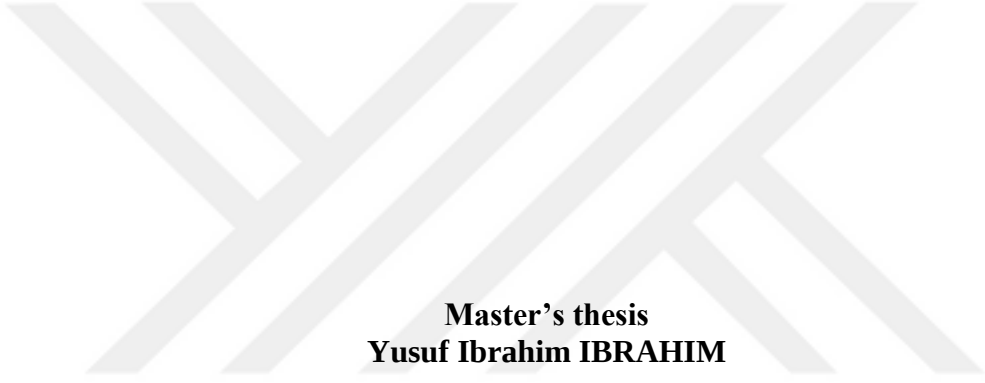


**REPUBLIC OF TURKEY
FIRAT UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

**QUANTITATIVE DETERMINATION OF RESVERATROL FROM DIFFERENT
FRUITS AND ISOLATION OF RESVERATROL FROM GRAPE SEEDS**



**Master's thesis
Yusuf Ibrahim IBRAHIM**

**Department of Chemistry,
Biochemistry**

Supervisor: Prof. Dr. Fikret KARATAŞ

April-2018

**REPUBLIC OF TURKEY
FIRAT UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

**QUANTITATIVE DETERMINATION OF RESVERATROL FROM DIFFERENT FRUITS
AND ISOLATION OF RESVERATROL FROM GRAPE SEEDS**

Master's thesis

Yusuf Ibrahim IBRAHIM

(152117108)

Date submitted to the Institute: 17 April 2018

Thesis defense Date: 03 May 2018

Thesis Supervisor : Prof. Dr. Fikret KARATAŞ (Fırat University)

Jury members : Prof. Dr. İsmet YILMAZ (İnönü University)

Prof. Dr. Süleyman AYDIN (Fırat University)

April-2018

ACKNOWLEDGEMENT

I am hereby wholeheartedly with all sense of respect appreciate the effort, sincerity and guide of my supervisor Prof. Dr. Fikret KARATAS will really remained indebted to him for all the patience and time he has for me during my course work as well as during my laboratory and thesis proceedings, I really learned a lot from him and this made me a better and sound person academically henceforth.

I would like to acknowledge the Firat University Scientific Research Center (FUBAP) for providing all financial implication to complete this project “Project Number FUBAP FF.17.09.”

I would like to take this opportunity to say warm thanks to all my beloved friends, who have been so supportive along the way of doing my thesis.

I owe profound gratitude to my wife, Sa’adatu Aliyu who is always by my side and at the same time invested very heavily in kind and patience wise to take care of some of my responsibilities while on study also for her constant encouragement and great sacrifice. I will like to thanks my parents for their lifelong support morally as well as financially toward achieving my goals in life. My counsellor and motivator Zulaiha G. Mukhtar for advised and suggestion, I will always remain appreciative for all her kindness.

CONTENTS

	Page Number
ACKNOWLEDGEMENT	II
CONTENTS	III
ÖZET	V
SUMMARY	VI
LIST OF FIGURES.....	VII
LIST OF TABLES.....	VIII
SYMBOLS AND ABBREVIATION.....	IX
1. GENERAL INTRODUCTION	1
1.1. RESVERATROL	2
1.1.1. Physical properties of Resveratrol.....	4
1.2. BIOACTIVITIES/IMPORTANCE OF RESVERATROL	4
1.2.1. Resveratrol as antioxidant	5
1.2.2. Resveratrol as Cardio-protection agent	6
1.2.3. Resveratrol in cancer prevention.....	6
1.2.4. Anti-inflammation Activities of Resveratrol.....	7
1.2.5. Resveratrol and diabetes.....	8
1.2.6. Resveratrol as Antimicrobial agent	8
1.2.7. Anti-aging property of Resveratrol	9
1.3 Main Aims and Objectives of This Research Work.....	9
2. MATERIALS AND METHOD	11
2.1. Materials.....	11
2.1.1. Equipment and Chemicals.....	11
2.2. Method	12
2.2.1. Sample preparation for resveratrol extraction and Isolation	12
2.2.2. Extraction of resveratrol.....	12
2.2.3. Extraction of resveratrol for HPLC analysis <i>cis/trans</i> resveratrol contents of from fruit/seeds sample	13
2.2.4. Soxhlet Extraction Method for Resveratrol	13
2.2.5. Hydrolysis and Liquid-Liquid extraction of resveratrol	15
2.2.6. TLC for the determination of best mobile phase for extract	15
2.2.7. Preparation of column using different column fillers (stationary phase).	17

2.2.7.1. Silica Gel.....	17
2.2.7.2. Alumina	17
2.2.7.3. DOWEX (cation exchange)	19
2.2.7.4. DOWEX (anion exchange).....	20
2.2.7.5. Florisil column preparation.....	22
2.2.8. HPLC method for the determination of resveratrol	23
2.2.9. Statistical analysis	24
3. RESULT	25
4. DISCUSSION.....	36
4.1. Amounts of resveratrol in some selected foods/fruits/herbs samples	36
4.2. Findings on Best mobile phase determination for resveratrol separation using TLC	37
4.3. Findings on Best column filler (stationary phase) for separation of resveratrol from crude extract.	38
LITERATURE.....	40

FARKLI MEYVE VE TOHURLARINDA RESVERATROL TAYİNİ İLE ÜZÜM ÇEKİRDEKLERİNDEN RESVERATROLÜN İZOLASYONU

ÖZET

Resveratrol, bitkilerde bulunan biyolojik en önemli stilojen sınıfı olarak sıralanmıştır. Resveratrol'ün antioksidan, antikanser, antiinflamatuvar, damar gevşetici, anti-diyabetik, yaşlanmayı geciktirici ve genel antimikrobiyal aktiviteleri olduğu kanıtlanmıştır. Bu araştırmada, resveratrolün siyah üzüm çekirdeğinde izole edilerek, saflaştırılması için ince tabaka kromatografisinde (TLC) değişik çözücü sistemleri kullanıldı. TLC'de tercih edilen çözücü sistemi kolon kromatografisi ve HPLC ile resveratrolün tayin edilmesinde kullanıldı. Elde ettiğimiz sonuçlarda resveratrolün, Vitis vinifera (üzüm) tohumunun ham ekstresinden ayrıştırılması için en iyi mobil fazın, 9: 1: 1 oranında kloroform: metanol: asetonitril olduğu, ince tabaka (TLC) kromatografisi ile belirlendi. Kolon kromatografisinin optimizasyonu için, silika jel, alümina, dowex katyon değiştirici, dowex anyon değiştirici ve florisil adsorbanların hepsi, TLC analizinden oluşturulan aynı en iyi mobil faz sistemini kullanarak, alümina hariç ham ekstraktan resveratrol ayırmak için uygun olduğu belirlendi. Resveratrolü izole etmek için Silika jel > Florisil > Dowex katyon değiştirici > Dowex anyon değiştirici, sırasıyla tercih edilebilir. Resvetrolü kaynağından çıkarmak için önce heksan: etilasetat (9: 1) çözücü sistemi kullanıldı ve daha sonra kloroform: metanol: asetonitril (9: 1: 1) çözücü sistemi kullanılarak ham bitki özünden resveratrol izole edildi. Cis ve trans resveratrol'ün belirlenmesinde Agilent 5 TC-C18 kolonu (25.0 cm x 4.60 mm x 5.0 µm) kullanıldı. Mobil faz olarak metanol: asetonitril: % 1.0 fosforik asit çözeltisi (6: 1: 3 v /v), 1.0 mL/dak akış hızına tercih edildi. Cis resveratrol ve trans resveratrolün maksimum absorbans yaptığı dalga boyunun ise 308 nm olduğu gözlemlendi. Ayrıca saflaştırılan resveratrolün NMR spektrumu da alınarak HPLC ile alınan sonuçlar ve yapı tayini test edilmiş oldu. Optimize edilmiş olan tüm bu şartlar uygulanarak cis ve trans formdaki esveratrolün HPLC ile kantitatif tayini için bazı gıda / meyve / bitki örneklerinden analizler gerçekleştirildi.

Anahtar Kelimeler : Resveratrol, HPLC, TLC, Kolon dolgu maddesi, Mobil faz

SUMMARY

Resveratrol has been ranked as the most biologically important class of stilbenoid found in plants. It has been proven to have antioxidant, anticancer, anti - inflammatory, vasorelaxer, anti-diabetic, anti-aging and general antimicrobial activities. This noble molecule resveratrol is investigated in this research work, with a special interest in isolating and purifying this molecule, in this end optimization methods for TLC, column chromatography as well as HPLC for resveratrol were investigated, at the same time presence of resveratrol qualitatively and quantitatively from some samples of food/fruits/herbs (twenty two of them) exclusively obtained from Turkey were also investigated. Our results and finding shows that the best mobile phase to separate resveratrol from crude extract of *Vitis vinifera* (grape) seed is chloroform: methanol: acetonitrile with proportion ratio of 9:1:1, this is on TLC plate coated with silica gel 60, for optimization of column chromatography, silica gel, alumina, dowex cation exchanger, dowex anion exchanger and florisil adsorbents were all found to separate resveratrol from crude extract with exception of alumina, using the same best mobile phase system established from TLC analysis, the sequence by which these column fillers can be used to isolate resveratrol is Silica gel > Florisil > Dowex cation exchanger > Dowex anion exchanger, gradient elution using two sets of solvent system of hexane:ethylacetate; 9:1, and then followed by chloroform: methanol: acetonitrile 9:1:1 was found to be the best way to isolate resveratrol from complex crude plant extract. Agilent 5 TC-C18 column (25.0 cm x 4.60 mm x 5.0 µm) was used in the determination of cis resveratrol and trans resveratrol. Methanol: Acetonitrile: 0.1% phosphoric acid solution (6:1:3 v/v) used as a mobile phase with the flow rate of 1.0 mL/min. The maximum absorbance of cis resveratrol and trans resveratrol was 308 nm. NMR analysis used in this work shows a spectra which confirmed the presence and purity of extracted resveratrol. This research work established presence of resveratrol from many foods/fruits/herbs which has never been reported before and also established a better column chromatographic condition to isolate resveratrol from any plant source.

Key words; Resveratrol, HPLC, TLC, Column filler, Mobile phase

LIST OF FIGURES

	Page Number
Figure 1.1. Synthesis of resveratrol in plant.....	3
Figure 1.2. Isomers of resveratrol	4
Figure 2.1. Showing soxhlet extraction setup used for solid liquid extraction of resveratrol from powdered grape seed	14
Figure 2.2. Showing silica gel column chromatographic set-up	18
Figure 2.3. Showing Alumina column chromatographic set-up	19
Figure 2.4. Showing Dowex cation exchange chromatographic set-up.	20
Figure 2.5. Showing Dowex anion exchange column chromatographic set-up.....	21
Figure 2.6. Showing Florisil column chromatographic set-up.....	22
Figure 3.1. 72 ppm <i>Trans</i> resveratrol chromatogram.....	25
Figure 3.2. 96 ppm <i>Cis</i> resveratrol chromatogram.....	25
Figure 3.3. 72 ppm <i>Trans</i> resveratrol chromatogram.....	26
Figure 3.4. 64 ppm <i>Cis</i> resveratrol chromatogram.....	26
Figure 3.5 36 ppm/32 ppm <i>Trans</i> and <i>Cis</i> Resveratrol chromatogram	27
Figure 3.6. Standard curve for <i>trans</i> -resveratrol standard solution.....	28
Figure 3.7. Standard curve for <i>cis</i> -resveratrol standard solution	28
Figure 3.8. Black pepper chromatogram	29
Figure 3.9. Clove sample resveratrol chromatogram	29
Figure 3.9.1. Showing pictures of silica gel coated TLC	31
Figure 3.9.2. Showing chromatogram of purified resveratrol collected from silica gel Column.....	32
Figure 3.9.3. Dowex cation exchanger Column eluate chromatogram	33
Figure 3.9.4. Dowex anion exchanger Column eluate chromatogram.....	34
Figure 3.9.5. Florosil Column eluate chromatogram	34
Figure 3.9.6. Resveretrol (400 MHz- DMSO-d ₆) ¹ H-NMR spectra.....	35

