methanol by using rotary evaporator. The results of phytochemical analysis of all plant parts are represented in Table 6.

The results of qualitative phytochemical analysis were presented in Table 1, which showed that crude powder of fruit and seeds of *W. filifera*. The phytochemical analysis revealed the presence of carbohydrates, flavonoids, saponins and tannins in all investigated parts of the plant. The crude fruit powder contains carbohydrates, flavonoids, saponins, tannins, and terpeneoids but not alkaloids and anthraquinones. On the other hand crude seed powders of the plant contain carbohydrates, flavonoids, saponins, tannins, and terpenoids but again not alkaloids and antraquinones. The comparison between in mature and immature seeds were done, the results were almost the same. These phytochemical results revealed that, there is not huge difference between mature and immature seeds of *W. filifera*. The presence of mentioned phytochemicals like flavonoids suggest that *W. filifera* may possess potential antibacterial activities against some pathogenic bacteria.

These results are only qualitative analysis about phytochemicals which give results due to colour changes. There is not any analysis about phytochemicals in *W. filifera*

to compare the results. However, there is a research about polyphenolic compound determination of *Washingtonia robusta* H. Wendl which is the second species of *Washingtonia* genus. According to phytochemical screening of *W. robusta* roots, rachis and leaflets, all the parts have flavonoids, tannins and polyphenols but not alkaloids [86]. The results of phytochemical analysis on both species are almost the same. Depending on the high flavonoid contents of all the parts, the antibacterial and antioxidant activities can be detected in *W. filifera*.

Table 6:Phytochemical analysis results on the crude powder of plant sample.

	Constituent	**Immature Fruit**	**Mature Fruit**	**Immature fruit's seed**	**Mature fruit's seed**
1	Alkaloids	**-**	**-**	**-**	**-**
2	Carbohydrates	**+**	**++**	**+**	**++**
3	Flavonoids	**++**	**+**	**++**	**++**
4	Saponins	**+**	**+**	**+**	**+**
5	Steroids	**-**	**-**	**-**	**-**
6	Tannins	**+**	**+**	**++**	**++**
7	Terpenoids	**-**	**-**	**++**	**++**
8	Anthraquinones	**-**	**-**	**-**	**-**

++= highly detected; + = detected; - = not detected

4.2 Total Phenols Test Results on *Washingtonia filifera* Plant Extracts

The total phenolic results of *W. filifera* fruit and seeds are represented in the Table 7,8 and 9. Immature seeds of *W. filifera* showed phenolic content with the extraction of Ethyl acetate (23.3±2.0 mg/g) which is the highest content of all extracts. Mature seeds of *W. filifera* extracted with 70% Methanol (18.5±0.1 mg/g) revealed high phenolic content as well. On the other hand, immature seeds were showed high phenolic content (16.7±0.3 mg/g) with the extraction of 70% Methanol which is the third highest phenolic content of the extracts. The fruits of *W. filifera* did not show

high phenolic content in contrast to seeds. If whole results were compared, extraction with Ethyl acetate of immature and mature seeds and mature fruits showed more phenolic content than all others.

Table 7:Total Phenol results from extracts of *W. filifera* Immature seeds.

		Total Phenol ($mg_{GAE}/g_{extract}$)
	Yield	*W. filifera* Immature seeds
(A)	56	0.58±0.2
(B)	20	23.3±2.0
(C)	43	16.7±0.3

(A): Hexane; (B): Ethyl acetate; (C): 70% Methanol. Values (mg/g) are expressed as means ± standard deviation. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material.

Table 8:Total Phenol results from extracts of *W. filifera* Mature seeds.

		Total Phenol ($mg_{GAE}/g_{extract}$)
	Yield	*W. filifera* Mature seeds
(A)	64	4.7±0.8
(B)	17	8.7±0.3
(C)	74	18.5±0.1

(A): Hexane; (B): Ethyl acetate; (C): 70% Methanol. Values (mg/g) are expressed as means ± standard deviation. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material.

Table 9:Total Phenol results from extracts of *W. filifera* Mature fruits.

		Total Phenol	**($mg_{GAE}/g_{extract}$)**
	Yield		*W. filifera* Mature fruits
(A)	15		1.3±0.2
(B)	10		6.9±0.5
(C)	102		1.5±0.05

(A): Hexane; (B): Ethyl acetate; (C): 70% Methanol. Values (mg/g) are expressed as means ± standard deviation. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material. Extract yields expressed as milligrams of extract per gram (dry weight) of aerial material.