LIST OF TABLES

Table 2.1. Showing list of solvent mixer/ratio carried out against TLC for best solvent.....	16
Table 3.1. The amounts of Cis and Trans Resveratrol in some foods	30
Table 3.2. Collected eluates from Silica gel, Chloroform: Methanol mobile phase.....	32
Table 3.3. Collected eluates from Dowex (Cation exchange column)	33
Table 3.4. Collected eluates from Dowex (anion exchange column).....	33
Table 3.5. Collected eluates from Florisil column chloroform/methanol mobile phase..	34
Table 3.6. Collected eluates from Florisil column.....	35
Table 3.7. Collected eluates from Alumina column chloroform/methanol mobile phas.....	35

SYMBOLS AND ABBREVIATION

DMSO	: Dimethyl Sulfoxide
HPLC	: High performance liquid chromatography
TLC	: Thin Layer Chromatography
CHD	: Coronary heart disease
MM	: Molecular Mass
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate
ROS	: Reactive Oxygen Species
Cgmp	: Cyclic Guanine Mono Phosphate
SOD	: Superoxide dismutase
CHD	: coronary heart disease
RT-PCR	: Western blot analysis
MMP-9	: MAPK and SIRT1 proteins
NOX	: Nitrous oxide
Nrf2	: Nuclear factor erythroid 2-related factor
ddH₂O	: Doubly distilled deionized water

1. GENERAL INTRODUCTION

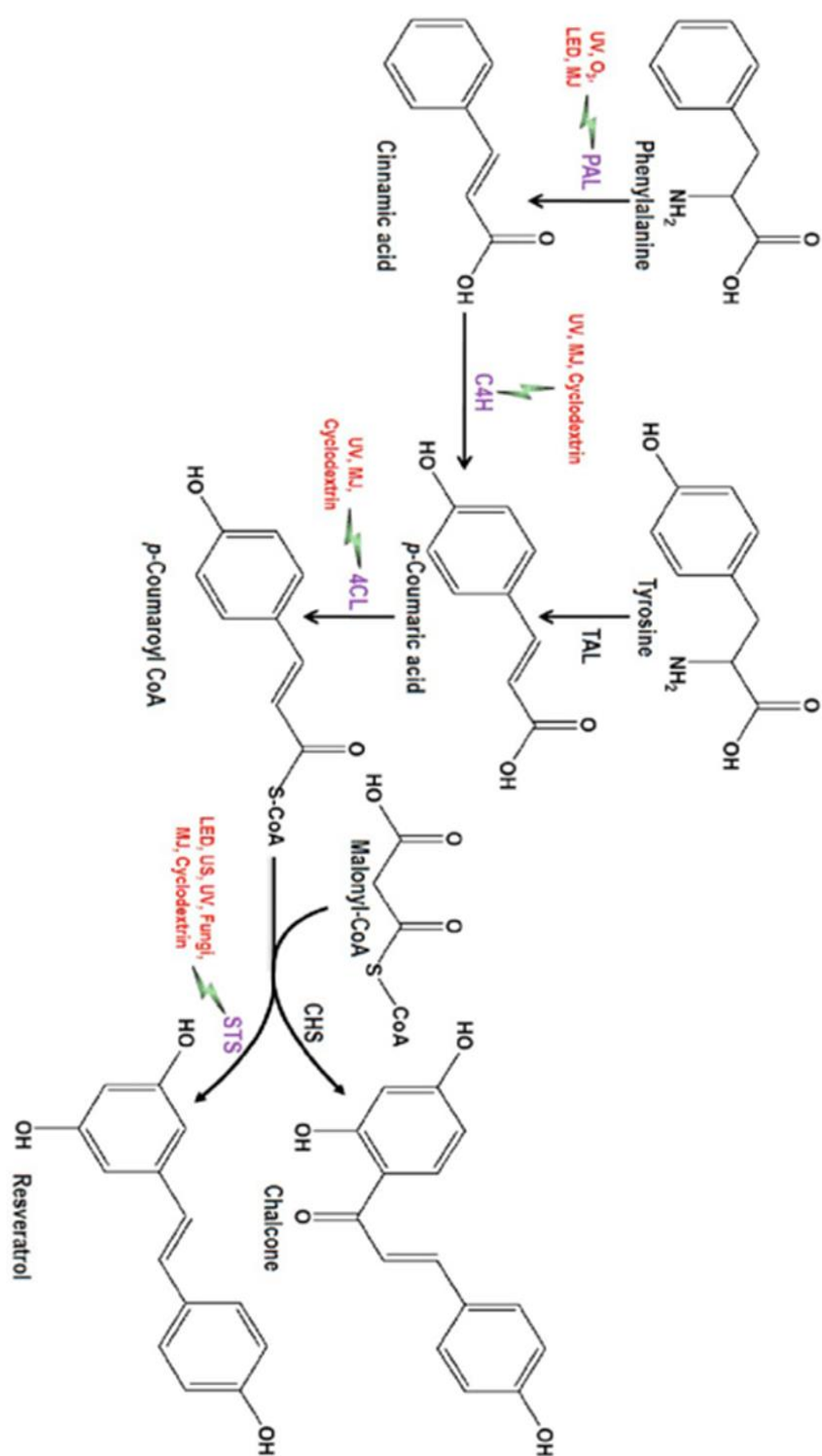
Fruit has been accepted as a noble source of vitamins and minerals, (these compounds are very essential for the maintenance of balanced homeostasis) and for their role in functioning as vitamins supplements and as well as treating deficiencies in Animals (human inclusive). Including enough amount of fruits in one's food improve the overall health and also helps to prevent many different diseases (and diseases conditions). It has been very well and accurately established that; fruits based diet is associated with lowering dangers of developing so many chronic diseases, these include, cancer and cardiovascular disease [1]. Adapting a consistent lifestyle with diet containing more proportion of vegetables and fruits can decelerate the risks of many type of cancers, heart failure, Alzheimer disease, cataracts, stroke, and some illnesses attacking peoples during old age [2]. In recent time from different class and walk of life consider fruit as part of their everyday food, this make fruit to be at a very high position in many family's menu worldwide, this is because of their excellent nutritional, useful and therapeutic potentials [3]. Dietitians and Nutritionists suggest that quantities of fruit/vegetables must be part well balance diet for healthy life. Many compound found naturally occurring in fruit and/or vegetables, such as; potassium, folate, vitamins, fiber, and other phenolic compounds are directly responsible for most of the health benefits that fruits gives, and also these compound including vitamins and antioxidants helps in slowing the attacks of decrease chronic diseases, (example. coronary heart disease (CHD), by means of their protective activities for example, antioxidant against free-radicals [4]. Resveratrol is one of compound found in fruits and maybe vegetables, it is a phenolic phytochemical it is said to be a very important molecule found in fruits, and plants' parts in general. Results of many scientific researches confirmed presence of resveratrol in many plans. Now more than 70 plant species distributed among 31 genera and 12 families containing resveratrol has been documented [5]. Large number of plants or fruits that are part of human diet, for example, grapes, wine, grape juice, cranberries, peanut etc. also contain some amount of resveratrol in them [6]. Based on the available scientific records so far, grape fruits have very much more quantity of resveratrol and hence is considered to be one of the most important human nutritional sources of resveratrol [7]. Both seeds and skins parts of grape are labeled as very rich sources of phytochemicals such as garlic acid, catechin, and epicatechin and (seed and skin of grape) are suitable raw materials for the production of antioxidative dietary supplements. Although both seed and skin of grape are source of antioxidative compound but there is difference in the number of phytochemicals

they contained and also differences in amounts of shared similar compounds. The differences in levels of the major monomeric flavanols and phenolic acids in seeds and skins from grapes of *Vitis vinifera* varieties Merlot and Chardonnay and in seeds from grapes of *Vitis vinifera* variety Muscadine were determined, and the antioxidant activities of these components were assessed in one of previous work [8].

1.1. RESVERATROL

Resveratrol has been ranked as the most biologically important class or compounds stilbenoid. Resveratrol; (with chemical name 3, 5, 4'-trihydroxy-*trans*-stilbene) can be call as polyphenol phytochemical, naturally synthesized by some species of plants in response to injury or stress, this can be seen as plant's own way of inflammatory response agent, antimalarial and antioxidant properties has been attributed to resveratrol. [9]. Available record indicated that; it was around the year nineteen ninety (1990s) that resveratrol was first time was discovered, red wine is the first sample in which presence of resveratrol was confirmed.

From that time (1990s) till today, a lot of investments has been made for interest in studying resveratrol and also trying to find its effect on health. Some important postulations suggested that; consumption of resveratrol from red wine might help explain the fact that French people have a comparatively low occurrence of coronary heart disease (CHD), ironically these people eat too much of high in saturated fat in their regular diets, a occurrence is what is termed as the "French Paradox" [10]. The plant biochemical pathway that lead to the anabolism of Resveratrol is known, in this pathway malonyl CoA and coumaroyl CoA are use as substrate in a reaction catalyze by an enzyme called resveratrol synthase, this process usually take place in response of an attack against resveratrol producing plant by disease causing microbes (otherwise called pathogens) [11]. Resveratrol dissolves easily in alcohols (eg ethanol or methanol) compound of resveratrol can exist in both *trans* and *cis* molecular configurations. Below is an illustration of the perceived pathway for resveratrol production in plants (Figure 1.1).



. b

Figure 1.1: Synthesis of resveratrol in plant

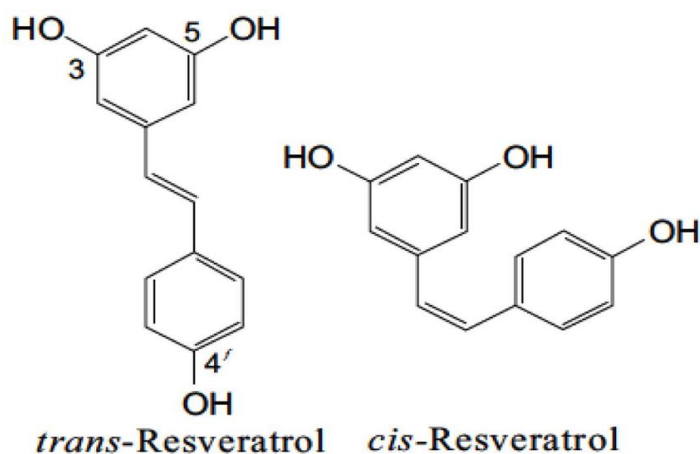


Figure 1.2. Isomers of resveratrol

1.1.1. Physical properties of Resveratrol

Resveratrol (with the chemical structure as depicted above) have a chemical or molecular formula of $C_{14}H_{12}O_3$ and molecular weight or molecular mass (MM) of $228.24 \text{ g}\cdot\text{mol}^{-1}$, the density of resveratrol is found to be exactly 1.359 g/cm^3 , ultrapure crystalized dried resveratrol is use to be off-white to tan powder in color (white powder with slight yellow cast). Flash Point and Refractive index of resveratrol are 222.3°C and 1.762 respectively, other physical characteristics of resveratrol are boiling point 449.1°C at 760 mmHg , resveratrol also start melting when heated at temperature between 261 to 263°C (502 to 505°F ; 534 to 536 K) [12]. Although resveratrol can proportionally dissolve in many solvents both polar and non-polar, but its solubility in the following seem much more relevant to its physical properties that is; 0.03 g/L in water, 16 g/L in DMSO and in ethanol. According to many literatures the maximum absorption wavelength of resveratrol is at UV-Vis light with 304nm for *trans*-resveratrol, and 286 nm for *cis*-resveratrol, both in water (Figure 1. 2) [13].

1.2. BIOACTIVITIES/IMPORTANCE OF RESVERATROL

A lot of in vivo and in vitro research work has been conducted and many more are under way to ascertain the all significant biological roles and properties of resveratrol, however, most of these investigations has been carried out using culture cells in test tubes (in vitro), and with relatively less number of studies conducted in animal subjects. Some of the latest publication on this issue is a paper review title “Resveratrol and clinical trials: the crossroad

from in vitro studies to human evidence” by Tomé-Carneiro et al, in which many related studies on biological activities of resveratrol was evaluated [14]. Recently, most of efforts and increasing interests on phenolic compounds found in grapes have focus on how these compound really affect biological activities, which can be of human benefits more especially on health and general wellbeing, this include their antioxidant properties, how do they protect cardiac problems/failures, how true is their anticancer properties (as widely reported), anti-inflammation effect, their ability to reduce or retard antiaging and other general antimicrobial properties [15].

Some major biological activity of resveratrol as widely reported are:-

Inhibition of lipid peroxidation; Chelation of copper; Free radical scavenging; Alteration of eicosanoid synthesis; Inhibition of platelet aggregation; Anti-inflammatory activity; vaso-relaxing activity; Modulation of lipid metabolism; Anticancer activity; Estrogenic activity [16].

1.2.1. Resveratrol as antioxidant

Vitamins and polyphenols are natural antioxidants that plays an important function in the prevention of diseases associated with free radicals. Resveratrol is found to reduces the rate of cytochrome c oxidation by hydroxyl radicals, produced as a result of the UV irradiation of hydrogen peroxide (H_2O_2), with an $IC_{50} = 33 \mu M$. Resveratrol is also very effective in scavenging hydroxyl radicals in an ethanolic solution of β -carotene [17]. Resveratrol contained in red wine has been confirmed as an antioxidant agent that prevent the development of heart-related illnesses, it has notable antioxidant and anti-inflammatory activities which have been considered to be responsible for the beneficial effects of red wine consumption on coronary heart disease, the evidence is based on observed less risk of developing cardiovascular diseases with people drinking red wine in their eating habits [18]. The effect of resveratrol on MMPs' activity was evaluated using gelatin zymography. Western blot analysis and RT-PCR assay were used to determine the effect of resveratrol on the expression level of MMP-9, MAPK and SIRT1 proteins and genes, respectively [19]. Some scientists believed that resveratrol activates or enhance the catalytic activities of some antioxidant enzymes, these are superoxide dismutase (SOD), thioredoxin, glutathione peroxidase-1, heme oxygenase-1, and catalase, and/or maybe is also responsible for the inhibition or blocking of reactive oxygen species (ROS) produce by nicotinamide adenine

dinucleotide phosphate (NADPH) oxidases (NOX) [20]. Although the reported biological information indicate that resveratrol is a highly promising cardiovascular protective agent, more researches are required to establish its bioavailability and *in vivo* cardio-protective effects, more especially *in vivo* and also in human subjects.

1.2.2. Resveratrol as Cardio-protection agent

Many studies conducted on disease prevention and control has it to say that; nutritional factors, as well as red wine consumption, can decrease the risk of developing many heart-related diseases, at least, this is partly because of the existence of polyphenols (resveratrol inclusive) in many food more especially fruit-based diet [21, 22]. Suggestion for cardio-protection of alcoholic drinks usually because of their polyphenols contents has been widely reported and established in many scientific literatures [19]. Studies suggest that resveratrol modulates vascular cell function, inhibits low density lipoprotein metabolism (oxidation in this case), suppresses platelet aggregation and reduces myocardial damage during ischemia-reperfusion, although the reported biological data indicate that resveratrol is a highly promising cardiovascular protective [19]. Resveratrol has been found to exert a number of protective properties on the cardiovascular system *in vitro*, including inhibition of both platelet activation and aggregation, but as always reported earlier, there is need for full *in vivo* research on all the claimed made on cardio-protective nature or resveratrol this must be now or in very near future [23-25]. At least for over twenty years now, scientists and researchers alike have studied the function of resveratrol in preventing, protecting or reversing the development of abnormalities which causes heart disease and heart failure. The most recent results from animal studies have been positive, and very recently, this knowledge has been translated into examining the efficacy of resveratrol in humans with heart disease/failure [26]

1.2.3. Resveratrol in cancer prevention

Resveratrol (RES) has many pharmacological activities in extending longevity and treatment of cardiovascular disease, it also links to treating diabetes and cancer [27]. Resveratrol has been suspected to be very good agent in prevention of the variety of human tumor cell lines, including those from breast, prostate, stomach, colon, pancreatic, and thyroid cancers [28],

many other in vitro and clinical studies have been conducted involving anticancer activity of resveratrol through many physiological and biochemical pathways [29, 30]. Resveratrol has been confirmed to increased reactive oxygen species (ROS) production [31-35], using an experimental animals rat specifically, different route of administration applied such as systemic (oral and injection), local (topical) in both cases the growth of chemically-induced tumor has been repressed by resveratrol at many sites, including stomach/intestine, hepatic organ, dermal, breast, prostate, and lungs [35, 36]. In one of the several research works conducted it has been demonstrated that M2 polarization of human monocyte derived macrophage (HMDMs) induced by the lung cancer cells conditioned medium was inhibited by Resveratrol. Additionally, Resveratrol exhibited inhibitory function in lung cancer cells co-cultured with human macrophages. The activity of signal transducer and activator of transcription 3 (STAT3) was significantly decreased by Resveratrol. Moreover, in a mouse lung cancer xenograft model, Resveratrol significantly repressed the tumor growth, which was associated with inhibition of cell proliferation and decreased expression of p-STAT3 in tumor tissues [27]. Resveratrol anti-cancer effects have been well documented in and this include variety of cancers, whereby it regulated cell division, and growth.

1.2.4. Anti-inflammation Activities of Resveratrol

Resveratrol in grapes, especially in grape seeds, have shown significant anti-inflammation activities this mostly demonstrated on rats, mice and even human being [37, 38], a reliable data show for the first time that in cytokine-challenged cells, cyanidin-3-glucoside and resveratrol induced Nrf2 activation, increased hemoxygenase-1 and glutamate cysteine ligase mRNA expression, enhanced reduced glutathione to oxidized glutathione ratio and inhibited reactive species production, at much lower concentrations than 5-ASA. Unlike cyanidin-3-glucoside, resveratrol and 5-ASA also increased nuclear levels of PPAR- γ in cytokine-stimulated cells. In conclusion, both polyphenols might be interesting as nutraceuticals, giving complementary benefits to conventional drugs against intestinal inflammation, typically present in patients with inflammatory bowel disease [39], another research conducted by; Sae Im Jeonga, et al [40], showed that resveratrol counteracted inflammation in visceral adipose tissue and in the brain and reduced stroke-induced brain injury and peripheral inflammation in aged female mice. Therefore, resveratrol

administration can be a valuable strategy for the prevention of age-associated and disease-provoked inflammation in postmenopausal women.

1.2.5. Resveratrol and diabetes

Diabetes mellitus is a common metabolic illness that is characterized by high amount of blood glucose above normal level as a result of the lack of insulin or the presence of insulin resistance in peripheral tissues or both. In animal studies, resveratrol has been shown to improve insulin recognition by cells, glucose tolerance, and lipid profiles in obese and/or metabolically abnormal animals [41], another present study suggested that oral supplementation of resveratrol might be a potential therapeutic strategy for the treatment and/or prevention of diabetic encephalopathy [42]. Resveratrol has also been found improves diabetes-associated erectile dysfunction (ED) in laboratory animals. Combination medication with resveratrol and sildenafil have been found to of synergistic effect in reversing ED. The mechanisms might be attributed to its anti-oxidative properties and NO–cGMP signaling pathway upregulation [43].

1.2.6. Resveratrol as Antimicrobial agent

Many research works established that red wine and resveratrol showed antimicrobial effect against foodborne pathogens. *Staphylococcus aureus* and *Escherichia coli*, were susceptible to wine treatment [44], Resveratrol also prevents the adherence of *Salmonella typhimurium*, *E. coli*, and *Listeria monocytogenes* to human colonic cells [45]. Derivatives of resveratrol (2-chloro-resveratrol and 2-bromo-resveratrol), have been reported as very effective antimicrobial agent, inhibiting the growth of Gram-positive bacteria, Gram-negative bacteria and fungi [46], another finding suggest that red wine and resveratrol can affect the foodborne viral surrogates at an early stage of infection. Red wine and resveratrol have the potential to be developed for control of norovirus-mediated foodborne disease [47], many other scientific investigations have reported inhibitory properties of resveratrol on viruses [48].

1.2.7. Anti-aging property of Resveratrol

It has been established that polyphenolics existing in foods can be of beneficial effect in reversing aging. This is because of their outstanding antioxidant activity, such as scavenging free radical, they could prevent organs and tissues from oxidative damage, and change the body negative mechanism of oxidation-reduction status. Resveratrol activates Sirtuin 1 (Sirt1), an NAD⁺-dependent protein deacetylase that is a key regulator of lifespan in several model organisms under metabolic stress [49]. Moreover, resveratrol demonstrates an anti-aging property by modulating various transcriptional factors, such as activating transcription factor 3 (ATF3) and activator protein 1 (AP-1), which typically form a complex involved in regulating cell apoptosis and survival [50].

1.3 Main Aims and Objectives of This Research Work

To obtain some selected fruits e.g.: Black grape, Blackberry, Mulberry, Figs, Cherry (sweet and sour) etc.

- To process/treat the sample, using a standard homogenization method.
 - To prepare column using different column fillers (silica gel, alumina, Dowex cation exchanger, Dowex anion exchanger and Florisil)
 - To formulate different mobile phase for separation/purification
 - To carryout column chromatographic isolation/purification of resveratrol from the samples
 - To carry out high performance liquid chromatography (HPLC) in determining quality, quantity and purity grade of the resveratrol using standard, against standard resveratrol HPLC chromatogram
 - To make calculations, data analysis, result presentations and recommendations.
- To isolate resveratrol from different fruits and their seeds
- To purify the isolated resveratrol
- To investigate the quality and quantity for isolated/purified resveratrol
- To elucidate the most suitable column filler (matrix) and/or mobile phase for isolation and purification of resveratrol
- To compare/contrast quality and quantity resveratrol from edible part of each fruit and its seed.
- To evaluate better optimum condition for both isolation and purification of resveratrol

material selected as samples for resveratrol determination

Pea nut, Hazel nut, Rosehip, Walnut, Almond kernel, Apricot kernel, Cherry seed, Garlic, pistachios, Red cherry, Black Cherry, Plum, Mountain fig, Dried plum, Goat horn, Sour cherry, Lentil, Hackberry, Sumac, Quince, Apple seed, Date fruit seed, Black carrot, Ginger, Black pepper, Red beet, Donut, Turmeric powder, Onion, Black grape seed, white grape seed, Pomegranate. All of these sample where obtained randomly from local market within Turkey,



2. MATERIALS AND METHOD

2.1. Materials

High performance liquid chromatograph (HPLC) auto sampler, rotary evaporator water bath, vortex mixer. Silica Gel G, for adsorption chromatography, florisil for adsorption chromatography, Dowex cation exchanger, Dowex anion exchanger and Alumina (all these are for stationary phase of column chromatography). Ethanol (analytical grade), ethyl acetate (analytical grade), acetone, n-Hexane, Chloroform, (analytical grade), methanol (analytical grade), acetonitrile (analytical grade), methanol (HPLC grade), Diethyl ether (analytical grade), Petroleum ether (analytical grade) and standard resveratrol (*cis* and *trans*) Sigma 99% will be used in the experiments. Other ancillary apparatus includes; Glass column for preparative chromatography, calibrated/standard pipettes, 10, 25, 50, 100, 150 and 250 mL conical flasks, funnels, beakers, automatic pipettes, Buchner flask/funnel, mortar and pestle.

2.1.1. Equipment and Chemicals

trans-Resveratrol and *cis*-resveratrol standards were purchased from Cayman Chemical Company (Ann Arbor, MI). Emodin and quercetin standards were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). HPLC-grade methanol and formic acid were purchased from Fisher Scientific Canada. Ultrapure water (18.2 MΩ cm) was obtained using a Millipore Milli-Q 10 water system for the preparation of the buffer solution (Billerica, MA). Standard. The working solution was prepared by diluting the stock solution to obtain appropriate concentrations for calibration curves. The chemicals and reagents used in this work were of analytical grade and purchased from Sigma Chemical Co. (Darmstadt, Germany). All glassware was acid washed and rinsed with doubly distilled deionized water (ddH₂O).

HPLC was performed with the Shimadzu Prominence-İ LC-2030C 3D Model with PDA detector.

Other materials for sampling are: Hazel nut (*Corylus*), Rosehip (*Rosa canina*), Almond (*Prunus dulcis*) kernel, Apricot (*Prunus armeniaca*) kernel, Garlic (*Allium sativum*), pistachios (*Pistacia vera*), Red cherry (*Prunus avium*), Black Cherry, Plum (*Prunus domestica*), Goat horn (*Ceratonia siliqua*), Lentil (*Lens culinaris*), Hackberry (*Celtis australis*) Sumac (*Rhus*), Quince (*Cydonia oblonga*), Date fruit (*Phoenix dactylifera*) seed, Black pepper (*Piper nigrum*), Red beet (*Beta vulgaris*), Black seed (*Nigella sativa*), Turmeric powder (*Curcuma longa*), Strawberry (*Fragaria × ananassa*), Cinnamon

(*Cinnamomum verum*). Samples were collected between February-March 2018. Samples collected were identified by Dr. Şemsettin CİVELEK faculty member, Biology department Firat University.