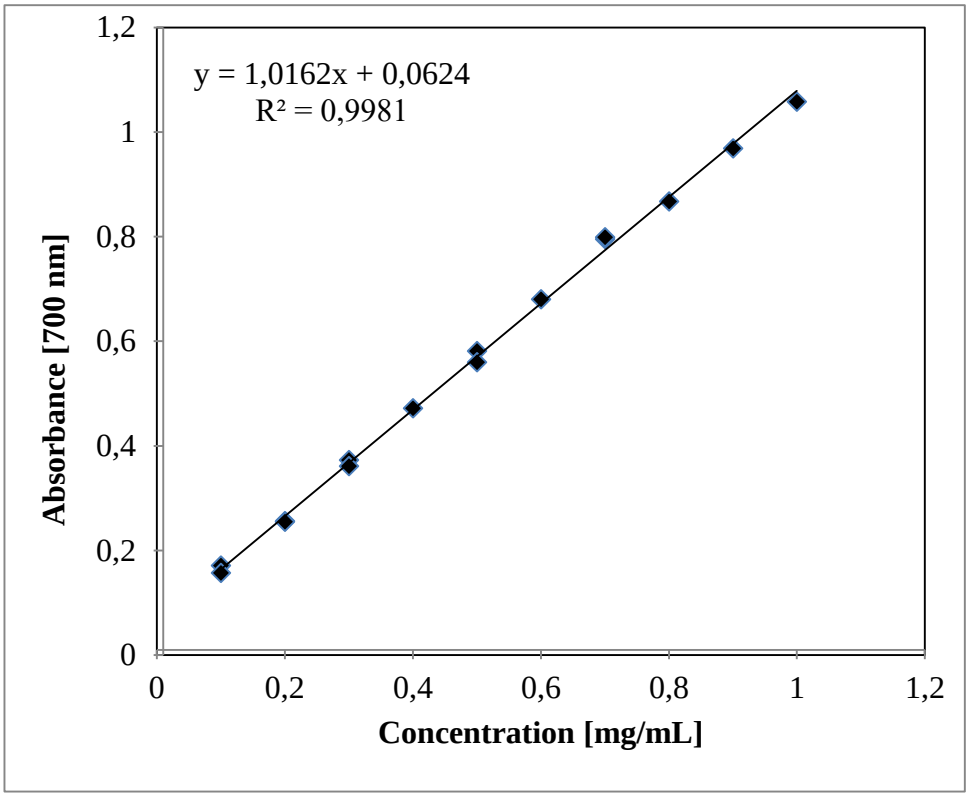


Figure 24:Calibration curve of gallic acid for total phenol assay.

4.3 Antioxidant Activity Analysis Results on *Washingtonia filifera* Plant Sample Extracts

4.3.1 $ABTS^{+\bullet}$ radical scavenging activities of *W. filifera* Extracts

The $ABTS^{+\bullet}$ radical scavenging activities results are represented in Figure 24. All the Hexane extracts could not reach the BTH. This is why, they were found inactive in $ABTS^{+\bullet}$ radical scavenging activity. H2A and H2C which were ethyl acetate extracts were found active but H2B was a little less active than BHT. In 70% Methanol extracts, H3A and H3C were found more active but H3B were not detected may be due to a mistake or contamination.

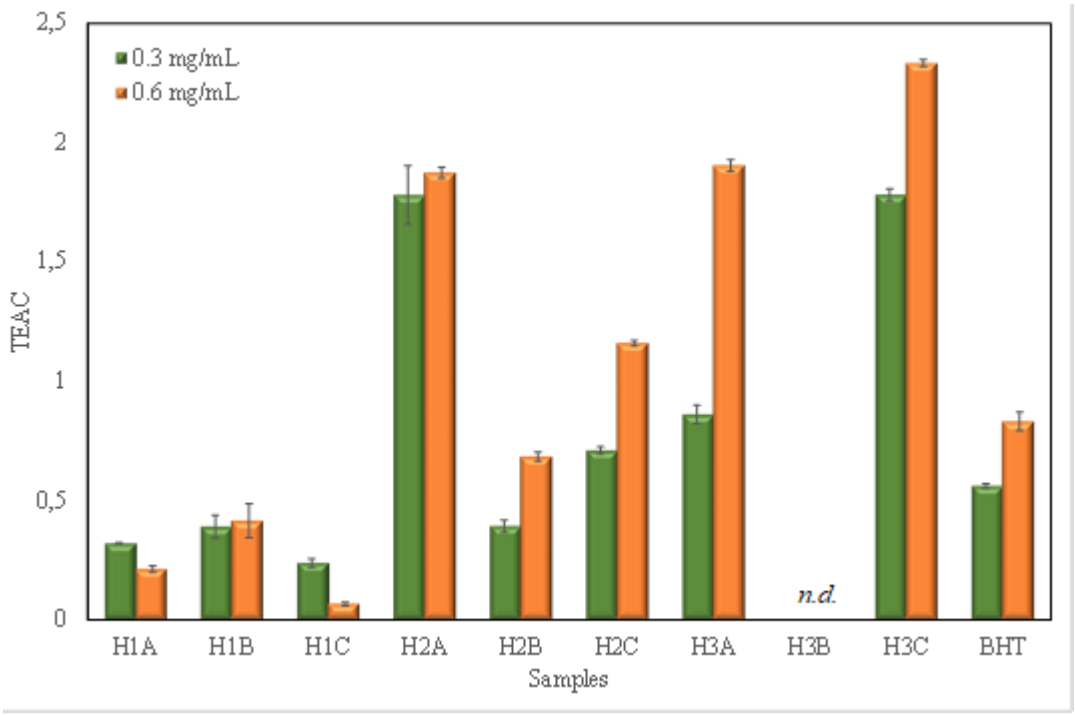


Figure 25:$ABTS^{+\bullet}$ radical scavenging activities of *W. filifera* Extracts.
(n.d) not detected, (BHT) Butylated hydroxytoluene as a control, (H1A) Hexane extract of immature seed, (H1B) Hexane extract of mature fruit, (H1C) Hexane extract of mature seed, (H2A) Ethyl acetate extract of immature seed, (H2B) Ethyl acetate extract of mature fruit, (H2C) Ethyl acetate extract of mature seed, (H3A) 70% Methanol extract of immature seed, (H3B) 70% Methanol extract of mature fruit, (H3C) 70% Methanol extract of mature seed.