2.2. Method

2.2.1. Sample preparation for resveratrol extraction and Isolation

The treatment of sample prior to analysis will comprise of several stages that include drying, homogenization, sieving, extraction, and then concentrating the extract [56], for the purpose of this research all edible fruit sample should be homogenize directly using a standard homogenizing machine coffee miller with mortar and pestle

Drying seeds is carried out inside hot air ovum, the ovum is set at temperature of 65°C after thoroughly washing the sample with clean water, it is then transferred into ovum and allow to stand for 3-6 hours depending on the moisture contents of the sample to be dried, after been well dried, sample are then keep refrigerated and away from direct sun light for onward procedure. All condition for stability of resveratrol (pH, temperature and light) should be maintained as according to previous literature [64]. The first activity conducted after obtaining grape seed is washing it with water, drying it in oven at a temperature of 65 °C for 3-6 hours is to use mortar and pestle in grounding the dried seeds into fine powder, sieving is also involve to obtained a relatively more finer granules, this is to increase the available surface area for better extraction.

2.2.2. Extraction of resveratrol

Extraction is the most important and critical stage in isolation/purification of natural products find in plants, this is to make sure that most if not all the target compound is within the extracting medium, several very important reason for these are: in order to obtain a maximum yield, also to know exact level in w/w, w/v or v/w of the target compound from the whole sample, again this would enable comparison with other work/research possible. Many work conducted on resveratrol has suggested several extraction method for resveratrol among with are supercritical extraction method [58], by Kinetics and modeling [68] multi-stage counter current extraction [69]

2.2.3. Extraction of resveratrol for HPLC analysis *cis/trans* resveratrol contents of from fruit/seeds sample

Sample of any of the material used for this study were first dried in hot air oven at temperature of 75 °C for 2 to 5 hours (according to the sample nature), a dried material is then made into powder by grinding with mortar and pestle, 0.5-1.5 g powder of each sample was measured into test tube, three milliliter (3 mL) of 80% ethanol (96% ethanol: distilled water; 8.5:1.5) was added into the test tube this is then mixed using vortex mixer for one minute after which all the test tube containing respective sample are transferred into water bath and stand for ten minutes at temperature of 60°C, after removal from water bath, one milliliter (1 mL) of n-hexane is then added to the test tube another mixing with vortex mixer is then followed for one minute again, this extraction mixture is then transferred into centrifuge machine set at 4500 rpm and allowed to run for ten minutes, when the machine stopped, all test tubes are removed and two layers aqueous ethanol and n-hexane are formed, the n-hexane layer is removed by the use of micropipette. One milliliter of the ethanolic extract is then measured into HPLC vials, and leveled against each sample, this is then read by HPLC auto sampler machine, and the area under retention time of both *cis* and *trans* resveratrol is then recorded for onward calculations. Mobile phase for HPLC runs was prepared by mixing Methanol, Acetonitrile and 0.1% phosphoric acid solution at the ratio of 6:1:3.

2.2.4. Soxhlet Extraction Method for Resveratrol

For the isolation of resveratrol from grape seed, the extraction method used is soxhlet this is in order to obtain an appreciable quantity of extract, several methods of extraction have been carried out to extract stilbene (resveratrol inclusive) in one of these researches [70], soxhlet proved to yield good quantity of resveratrol. Polyphenols especially resveratrol have also been extracted via soxhlet extraction method on this work [71] extract resveratrol particularly from grape seed, similar to this work.

The procedure of soxhlet is as follows; 20 g dried powder of grape seed is measured into thimble (specially made from a thick filter paper), the thimble containing sample is placed in the soxhlet extractor chamber, about 150 mL of 96 % ethanol is measured into round bottom flask, the extractor is put fitted at the mouth of the flask and the flask is then mounted onto a heating mantle apparatus (as shown in the figure 2.1), the setup is now completed by attaching a condenser to it.



Figure 2.1. Showing soxhlet extraction setup used for solid liquid extraction of resveratrol from powdered grape seed.

Two or three cycles of extraction are carried out per 20 g sample, this is because according to [70] after two cycles of extraction resveratrol amount that may still be present in the residue is very negligible. After collecting all the ethanolic extract, the solvent (ethanol) is then removed using rotary evaporator, the remaining what appears to be a very sticky powder is washed/dissolved in methanol, liquid-liquid extraction is then conducted with n-Hexane, the n-hexane layer is separated from the methanol layer (resveratrol is not soluble in hexane), the hexane layer now removed any hydrophobic contaminants including oils and greases, the methanol phase is then dried at 60°C in an oven to dryness, after which the leftover powder is collected for onward process.

2.2.5. Hydrolysis and Liquid-Liquid extraction of resveratrol

The collected powder from the extraction process is dissolved in distilled water and pH is adjusted to 1 using hydrochloric acid, this is then heated under reflux at 57°C for 8 hours, this is to enable any dimer or trimer and also glycosides of resveratrol to be hydrolyzed, after the hydrolysis an extracting solvent made by mixture of ethyl acetate: ether is used to extract resveratrol from the aqueous hydrolyzing solution by liquid-liquid extraction in a separation funnel (pH adjusted to 7 using sodium carbonate before liquid-liquid extraction). The extract obtained here is used for TLC analysis as well as Column chromatography analysis.

2.2.6. TLC for the determination of best mobile phase for extract

Thin layer chromatography was conducted using several mixture/ratio of solvents, this is to determine the best solvent system(s) that can be used to separate resveratrol by column chromatography from our extract (found in 2.2.3 above) according to literature some TLC system of resveratrol has been developed [72] in this an attempt is made to randomly select different solvent mixture and run TLC plate (silica gel coated, aluminum) then the best system that gives better separation and highest number of visible spots will be chosen, some of this solvent system are modification of some previous work e.g. [73].

The different ratio/combinations of solvent screened for this work has been tabulated below, total of 43 solvent systems was developed (Table 2. 1).

Table 2. 1: Showing list of solvent mixer/ratio carried out against TLC for best solvent system determination

TLC plate number	Solvent System / Mixture	Solvent ratio
1	Chloroform: Ethanol	4:1
2	Chloroform: Ethanol	2:1
3	Chloroform: Methanol	7:3
4	Chloroform: Methanol	4:1
5	Chloroform: Ethanol	7:2
6	Chloroform: Ethanol	7:3
7	n-Hexane: Ethyl-acetate	4:1
8	n-Hexane: Ethyl-acetate	2:1
9	Chloroform: Ethanol	7:1
10	Chloroform: Methanol	3:1
11	n-Hexane: Ethyl acetate	9:1
12	Chloroform: Methanol :	7:1.5
13	n-Hexane: Ethyl-acetate	3:1
14	n-Hexane: Ethyl-acetate	3.5:1
15	n-Hexane: Ethyl-acetate	5:1
16	n-Hexane: Ethyl-acetate	9:1
17	n-Hexane: Ethyl-acetate: Acetonitrile	9:1:1
18	n-Hexane: Ethyl-acetate: Acetonitrile	7:2:1
19	Chloroform: Methanol: Acetonitrile	9:1:1
20	n-Hexane: Ethyl-acetate: Acetonitrile	9:2:1
21	n-Hexane: Ethyl-acetate: Acetonitrile	8:2:1
22	n-Hexane: Ethyl-acetate: Acetonitrile	8:1:2
23	n-Hexane: Ethyl-acetate: Acetonitrile	10:1:1
24	n-Hexane: Ethyl-acetate: Acetonitrile	10:1:2
25	n-Hexane: Ethyl-acetate: Acetonitrile	10:2:1
26	n-Hexane: Ethyl-acetate: Methanol	10:2:1
27	n-Hexane: Ethyl-acetate	6:1
28	n-Hexane: Ethyl-acetate	4:1
29	n-Hexane: Ethyl-acetate	4:1.5
30	n-Hexane: Ethyl-acetate	4.5:1
31	n-Hexane: Ethyl-acetate	5:1
32	Chloroform: Methanol	7:2.5
33	Chloroform: Methanol	7:1.5
34	Chloroform: Methanol	1:1
35	n-Hexane: Ethyl-acetate: Ethanol	9:2:1
36	n-Hexane: Ethyl-acetate: Ethanol	9:1:2
37	n-Hexane: Ethyl-acetate: Ethanol	9:2:2
38	n-Hexane: Ethyl-acetate: Ethanol	7:2:1
39	n-Hexane: Ethyl-acetate: Ethanol	7:1:2
40	n-Hexane: Ethyl-acetate: Methanol	9:2:2
41	n-Hexane: Ethyl-acetate: Methanol	9:3:2
42	n-Hexane: Ethyl-acetate: Ethanol	4.5:1:0.5
43	n-Hexane: Ethyl-acetate: Ethanol	4.5:1:1.5

After running these several tests, result was obtained, the figure below depicted some of TLC chromatogram, the best solvent system can easily be identified with many number of spots and also with better resolution,

2.2.7. Preparation of column using different column fillers (stationary phase).

2.2.7.1. Silica Gel

40 g of Silica Gel G was measured, and it then heated at 105°C for 30 min, then is was activated by continuous heating for another 30 min at 180°C in a drying oven. The prepared Silica Gel G will be pack into chromatographic column using wet packing method [74]. The column is loaded with 0.2-0.5 g of ethanolic extract of grape seed, gradient elution using two type of mobile phase (solvent system is used) these are; n-Hexane: Ethyl-acetate: Acetonitrile (9:1:1) and Chloroform: Ethanol (7:2) at a rate of 5 mL/min, collecting the eluate 10 mL/bottle. The eluates are then vaporized, the remainder was dissolved in 10 mL methanol, then the samples were taken for HPLC analysis, in this total of 10 eluates was collected from first mobile phase system hexane: ethyl acetate: acetonitrile and total of thirty eluates was collected from chloroform: Ethanol mobile phase (Figure 2.2).

2.2.7.2. Alumina

50 g of basic alumina was measured, this alumina is then packed into chromatographic column using wet packing method (Figure 2.3), this method is also modification of [74]. The column is loaded with 0.2-0.5 g of ethanolic extract of grape seed, gradient elution using two type of mobile phase (solvent system is used) these are; n-Hexane: Ethyl-acetate: Acetonitrile (9:1:1) and Chloroform: Ethanol (7:2) at a rate of 5 mL/min, collecting the eluate 10 mL/bottle. The eluates are then vaporized, the remainder was dissolved in 10 ml methanol, then the samples were taken for HPLC analysis, in this total of 10 eluates was collected from first mobile phase system hexane: ethyl acetate: acetonitrile and total of thirty eluates was collected from chloroform: Ethanol mobile phase.



Figure 2.2. Showing silica gel column chromatographic set-up



Figure 2.3. Showing Alumina column chromatographic set-up

2.2.7.3. DOWEX (cation exchange)

The concentrated extract obtained from grape seed was loaded onto column to be chromatographed over a cation-exchange resin column (DOWEX, Sigma-Aldrich), the column is prepared by measuring 30 g of DOWEX resin into a beaker and packed to a column using wet packing method (Figure 2.4), the column is firstly washed with distilled water to remove any trace of sugars and other water soluble substances, and the finally eluted with 75 % (v/v) aqueous ethanol, only the ethanolic eluates are collected, as water

eluate prove not to elute resveratrol from Dowex column [75] total of twenty eluates were collected, and 1mL of each is transferred into HPLC vial for analysis of resveratrol.



Figure 2.4. Showing Dowex cation exchange chromatographic set-up

2.2.7.4. DOWEX (anion exchange)

The concentrated extract obtained from grape seed was loaded onto column to be chromatographed over a anion-exchange resin column (DOWEX, Sigma-Aldrich), the column is prepared by measuring 30 g of DOWEX resin into a beaker and packed to a column using wet packing method (Figure 2.5), the column if firstly washed with distilled water to removed any trace of sugars and other water solubale ubstances, and the finally

eluted with 75 % (v/v) aqueous ethanol, only the ethanolic eluates are collected, as water eluate prove not to elute resveratrol from Dowex column, the same way as it was conducted with cation Dowex [75] total of twenty eluates were collected and 1 mL of each is transferred into HPLC vial for analysis of resveratrol.



Figure 2.5. Showing Dowex anion exchange column chromatographic set-up

2.2.7.5. Florisil column preparation

Florisil column is prepared by weighing exactly 30g of the adsorbent into column (it was packed according to wet method (Figure 2.6), the column if prepared using hexane: ethyl acetate (9:1) mobile phase, the column is then loaded with the crude extract obtained from grape seed, the column is washed with this mobile phase, the eluates are collected at 10mL per bottle, after collecting 15 eluates from this mobile phase, new mobile phase is then introduce this is chloroform: methanol, in this also 15 eluates was collected at 10mL per bottle, the eluates from both mobile phases are then taken for HPLC analysis of resveratrol.