4.3.2 DPPH• scavenging activities of *W. filifera* Extracts

The result of DPPH• scavenging activity assay is represented in figure 25. Hexane extracts (H1A, H1B and H1C) were found inactive in DPPH• scavenging activity which means they are not detected. H2A and H2C which were ethyl acetate extracts did not show a good activity. Because if an antioxidant shows a low value than BHT which is a reference, that indicates a strong radical scavenger. H2B which was ethyl acetate extract of mature fruit was found as the most active of all extracts, it showed less activity than BHT. Methanol (70%) extracts (H3A, H3B, H3C) did not give a good activity as well. But H3A showed a little less activity than BHT. Most of the extracts did not reveal a good activity in certain concentration which was 0.1 mg/mL, except H2B which was ethyl acetate extract of mature fruit.

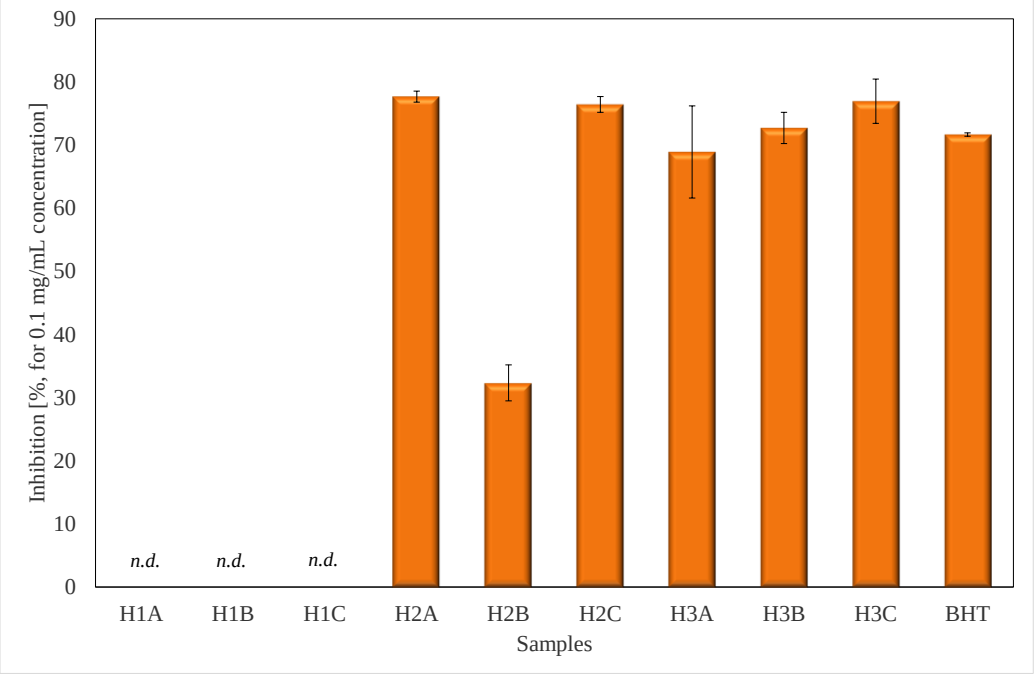


Figure 26:DPPH• scavenging activity assay for 0.1 mg/mL concentration.
(n.d.) not detected, (BHT) Butylated hydroxytoluene as a control, (H1A) Hexane extract of immature seed, (H1B) Hexane extract of mature fruit, (H1C) Hexane extract of mature seed, (H2A) Ethyl acetate extract of immature seed, (H2B) Ethyl acetate extract of mature fruit, (H2C) Ethyl acetate extract of mature seed, (H3A) 70% Methanol extract of immature seed, (H3B) 70% Methanol extract of mature fruit, (H3C) 70% Methanol extract of mature seed.

4.4 Antibacterial activity of various extracts of different parts of *Washingtonia filifera* against bacterial species tested by agar well diffusion

The antibacterial activity results are represented in Tables 10, 11 and 12. There were three different extracts which were hexane, ethyl acetate, 70% methanol for each plant sample. It was detected that all extracts did not show any activity against *E. coli* Gram-negative pathogenic bacteria. H1A, H1B, H1C, H2B and H3B were revealed no activity against *S. aureus* Gram-positive pathogenic bacteria. H2A which was ethyl acetate extract of immature seeds were revealed 11 mm zone inhibition against *S. aureus* Gram-positive bacteria at 80 mg/mL highest concentration (Table 10 and Fig.27). H3A which was 70% methanol extract of immature seeds were revealed 13 mm zone inhibition against *S. aureus* Gram-positive at 80 mg/mL highest concentration (Table 10).

Mature seeds which was extracted with ethyl acetate, H2C were revealed 13 mm zone inhibition against *S. aureus* Gram-positive at 80 mg/mL highest concentration (Table 11 and Fig.27). H3C, the mature seeds which were extracted with 70% methanol were revealed the highest inhibition zone of all extracts, 17 mm at 80 mg/mL (Table 11 and Fig. 28).

All extracts did not show activity to *E. coli* and some extracts did not show any activity to *S. aureus* as well. The reason that activity did not observed on these extracts is can be due to a contamination that have occurred during the experimental process.

The highest inhibition was 17 mm at 80 mg/mL concentration. In general, ethyl acetate and methanol (70%) extracts revealed antibacterial activity against only to Gram-positive *S. aureus* test microorganism. So, ethyl acetate and 70% methanol extracts were found to be more effective antimicrobial agents than the hexane extracts.

Table 10:Zone Inhibition of Different Concentrations of Immature plant seed extracts against *S. aureus* and *E. coli.*

	Hexane Extracted Immature Seed (H1A)						**Ethyl acetate Extracted Immature Seed (H2A)**						**70% Methanol Extracted Immature Seed (H3A)**					
Concentration (mg/mL)	2.5	5	10	20	40	80	2.5	5	10	20	40	80	2.5	5	10	20	40	80
Concentration (mg/mL)*S. aureus* (mm)	0	0	0	0	0	0	0	0	0	8	8	11	7	9	10	11	12	13
Zone of inhibition for *E. coli* (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(H1A) Hexane extract of immature seed, (H2A) Ethyl acetate extract of immature seed, (H3A) 70% Methanol extract of immature seed.

Table 11:Zone Inhibition of Different Concentrations of Mature plant seed extracts against *S. aureus* and *E. coli.*

	Hexane Extracted Mature Seed (H1C)						**Ethyl acetate Extracted Mature Seed (H2C)**						**70% Methanol Extracted Mature Seed (H3C)**					
Concentration (mg/mL)	2.5	5	10	20	40	80	2.5	5	10	20	40	80	2.5	5	10	20	40	80
Zone of inhibition for *S. aureus* (mm)	0	0	0	0	0	0	0	0	8	10	11	13	0	0	13	15	16	17
Zone of inhibition for *E. coli* (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(H1C) Hexane extract of mature seed, (H2C) Ethyl acetate extract of mature seed, (H3C) 70% Methanol extract of mature seed.

Table 12:Zone Inhibition of Different Concentrations of Mature plant fruit extracts against *S. aureus* and *E. coli*.

	Hexane Extracted Mature Fruit (H1B)						**Ethyl acetate Extracted Mature Fruit (H2B)**						**70% Methanol Extracted Mature Fruit (H3B)**					
Concentration (mg/mL)	2.5	5	10	20	40	80	2.5	5	10	20	40	80	2.5	5	10	20	40	80
Zone of inhibition for *S. aureus* (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zone of inhibition for *E. coli* (mm)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(H1B) Hexane extract of mature fruit, (H2B) Ethyl acetate extract of mature fruit, (H3B) 70% Methanol extract of mature fruit.

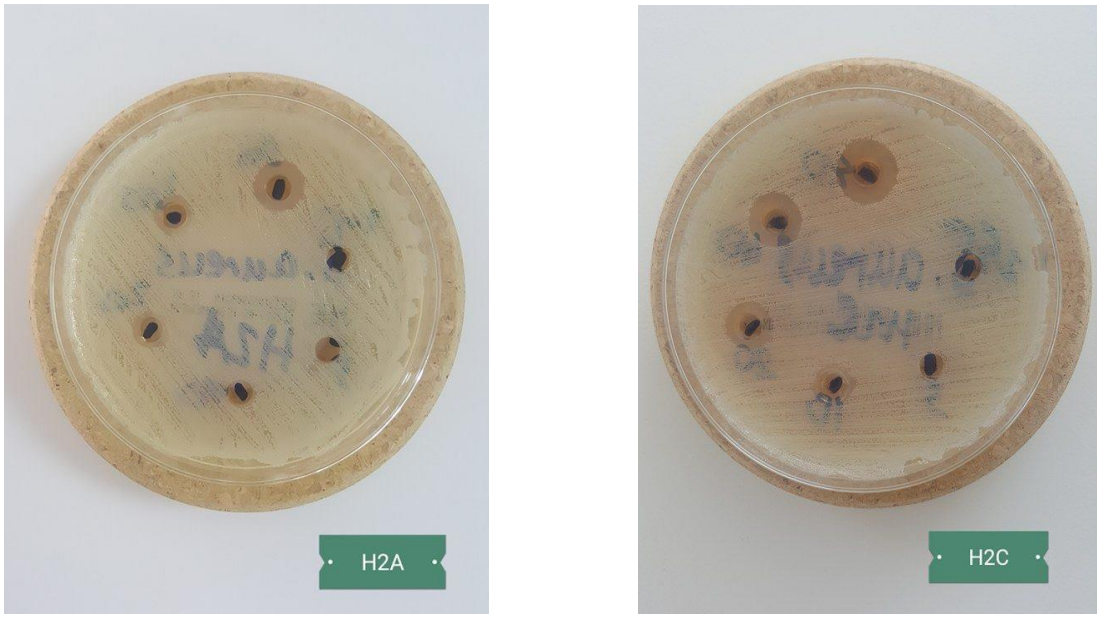
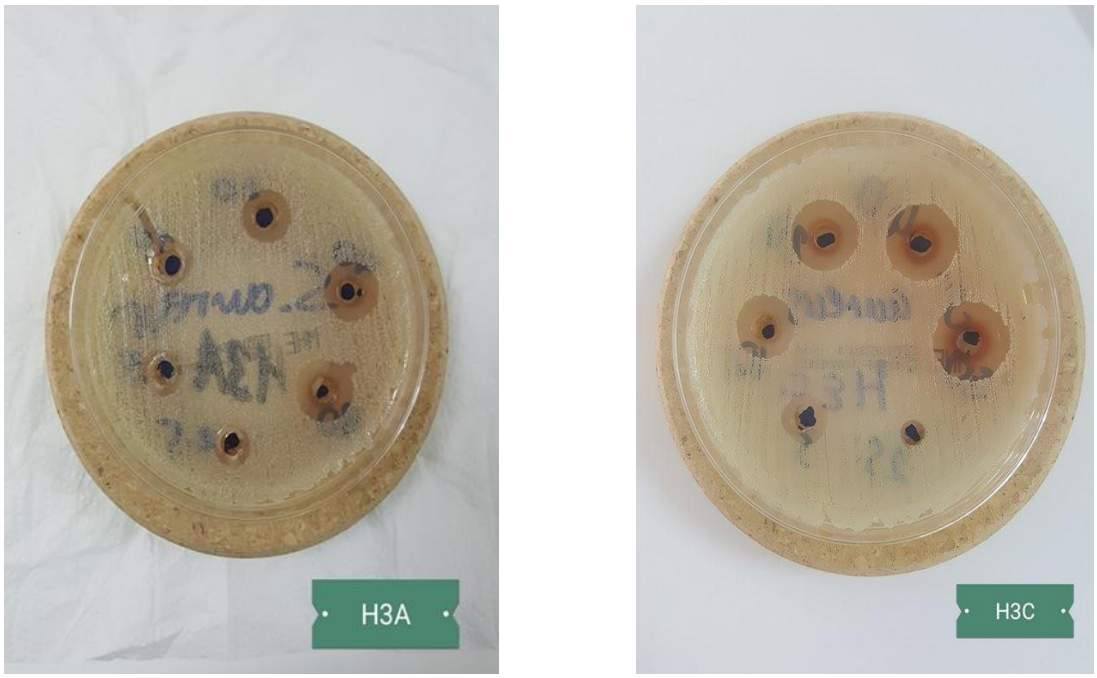