Figure 2.6. Showing Florisil column chromatographic set-up

2.2.8. HPLC method for the determination of resveratrol

The amount of resveratrol in the eluents is to be determine using high-performance liquid chromatography (HPLC) according to Zhao's method [76]. 1mL of the ethanolic extract was measured into HPLC vial for analysis. The Agilent 5 TC-C18 (2) column (25.0 cm x 4.60 mm x 5.0 μ m) was used in the determination of *cis* resveratrol and *trans* resveratrol. Methanol: Acetonitrile: 0.1% phosphoric acid solution (6:1:3 v/v) and methanol: water (98:2) are used as a mobile phase with the flow rate of 1.0 mL/min and also 1.5mL/min, also PDA detector was used for *cis*- resveratrol and *trans* resveratrol at 308 nm. Preparation. Stock solutions of each standard (*trans*-/ *cis*-resveratrol) were prepared by dissolving each analyte (1.0 mg/mL) in pure 96 % ethanol. The working solution was prepared by diluting the stock solution to obtain appropriate concentrations for calibration curves (see figure 3.1 and 3.2). To plot a standard calibration curve, 0.02 g of standard resveratrol sample will weigh and then place in a 50 mL brown volumetric flask, methanol (other mobile phase) solution will be added to scale, followed by regular shaking, then got the storing liquid to be 400 mg/L; diluting the stock solution to 16 (μ g/mL) ppm, 32 ppm, 48ppm, 64 ppm (see figure 3.4) and 96 ppm for *cis* resveratrol standard solutions, then 18 ppm, 36 ppm, 54 ppm, 72 ppm (see figure 3.3) and 90 ppm for *trans* resveratrol, The injection volume from HPLC auto sampler is 20 μ L. A standard curve is drawn where the ordinate represents the peak area, and the abscissa represents the mass concentration of resveratrol. To determine resveratrol in the samples. Resveratrol identification is to be made by comparing the relative retention times of sample peaks with standards. And the calibration curve will be use in determining amount of resveratrol in each and every sample. The following equations were used to calculate the amounts of *cis* and *trans* resveratrol in each sample. The calculations for all concentrations of *cis* and *trans* resveratrol were con ducted using standard calibration curves. The right equations deducted from result section is (Figure 3.6 and Figure 3.7 given in the results section)

Trans resveratrol determination used this formula $y = 89425x - 65612$ $R^2 = 0,9955$

Cis resveratrol determination used this formula $y = 1458.5x + 2172.7$ $R^2 = 0,9965$

2.2.9. Statistical analysis

All measurements were triplicated. The statistical analysis of results obtained was performed the PC running on Windows 10 with SPSS 10.0 and for the variance analysis and LSD, multiple. comparison tests were performed in between $p < 0.05$ to $p < 0.005$ level.



3. RESULT

Below are samples of chromatograms obtained from various stages of our investigations, as the main aim of this project suggested we need to establish an optimum condition both, Column and HPLC separation of resveratrol, here samples of chromatograms from two different mobile phases are presented, and thereby discussed.

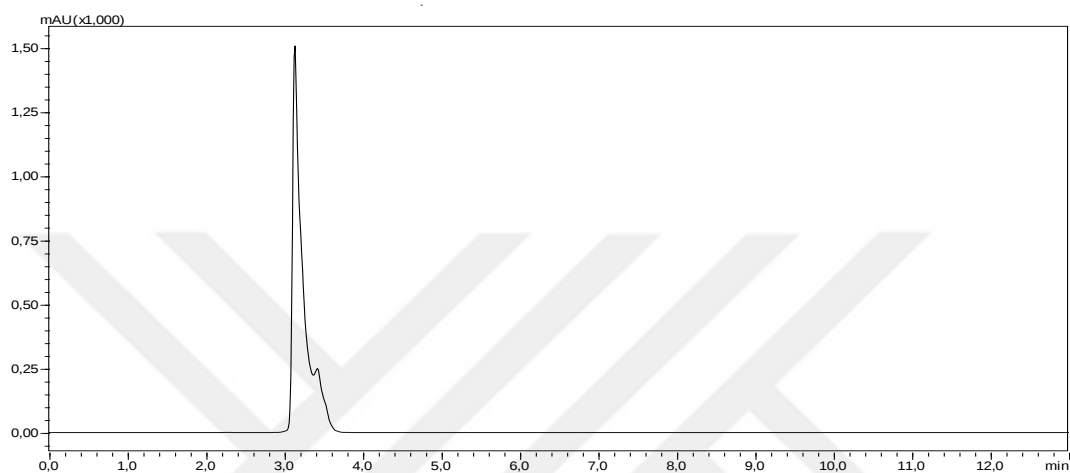


Figure 3.1. 72 ppm *Trans* resveratrol chromatogram (Retention time 3.1 -3.4 minutes ranged) : Mobil Phase Methanol :Water (98:2 v/v) ; Flow rate :1.5 mL/min Wavelength : 308 nm

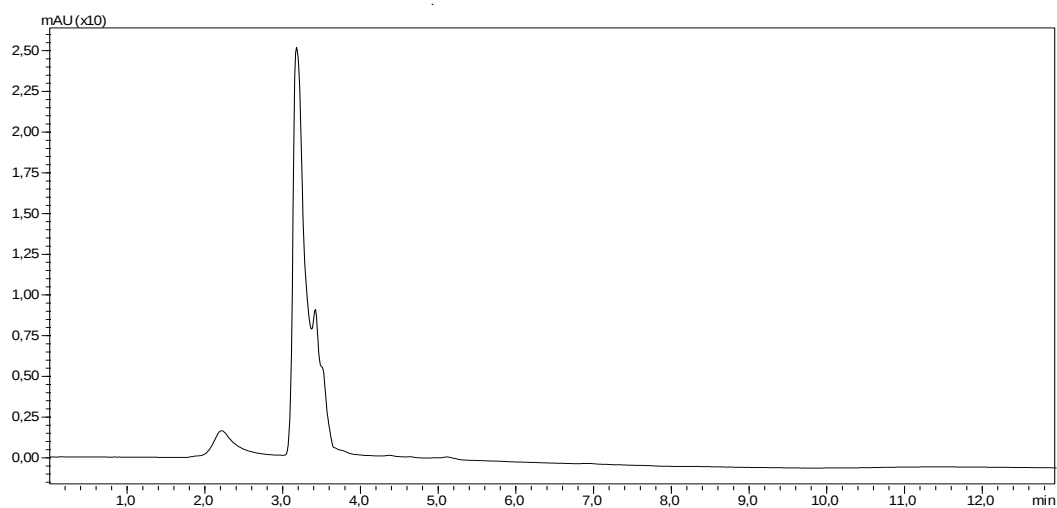


Figure 3.2. 96 ppm *Cis* resveratrol chromatogram (Retention time 3.1 -3.4 minutes ranged) Mobil Phase Methanol :Water (98:2 v/v) ; Flow rate :1.5 mL/min Wavelength : 308 nm

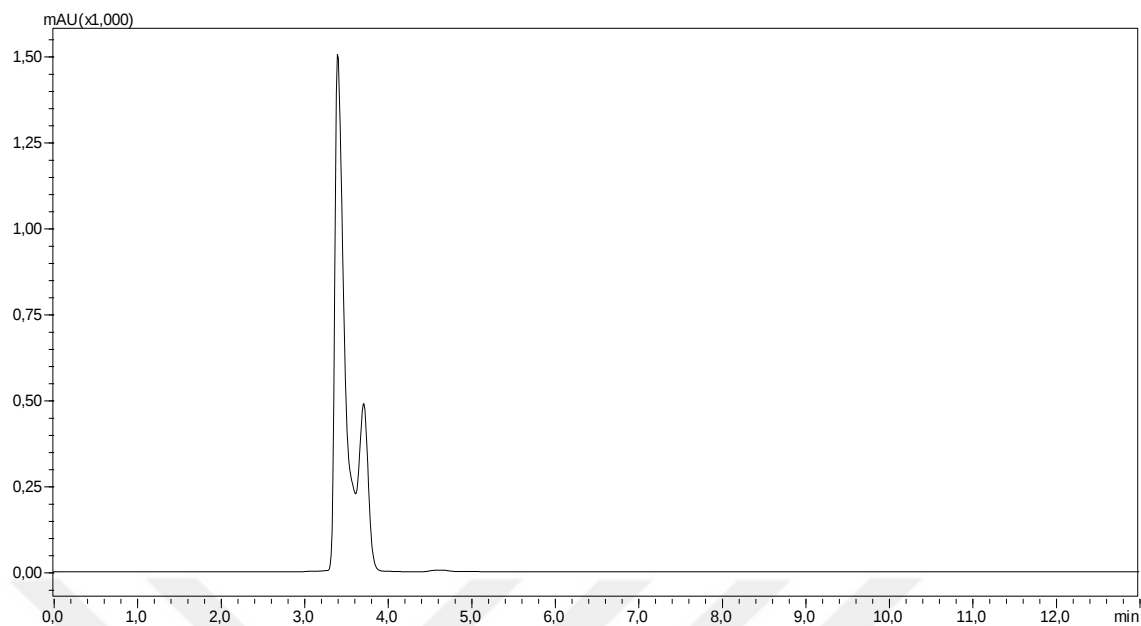


Figure 3.3. 72 ppm *Trans* resveratrol chromatogram. Retention time ranged between 3.42 -3.72 minutes. Mobil Phase; Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v)) Flow rate :1.5 mL/min Wavelength : 308 nm

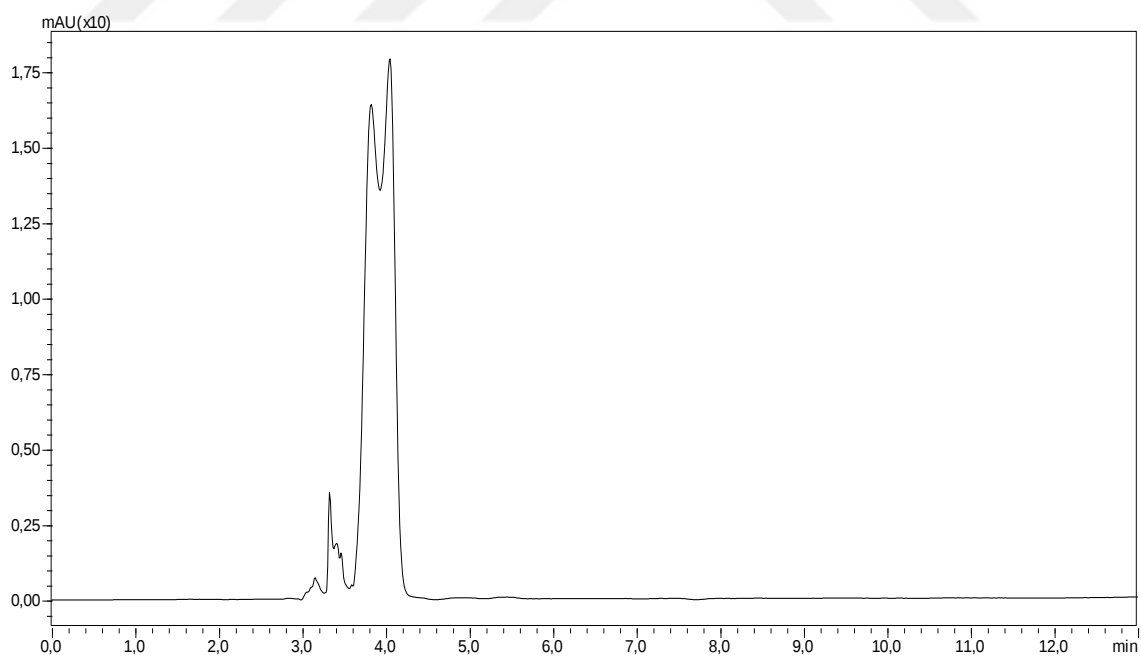


Figure 3.4. 64 ppm *Cis* resveratrol chromatogram. Retention time ranged between 3.82 -4.05 minutes. Mobil Phase; Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v)) Flow rate :1.5 mL/min Wavelength : 308 nm.