Figure 27:The results of Antibacterial Activity of H2A and H2C.
1: H2A Ethyl acetate extract of immature seed, 2: H2C Ethyl acetate extract of mature seed.



3 **4**

Figure 28:The results of Antibacterial activity of H3A and H3C.
3: H3A 70% Methanol extract of immature seed, 4: H3C 70% Methanol extract of mature seed.

CONCLUSION

It can be concluded that some extracts of seed and fruit extracts of *Washingtonia filifera* possess antibacterial and antioxidant activity.

The results also suggest that the fruit of *Washingtonia filifera* can serve as potential source of bioactive healthy compounds in the diet and the consumption could be useful in the prevention of some of the diseases. However, toxicity tests on the fruits and seeds are required for diet consumption.

Relaying upon the results that are obtained from seeds and fruits of *Washingtonia filifera*, possess antibacterial and antioxidant activities. Further research is needed for the identification and isolation of more active principles which are present in the extracts that could be used for pharmaceutical use. It may be concluded that the active antioxidant and antibacterial compounds can be isolated and could be useful in treating diseases like infections and oxidative stress related diseases. However, a better study would be needed to extrapolate laboratory results into hospital settings.

REFERENCES

[1] Motaleb, M.A. (2011). *Selected medicinal plants of Chittagong Hill Tracts*,IUCN(International Union for Conservation of Nature), Dhaka, Bangladesh, pp xii + 116.

[2] Alviano, D.S., Alviano, C.S. (2009). *Plant extracts: search for new alternatives to treat microbial diseases*. Current Pharmaceutical Biotechnology, 106-21.

[3] Infection. *Journal of Infectious Diseases & Therapy*, 2332-0877. Retrieved from https://www.esciencecentral.org/journals/infectious-diseases-and-therapy.php

[4] Khan, U. A., Rahman, H., Niaz, Z., Qasim, M., Khan, J. T., Rehman, B. (2013). Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. *European Journal of Microbiology & Immunology*, 272–274. doi: 10.1556/EuJMI.3.2013.4.6

[5] Zhang, R., Eggleston, K., Rotimi, V., and Zeckhauser, R.J. (2006). *Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States*. Global Health, doi:10.1186/1744-8603-2-6

[6] Paul, C., Chikezie, Ibegbulem, C. O. and Mbagwu F.N. (2015). Bioactive Principles from Medicinal Plants. *Research Journal of Phytochemistry*, 9 (3): 88-115.

[7] Okwu, D.E. (2005). Phytochemicals, Vitamins and Mineral Contents of Two Nigerian Medicinal Plants. *International Journal of Molecular Medicine and Advance Sciences*, 1(4): 375-381.

[8] Shakya, A. K. (2016). Medicinal plants: Future source of new drugs. *International Journal of Herbal Medicine*, 4(4): 59-64

[9] Yadav, R.N.S., and Agarlawa, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology*, Dibrugarh University, India, 3(12): 10-14.

[10] Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, Vol. 113, Issue 9, Supplement 2, Pages 71–88.

[11] Irchhaiya, R., Kumar, A., Yadav, A., Gupta, N., Kumar S., Kumar, S., Yadav, V., Prakash, A. and Gurjar, H., (2014). Metabolites in Plants and Its Classification. *World Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 4, Issue 1, 287-305.

[12] Liu, R. H. (2003). *Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals*. American Society for Clinical Nutrition, Vol. 78, no.3 517S-520S.

[13] Ördög, V., Zoltán, M. (2011). *Secondary metabolites in plant defences*. Plant Physiology. Retrieved from http://www.tankonyvtar.hu/en/tartalom/tamop425/0010_1A_Book_angol_01_novenyelettan/ch03s05.html

[14] Fattorusso, E., and Taglialatela-Scafati, O. (2007). *Bioactive Alkaloids: Structure and Biology and Ecological Roles of Alkaloids. Modern Alkaloids.* WILEY-VCH Verlag GmbH & Co. KGaA.

[15] *Morphine*. Compound Summary for CID 5288826. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/morphine#section=2D-Structure

[16] *Andrographolide*. Compound Summary for CID 5318517. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Andrographolide#section=2D-Structure

[17] *Resveratrol*. Compound Summary for CID 445154. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/resveratrol#section=2D-Structure

[18] *Diosgenin*. Compound Summary for CID 99474. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/diosgenin#section=2D-Structure

[19] *Artemisinin*. Compound Summary for CID 452191. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/452191#section=2D-Structure

[20] *Rhein*. Compound Summary for CID 10168. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/Rhein#section=2D-Structure

[21] Vermerris, W., and Nicholson, R. (2006). *Phenolic Compound Biochemmistry*. Springer, Dordrecht, Netherlands.

[22] Kozłowska, A., Szostak-Węgierek, D. (2014). *Flavonoids-Food Sources and Health Benefits.* Rocz Panstw Zakl Hig, 65(2):79-85.

[23] Stalikas, C.D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*, Doi: 10.1002/jssc.200700261.

[24] Tomás-Barberán, F.A., and Gil., M.I. (2008). *Improving the Health-Promoting Properties of Fruit and Vegetable Products*. Woodhead Publishing Limited, Cambridge, England.

[25] Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). *Polyphenols: food sources and bioavailability*. American Society for Clinical Nutrition, vol. 79 no. 5 727-747.

[26] Hollman, P.C.H., and Arts, I.C.W. (2000). Flavonols, flavones and flavanols – nature,occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, 80:1081±1093.

[27] Kwang-II Parka, Hyeon-Soo Parkb, Mun-Ki Kimb, Gyeong-Eun Hongb, Nagappanb, A., Ho-Jeong Leeb, Yumnamb, S., Won-Sup Leec, Chung-Kil Wonb, Sung-Chul Shind, Gon-Sup Kim. (2014). Flavonoids identified from Korean Citrus aurantium L. inhibit Non-Small Cell Lung Cancer growth in vivo and in vitro. *Journal of Functional Foods*, 287 – 297.

[28] Fraga, C.G. (2010). *Flavonoids-Structure and Their Dietary Occurrence*. Phenolics and Human Health, Biochemistry, Nutrition, and Pharmacology. A John Wiley & Sons, Inc., Publication.

[29] Kumar, S., and Pandey, A.K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. Hindawi Publishing Corporation, *The Scientific World Journal*, Article ID 162750.

[30] Breitmaier, E. (2006). *Terpenes: Importance, General Structure, and Biosynthesis.* Wiley-VCH Verlag GmbH & Co. KGaA, Tübingen, Germany, Doi: 10.1002/9783527609949.ch1.

[31] Zeiger., L., and Taiz E., (2003). *Plant physiology*. Annals of Botany, 91(6): 750–751, doi:10.1093/aob/mcg079.

[32] Kennedy, D.O., and Wightman, E.L., (2011). Herbal Extracts and Phytochemicals: Plant Secondary Metabolites and the Enhancement of Human Brain Function. *Advances in Nutrition an International Review of Journal*, vol. 2: 32-50, doi: 10.3945/an.110.000117.

[33] Bernhof, A. (2010). *Bioactive compounds in plants – benefits and risks for man and animals*. The Norwegian Academy of Science and Letters, Novus forlag, Oslo.

[34] *Phytochemicals, Saponins*. Retrieved from http://www.phytochemicals.info/phytochemicals/saponins.php

[35] Yazdani, D., Tan, Y.H., Zainal Abidin, M. A., and Jaganath, I. B. (2011). A review on bioactive compounds isolated from plants against plant pathogenic fungi. *Journal of Medicinal Plants Research*, Vol. 5(30), pp. 6584-6589, Doi:10.5897/JMPR11.485

[36] *The Healing Power of Essential Oils*. *Aromatic Intelligence*. Retrieved from http://www.floracopeia.com/site/pdf/Floracopeia-Essential-Oil-eBook.pdf

[37] Dillard, C.J. and Bruce German, B.J. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture*, Doi:10.1002/1097-0010(20000915)80:12<1744::AID-JSFA725>3.0.CO;2-W.

[38] Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E. Hilpert, K.F., Griel, A.E. and Etherton, T.D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, Vol. 113, Issue 9, Supplement 2, Pages 71–88.