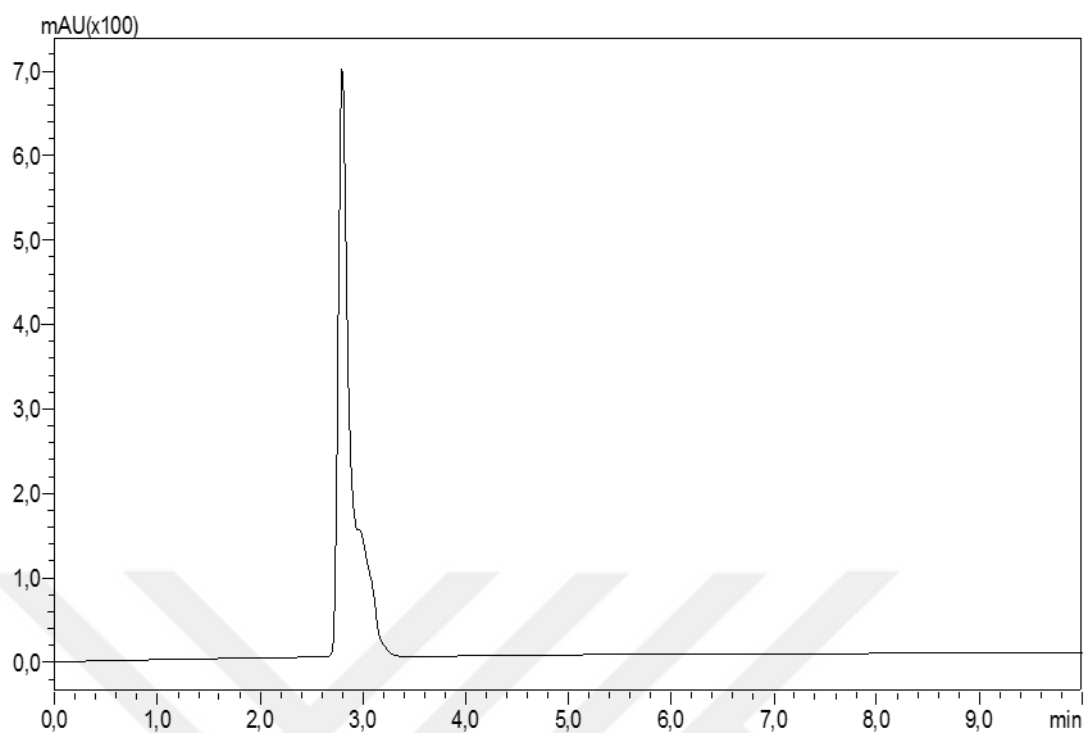


Figure 3.5a. 36 ppm *trans* Resveratrol. Retention time 2.7 minutes, Mobile phase methanol: water (98:2), Flow rate:1.0 mL/min Wavelength : 308 nm

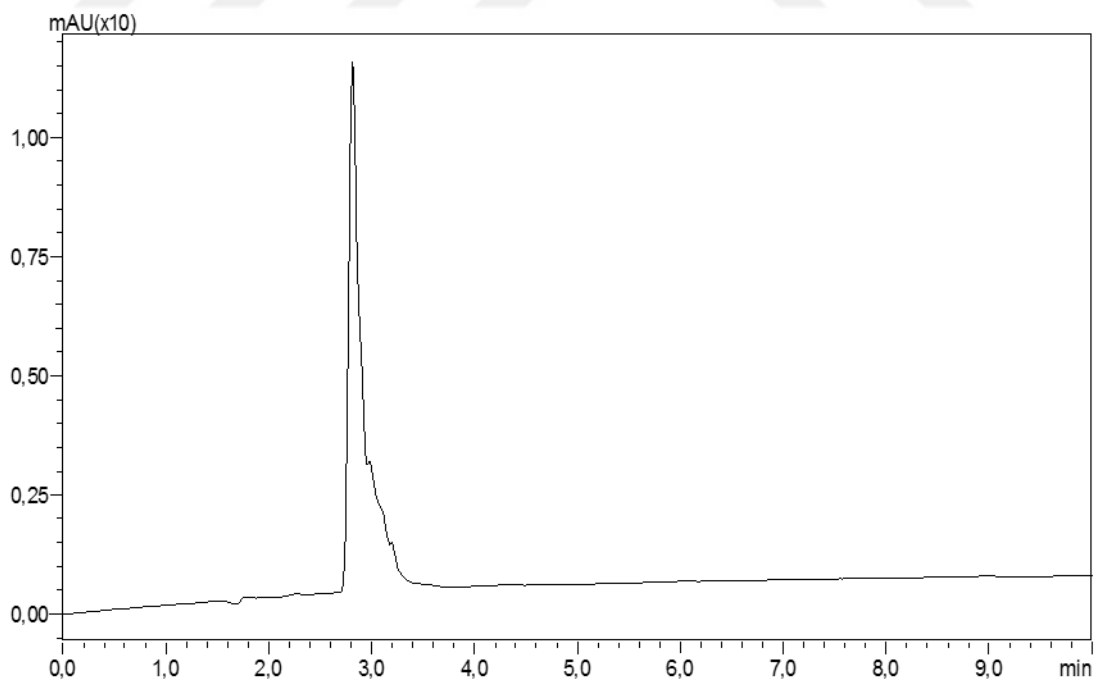


Figure 3.5b. 32 ppm *trans* Resveratrol. Retention time 2.8 minutes, Mobile phase methanol: water (98:2), Flow rate:1.0 mL/min Wavelength

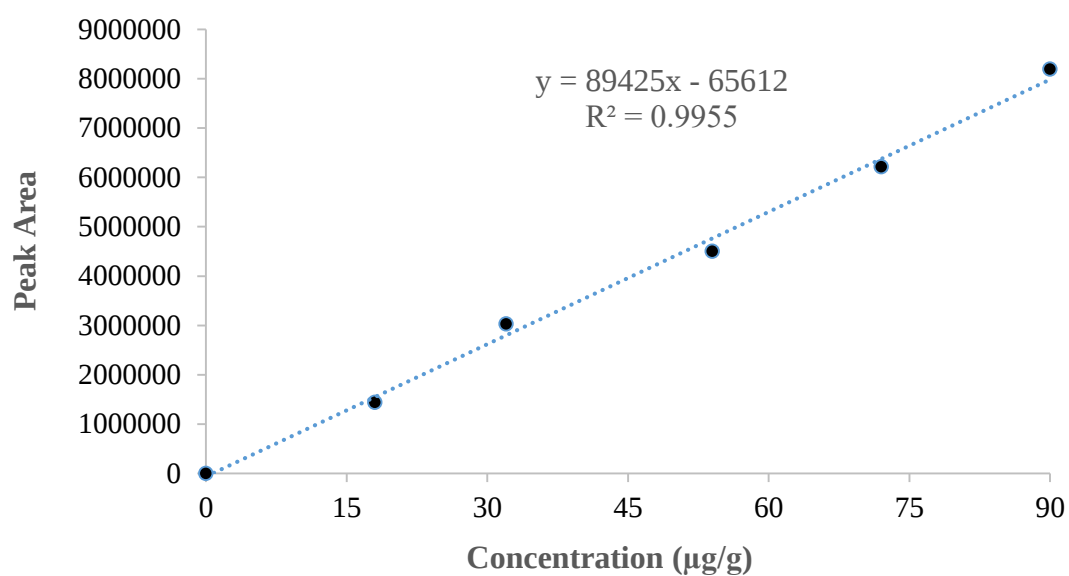


Figure 3.6. Standard curve for *trans*-resveratrol standard solution

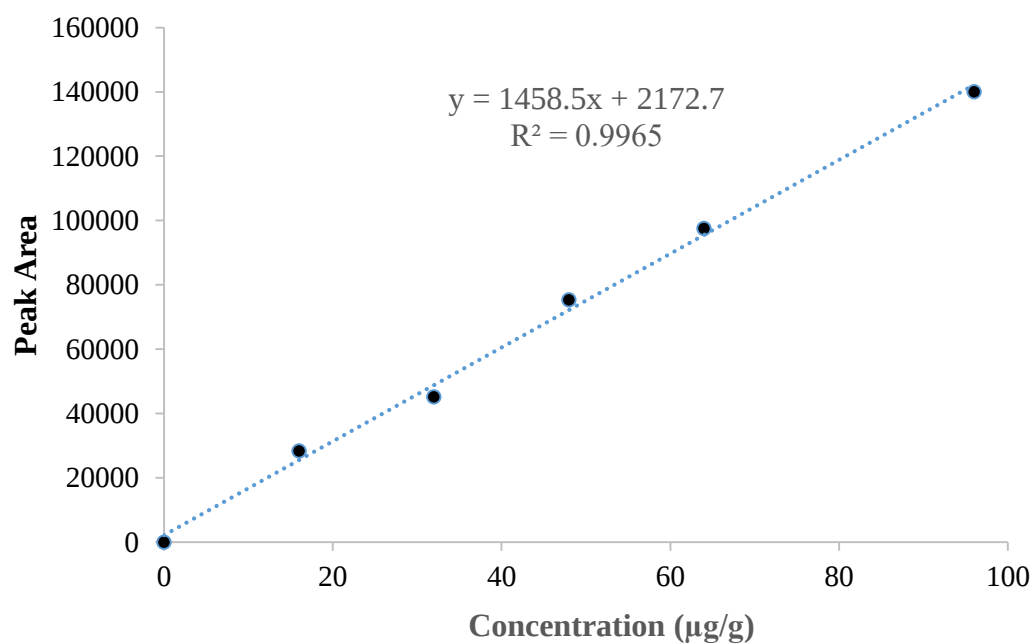


Figure 3.7. Standard curve for *cis*-resveratrol standard solution

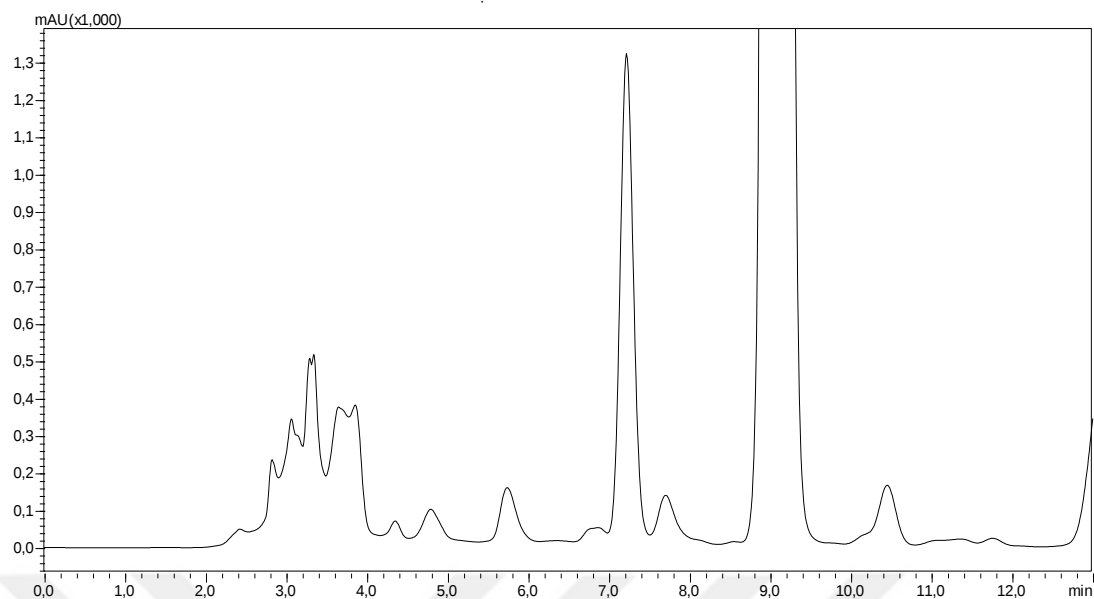


Figure 3.8. Black pepper chromatogram showing *cis* and *trans* resveratrol peak, showing both *trans* and *cis* Resveratrol peak, Retention time ranged between 3.42 -3.72 minutes and 3.82 -4.05 minutes for *trans* and *cis* resveratrol respectively, Mobil Phase; Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v)) Flow rate :1.5 mL/min Wavelength : 308 nm.