[39] Scalbert. A., Manach, C., Morand, C., Rémésy, C., Jiménez L. (2005). *Dietary polyphenols and the prevention of diseases.* Critical Reviews in Food Science and Nutrition, Vol. 45, Issue 4, Pages 287-306.

[40] Xiao, Z.P., Peng, Z.Y., Peng, M.J., Yan, W.B., Ouyang, Y.Z., Zhu, H.L. (2011). *Flavonoids Health Benefits and Their Molecular Mechanism*. Mini Reviews in Medicinal Chemistry, Vol. 11, Number 2, pp. 169-177.

[41] Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, 47(10):3954-62.

[42] Rates, S.M.K. (2000). *Plants as source of drugs.* Toxicon 39, 603–613.

[43] *Succes Story - Taxol® (NSC 125973)*. (1991). Developmental Therapeutics Program. Retrieved from https://dtp.cancer.gov/timeline/flash/success_stories/s2_taxol.htm

[44] Frusciantea, L., Baroneb, A., Carputob, D., Ercolanoa, M.R., Francesco della Roccaa, and Esposito, S. (2000). *Evaluation and use of plant biodiversity for food and pharmaceuticals*. Fitoterapia, Vol. 71, Supplement 1, Pages S66–S72.

[45] Handa, S.S., Rakesh, D.D., and Vasisht, K. (2006). *The Status of Medicinal and Aromatic Plants, Compendium of Medicinal and Aromatic Plants ASIA*. United Nations Industrial Development Organization and the International Centre for Science and High Technology.

[46] Khan, M.A. (2016). *Introduction and Importance of Medicinal Plants and Herbs*. National Health Portal. Retrieved from https://www.nhp.gov.in/introduction-and-importance-of-medicinal-plants-and-herbs_mtl

[47] Hassan, W., Noreen, H., Rehman, S., and Gul, S. (2016). *Antimicrobial Efficacies, Antioxidant Activity and Nutritional Potentials of Trachyspermum ammi*. Vitamins & Minerals, 5:145. doi: 10.4172/2376-1318.1000145.

[48] Lobo, V., Patil, A., and Chandra, N. (2010). *Free radicals, antioxidants and functional foods*: Impact on human health. Pharmacognosy Reviews, 4(8): 118–126.

[49] *Flora of Cyprus*. (2004-2017). Department of Forests. Retrieved from http://www.moa.gov.cy/moa/fd/fd.nsf/DMLflora_en/DMLflora_en?OpenDocument#1

[50] Georgiou, G., and Gavrilides, A. *The cultivation of aromatic and medicinal plants in Cyprus.* Medicinal, Culinary and Aromatic Plants in the Near East, Deparment of Agriculture.

[51] Zong lü ke, Shengji, P., Sanyang, C., Lixiu, G., and Henderson, A. (1991). *ARECACEAE (PALMAE).* Fl. Reipubl. Popularis Sin. 13(1): 1–172.

[52] Baill, J.C. (Mal.). *Arecaceae (Palmae)*. Retrieved from http://www.dipbot.unict.it/palms/Arec_fam.html

[53] *Classification for Kingdom Plantae Down to Family Arecaceae*. Plants Database. *United States Department of Agriculture (USDA).* Retrieved from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=Arecaceae

[54] *Classification for Kingdom Plantae Down to Genus Washingtonia H. Wendl.* Plants Database. *United States Department of Agriculture (USDA).* Retrieved from https://plants.usda.gov/java/ClassificationServlet?source=display&classid=WASHI

[55] El-Sayed, N.H., Ammar, N.M., Al-Okbi, S.Y., Abou El-Kassem, L.T. and Mabry, T.J. (2006). *Antioxidant activity and two new flavonoids from Washingtonia filifera*. Natural Product Research, Formerly Natural Product Letters ;20(1):57-61.

[56] *Washingtonia filifera (Linden ex André) H. Wendl. - California fan palm.* United States Departmnet of Agriculture-National Resources Conservation Service PLANTS Database, Image Gallery. Retrieved from https://plants.usda.gov/core/profile?symbol=WAFI

[57] Horward, J.L. (2017). *Botanical and Ecological Characteristics-Species: Washingtonia filifera.* Index of Species Information, United States Department of Agriculture.

[58] Watson, E.F.G., and Dennis, G. (1994) *Washingtonia filifera*. Fact Sheet ST-669. Retrieved from http://hort.ufl.edu/database/documents/pdf/tree_fact_sheets/wasfila.pdf

[59] Starr, F., Starr, K., and Loope, L. (2003). *Washingtonia spp. Mexican fan palm and California fan palm Arecaceae*. United States Geological Survey--Biological Resources Division, Haleakala Field Station, Maui, Hawai'i. Retrieved from http://www.hear.org/Pier/pdf/pohreports/washingtonia_spp.pdf

[60] Miller, V.J. (1983). *Desert Plants*. The University of Arizona (Tucson, A.Z.), Vol. 5, Number 3.

[61] *Washingtonia filifera. Plants and Flowers*, Comprehensive Plants and Flowers Database. Retrieved from http://www.plantsrescue.com/washingtonia-filifera/.

[62] Nehdi, I.A. (2011). *Characteristics and composition of Washingtonia filifera (Linden ex André) H. Wendl. seed and seed oil*. Food Chemistry Vol. 126, Issue 1, Pages 197-202.

[63] Hemmati, A.A., Kalantari, H., Siahpoosh, A., Ghorbanzadeh, B., Jamali, H. (2014). *Anti-inflammatory Effect of Hydroalcoholic Extract of the Washingtonia filifera Seeds in Carrageenan-Induced Paw Edema in Rats*. Jundishapur J Nat Pharm Prod. 10(1): e19887, Doi: 10.17795/jjnpp-19887.

[64] Murray, P.R., Rosenthal, K.S., and Pfaller, M.A. (2016). *Microbial Disease*. Elsevier.

[65] Mahady, G.B. (2005). *Medicinal Plants for the Prevention and Treatment of Bacterial Infections*. Current Pharmaceutical Design, 11: 2405-2427.

[66] *Bacterial Infections.* Journal of Bacteriology & Parasitology. Retrieved from https://www.omicsonline.org/scholarly/bacterial-infections-journals-articles-ppts-list.php

[67] Amils, R., Cernicharo Quintanilla, J., Cleaves, H.J., Irvine, W.M., Pinti, D., Viso, M. (2011). *Encyclopedia of Astrobiology*, pp 685-685

[68] Baron, S. (1996). *Medical Microbiology.* University of Texas Medical Branch at Galveston, Galveston, Texas.

[69] Maged, N.Q.A., and Abbas, N.A. (2013). *Antibacterial activity of Phoenix dactylifera L. leaf extracts against several*. Kua Journal for Veterinary Medical Sciences, Vol.4 No.2, 45-50.

[70] Perveen, K., Bokhari, N.A., and Soliman, D.A.W. (2012). Antibacterial activity of Phoenix dactylifera L. leaf and pit extracts against selected Gram negative and Gram positive pathogenic bacteria. *Journal of Medicinal Plants Research*, Vol. 6(2), pp. 296-300.

[71] Nwokonkwo, D.C. (2014). The Phytochemical Study and Antibacterial Activities of the Seed Extract. *American Journal of Scientific and Industrial Research*, doi:10.5251/ajsir.2014.5.1.7.12

[72] Usman, H., Abdulrahman, F.I. and Usman, A. (2009). *Qualitative Phytochemical Screening and In Vitro Antimicrobial Effects of Methanol Stem Bark Extract of Ficus Thonningii (Moraceae)*. Afr J Tradit Complement Altern Med. 6(3): 289–295.

[73] Trease, G.E., Evans, W.C. (2002). *Pharmacognosy*. Saunders Publishers, London.

[74] Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, Vol. 2 Issue 3: 365-372.

[75] Koşar, M., Göger, F., Can Başer, K.H. (2008). In vitro antioxidant properties and phenolic composition of Salvia virgata Jacq. from Turkey. *Journal of Agricultural and Food Chemistry,* 56 (7), pp 2369–2374.

[76] *Hexane.* Compound Summary for CID 8058. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/hexane

[77] *Classification of Solvents.* Retrieved from http://www.idc-online.com/technical_references/pdfs/chemical_engineering/Classification_of_solvents.pdf

[78] *Ethyl Acetate*. Compound Summary for CID 8857. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/ethyl_acetate

[79] *Methanol.* Compound Summary for CID 887. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/methanol

[80] Akkola, E.K., Göger, F., Koşar, M., K., and Başer, H.C. (2007). *Phenolic composition and biological activities of Salvia halophila and Salvia virgata from Turkey*. Food Chemistry, Vol. 108, Issue 3, Pages 942–949.

[81] Liu, Zai-Qun. (2010). *Chemical Methods To Evaluate Antioxidant Ability*. Chemical Reviews, 110, 5675–5691, Doi: 10.1021/cr900302x.

[82] Cos, P., Calomme, M., Pieters, L., Vlietinck, A.J., and Berghe, D.V. (2000). *Structure-Activity Relationship of Flavonoids as Antioxidant and Pro-oxidant Compounds.* Studies in Natural Products Chemistry, Volume 22, Part C, Pages 307-341.

[83] Rea, R., Pellegrinia, N., Proteggentea, A., Pannalaa, A., Yanga, M., and Rice-Evans, C. (1999). *Antioxidant activity applying an improved ABTS$^{+\bullet}$ radical cation decolorization assay*. Free Radical Biology and Medicine, Vol. 26, Issues 9–10, Pages 1231–1237.

[84] Gyamfia, M.A., Yonaminea, M., and Aniya, Y. (1999). *Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries.* General Pharmacology: The Vascular System, Vol. 32, Issue 6, Pages 661–667.

[85] Nutrient Broth Medium. HIMEDIA, Technical Data. Retrieved from http://www.himedialabs.com/TD/MM244.pdf

[86] Benahmed-Bouhafsoun, A., Djebbar, H., and Kaid-Harche, M. (2015). *Determination of Polyphenolic Compounds of Washingtonia robusta H. Wendl Extracts*. ACTA PHYSICA POLONICA A, Vol. 128, No. 2-B.

[87] Gram-positive vs. Gram-negative Bacteria, Retrieved from http://www.diffen.com/difference/Gram-negative_Bacteria_vs_Gram-positive_Bacteria