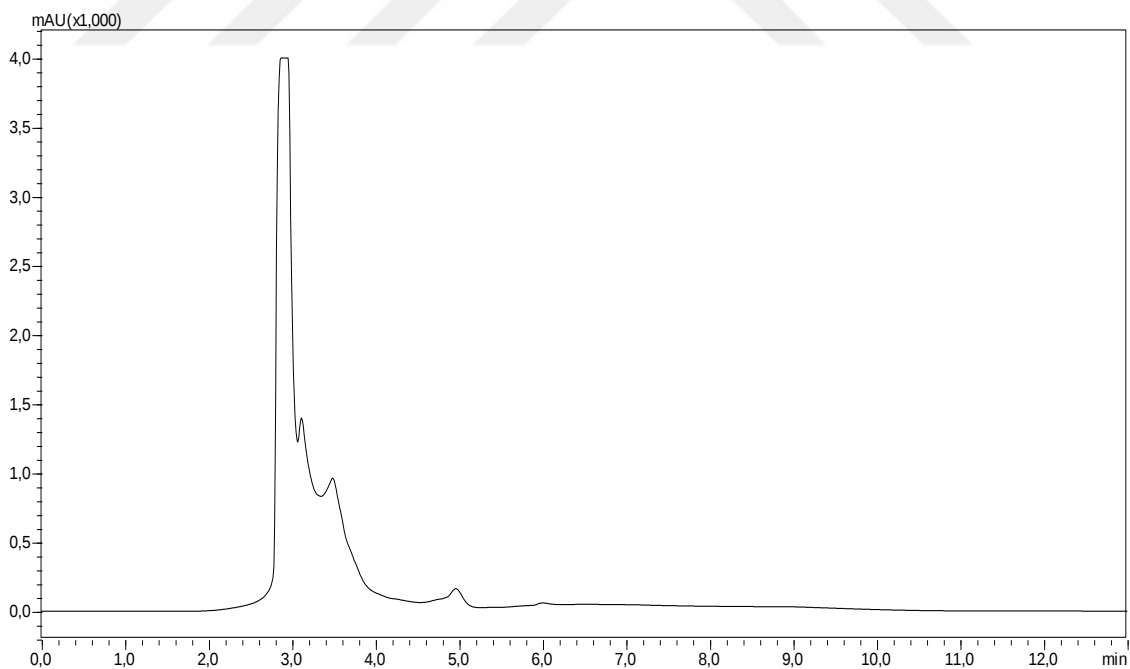


Figure 3.9. Clove sample resveratrol chromatogram(Retention time 3.1 -3.4 minutes ranged), Mobil Phase Methanol :Water (98:2 v/v)) ; Flow rate :1.5 mL/min Wavelength : 308 nm

Table 3.1. The amounts of *Cis* and *Trans* Resveratrol in some foods

Food sample	<i>Trans</i> Resveratrol (µg/g)	<i>Cis</i> Resveratrol (µg/g)
Hazelnut (<i>Corylus</i>)	23.95 ± 1.16	903.52 ± 7.29
Rosehip (<i>Rosa canina</i>)	42.84 ± 1.18	
Almond kernel (<i>Prunus dulcis</i>)	03.92 ± 0.12	
Apricot kernel (<i>Prunus armeniaca</i>)	02.71 ± 0.09	
Sumac (<i>Rhus</i>)	9.97 ± 0.79	642.99 ± 13.43
Cherry black (<i>Prunus avium</i>)	30.34 ± 2.47	
Pistachios (<i>Pistacia vera</i>)		236.18 ± 9.98
Cherry red (<i>Prunus avium</i>)	30.35 ± 0.79	
Red beet (<i>Beta vulgaris</i>)		15.07 ± 4.50
Plum (<i>Prunus domestica</i>)	20.77 ± 1.50	
Lentil (<i>Lens culinaris</i>)		277.22 ± 33.69
Black pepper (<i>Piper nigrum</i>)	374.75 ± 3.40	164.01 ± 2.67
Quince kernel (<i>Cydonia oblonga</i>)		2.58 ± 0.085
Garlic (<i>Allium sativum</i>)		271.67 ± 12.71
Goat horn (<i>Ceratonia siliqua</i>)		141.79 ± 3.38
Hackberry (<i>Celtis australis</i>)		281.62 ± 8.07
Cinnamon (<i>Cinnamomum verum</i>)	7.06 ± 0.04	
Date fruit seed (<i>Phoenix dactylifera</i>)	04.71 ± 0.50	
Donut (<i>Nigella sativa</i>)		174.12 ± 4.05
Turmeric powder (<i>Curcuma longa</i>)	384.32 ± 7.89	
Strawberry (<i>Fragaria ananassa</i>)	45.67 ± 2.33	
Clove (<i>Dianthus chinensis</i>)	443.66 ± 9.12	321.81 ± 5.24

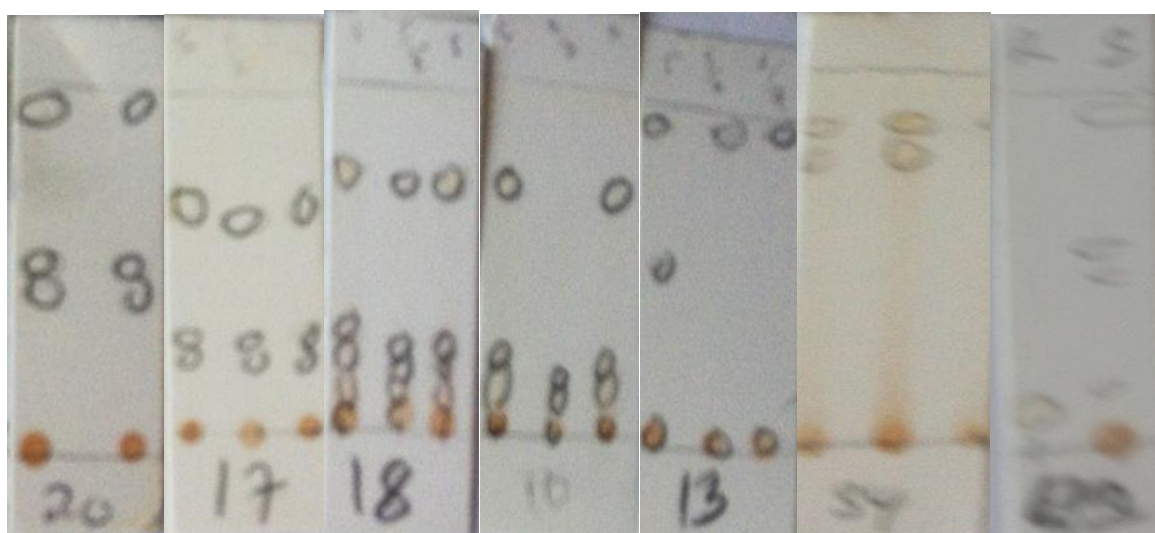
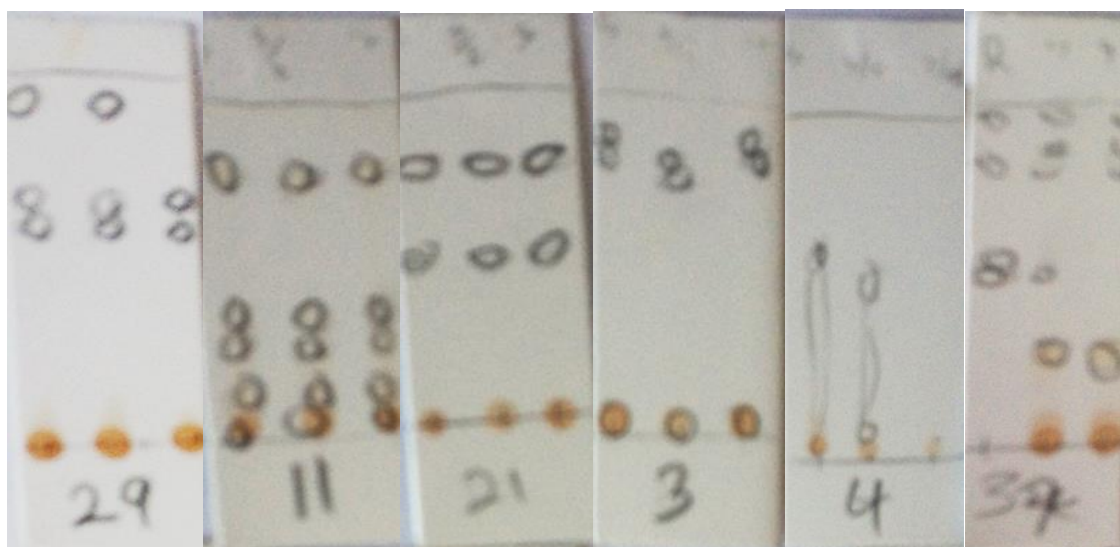


Figure 3.9.1. Showing pictures of silica gel coated TLC for determination of best solvent system for separation of resveratrol from crude mixer.

Table 3.2. Collected eluates from Silica gel, Chloroform: Methanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Presence of resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Eluent number	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Presence of resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Eluent number	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Presence of resveratrol	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Eluent number	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Presence of resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

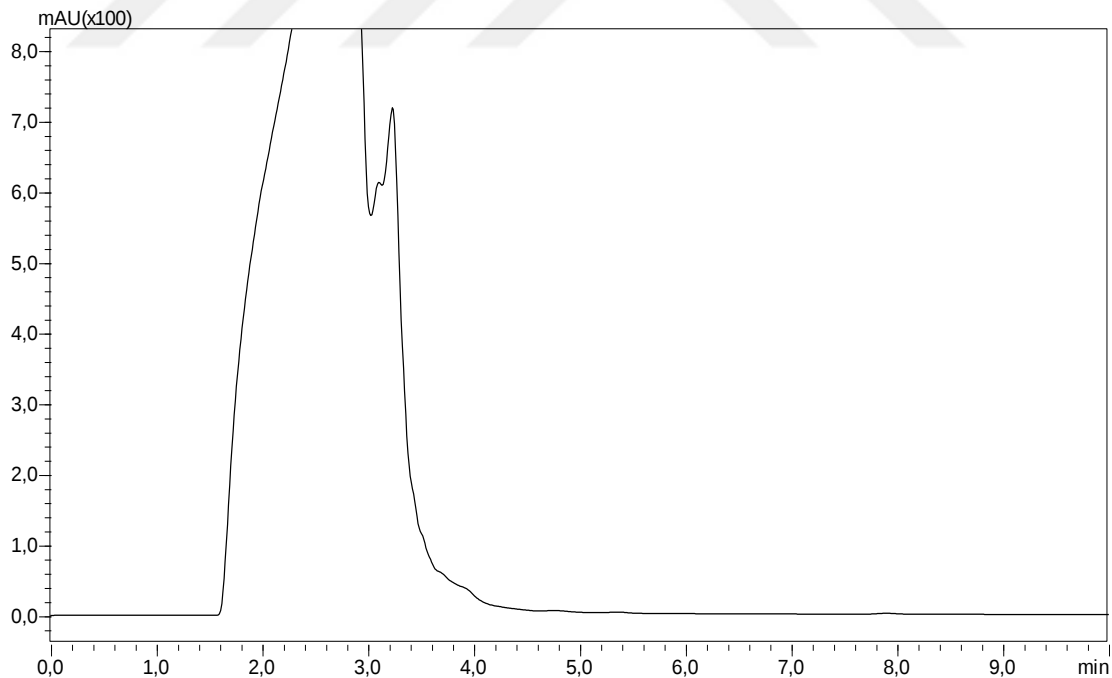
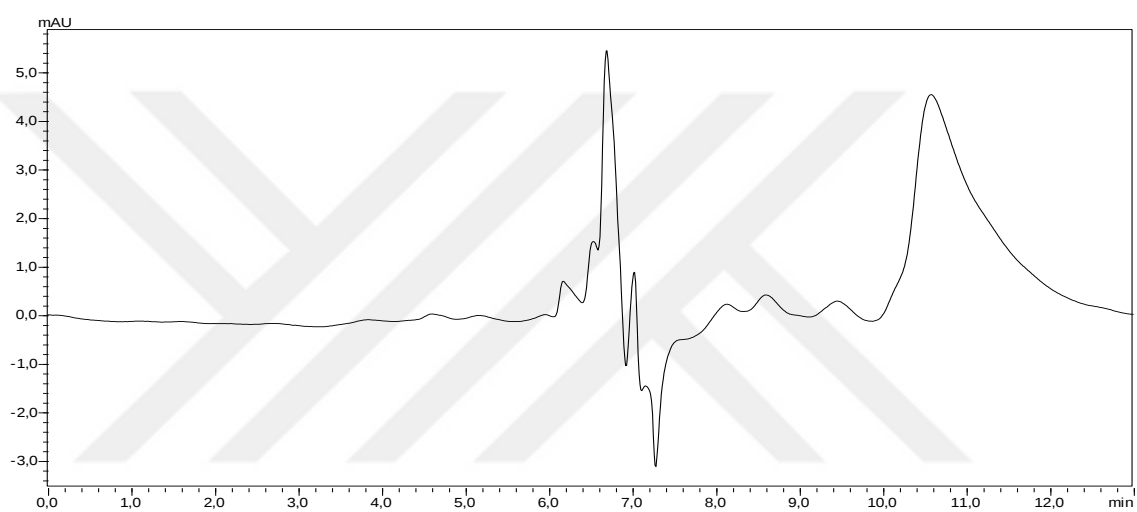


Figure 3.9.2. Showing chromatogram of purified resveratrol collected from silica gel column. Mobil Phase; Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v)) Flow rate :1.5 mL/min Wavelength: 308 nm.

Table 3.3: Collected eluates from Dowex (Cation exchange column), 70% ethanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Presence of res-veratrol	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	–	–	–	–	–

**Figure 3.9.3.** Dowex cation exchanger Column eluate chromatogram, Retention time 6.8-7.3minutes. Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v), Flow rate:1.0 mL/min Wavelength : 308 nm**Table 3.4.** Collected eluates from Dowex (anion exchange column), 70% ethanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Presence of resveratrol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–

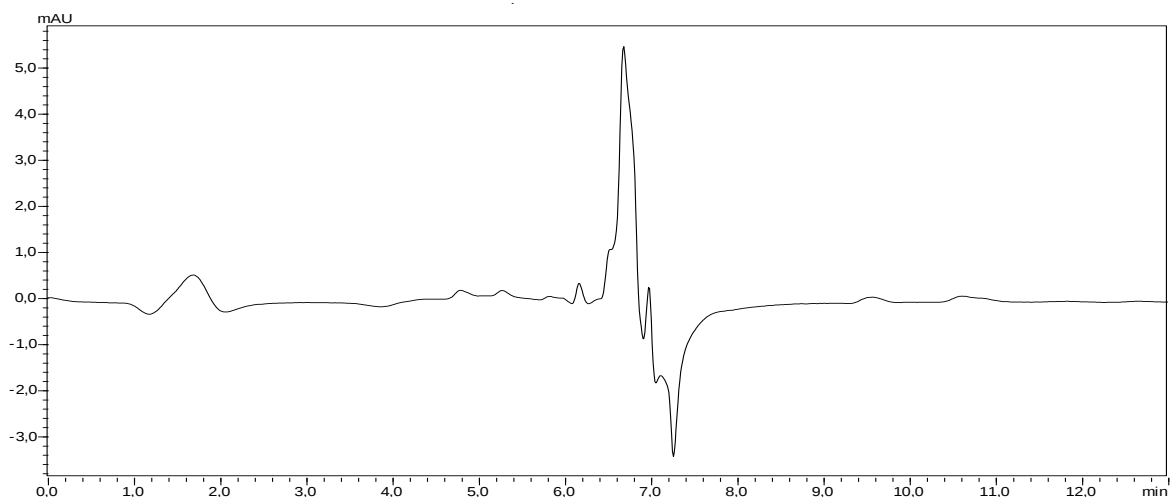


Figure 3.9.4. Dowex anion exchanger column eluate chromatogram, Retention time 6.8-7.3minutes.
Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v), Flow rate:1.0 mL/min Wavelength : 308 nm

Table 3.5: Collected eluates from Florisil column chloroform/methanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Presence of resveratrol	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+	—	—	—

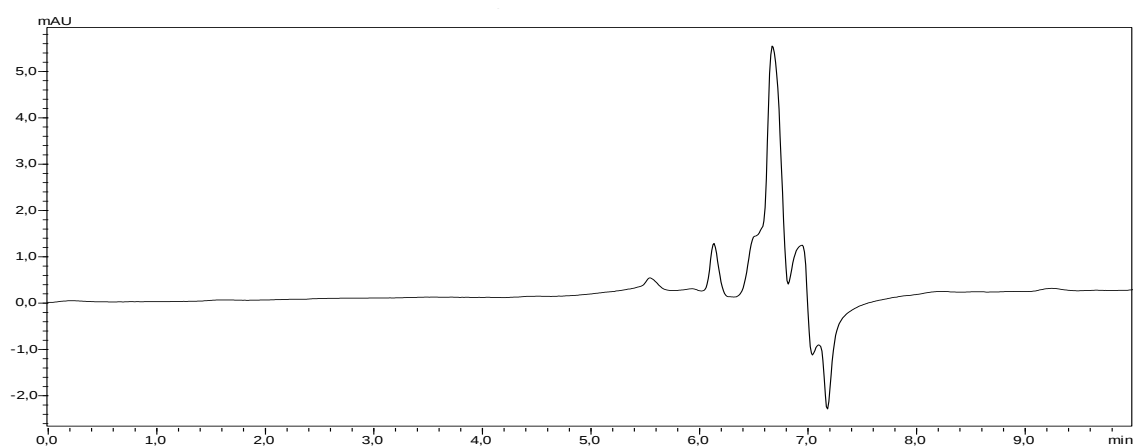


Figure 3.9.5. Florosil column eluate chromatogram, Retention time 6.8-7.3minutes

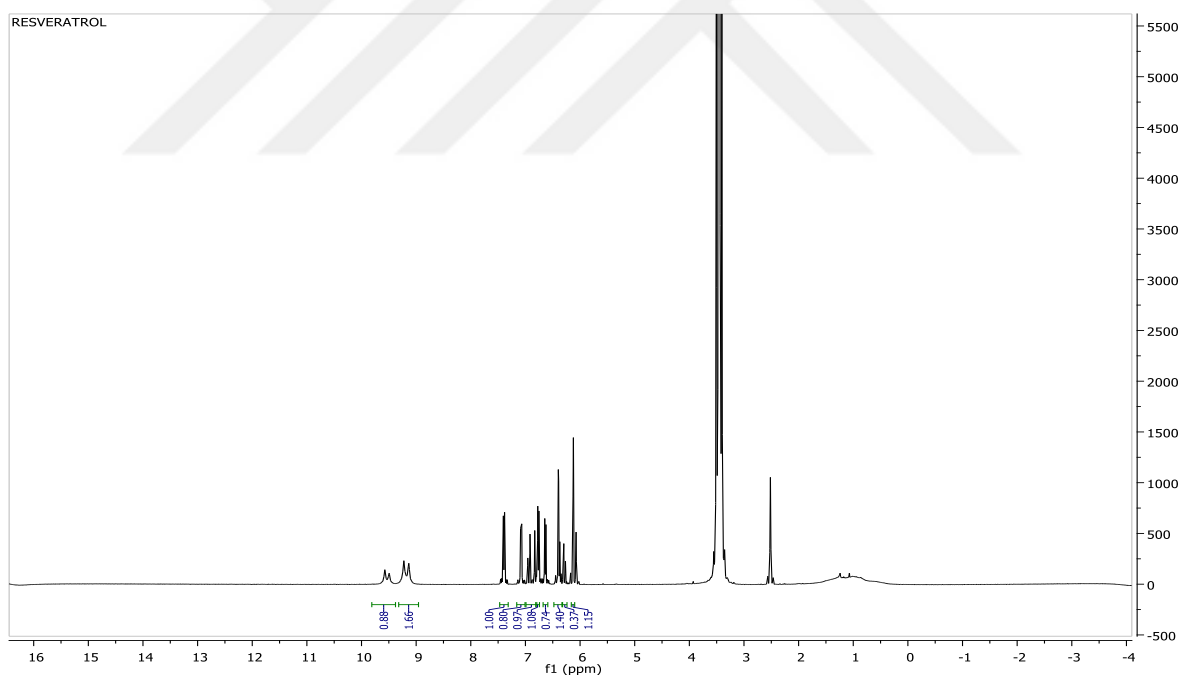
Analysis condition; Methanol: Acetonitrile: 0.1% Phosphoric acid (60:30:10 v/v), Flow rate:1.0 mL/min
Wavelength : 308 nm

Table 3.6: Collected eluates from Florisil column 70% ethanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Presence of resveratrol	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.7: Collected eluates from Alumina column chloroform/methanol mobile phase

Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Presence of resveratrol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eluent number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Presence of resveratrol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

**Figure 3.9.6** Resveratrol (400 MHz- DMSO- d_6) ^1H -NMR spectra, purified from black grape seed by column chromatography.

The peaks at 2.56 and 3.30 ppm belong to proton residues in the solvent. Olefinic and aromatic proton peaks were observed at 6.80-7.40 ppm while the peak belonging to phenolic OH protons were observed at 9.18 and 9.51 ppm.

4. DISCUSSION

4.1. Amounts of resveratrol in some selected foods/fruits/herbs samples

Resveratrol has been proven by different and diverse scientific researches and investigation to have a very good biological activity [8]. This research tries to investigate the presence of resveratrol in some food/fruits grown in Turkey, several samples were screened for presence of resveratrol qualitatively and quantitatively (almost 50 different samples) at the end total of twenty-two samples were confirmed to have presence of resveratrol in them, both *cis* and *trans*-resveratrol levels was determined. In comparison base on our result it can be noted that; Black pepper, Hazelnut, Sumac and clove are the only samples containing both *trans* and *cis* isomers of resveratrol with levels of *trans/cis* isomers of $374.75 \pm 3.40 \mu\text{gg}^{-1} / 164.01 \pm 2.67 \mu\text{gg}^{-1}$, $23.95 \pm 1.16 \mu\text{gg}^{-1} / 903.52 \pm 7.29 \mu\text{gg}^{-1}$, $9.97 \pm 0.79 \mu\text{gg}^{-1} / 642.99 \pm 13.43 \mu\text{gg}^{-1}$ and $443.66 \pm 9.12 \mu\text{gg}^{-1} / 321.81 \pm 5.24 \mu\text{gg}^{-1}$ for Black pepper (see figure 3.8), Hazelnut, Sumac and clove (see figure 3.9) respectively (Table 3.1), this is in compatibility with many finding that not all the two isomers are usually found in single source [77]. Also, it was discovered that samples with *cis* isomer happened to have large store of resveratrol. Samples that were found to contained only *trans* resveratrol in our studies are: Rosehip $42.84 \pm 1.18 \mu\text{gg}^{-1}$, Almond kernel $03.92 \pm 0.12 \mu\text{gg}^{-1}$, Cherry black $30.34 \pm 2.47 \mu\text{gg}^{-1}$, Cherry red $30.35 \pm 0.79 \mu\text{gg}^{-1}$, Plum $20.77 \pm 1.50 \mu\text{gg}^{-1}$, Cinnamon $7.06 \pm 0.04 \mu\text{gg}^{-1}$, Date fruit seed $4.71 \pm 0.50 \mu\text{gg}^{-1}$, Turmeric powder $384.32 \pm 7.89 \mu\text{gg}^{-1}$ and Strawberry $45.67 \pm 2.33 \mu\text{gg}^{-1}$. And samples that were found to contained *cis* resveratrol only are: Pistachios $236.18 \pm 9.98 \mu\text{gg}^{-1}$, Red beet $15.07 \pm 4.50 \mu\text{gg}^{-1}$, Lentil $277.22 \pm 33.69 \mu\text{gg}^{-1}$, Quince kernel $2.58 \pm 0.085 \mu\text{gg}^{-1}$, Garlic $271.67 \pm 12.71 \mu\text{gg}^{-1}$, Goat horn $141.79 \pm 3.38 \mu\text{gg}^{-1}$, Donut $174.12 \pm 4.05 \mu\text{gg}^{-1}$, and Donut $174.12 \pm 4.05 \mu\text{gg}^{-1}$. Many works from previous literature presented resveratrol of some fruit/vegetable/food for example resveratrol has been reported with concentration of $18.4 \pm 1.6 \mu\text{gg}^{-1}$ [78] which is very lower than some of our findings (e.g. Black pepper $374.75 \pm 3.40 \mu\text{gg}^{-1}$) and also higher than some (e.g. Apricot kernel $02.71 \pm 0.09 \mu\text{gg}^{-1}$). *Trans*-resveratrol reported to be detected blueberry and bilberry samples, with level of $140.0 \pm 29.9 \text{ pmol/g}$ and $71.0 \pm 15.0 \text{ pmol/g}$ for blueberry and bilberry from Poland respectively [79] this is really different with our finding by comparing a sister fruit strawberry with content of $45.67 \pm 2.33 \mu\text{gg}^{-1}$ also like other berries only *trans*-isomer detected. Another different worked found resveratrol in strawberry to be $1.87 \pm 0.08 \mu\text{gg}^{-1}$ [80] a very low compare to this our work. Looking at our result presented here it can be

easily said that; a major finding as far as resveratrol is concern has been obtained, and a relatively high amount of this molecule is established because throughout literature only one sample we came across to have high resveratrol content close to some of our finding here the said finding reported that *Polygonum cuspidatum* Sied. et Zucc. Contained 1.556mg/g and Zijin capsule with 75.93 mg/g [81] but these are resveratrol supplement capsule, the former is within the range of our finding but the latter is very higher with average results of our work. Another similar work try to assess level of resveratrol in different species of berry fruits [82] but none of the amounts found there are higher than our average finding. Highest amount of resveratrol reported in Japanese Plum is $6.2 \mu\text{gg}^{-1}$ [83] which lower than our finding of $20.77 \pm 1.50 \mu\text{gg}^{-1}$, based on our investigation resveratrol has not been reported in Hazel nut but here we find it to contain an appreciable amount of $23.95 \pm 1.16 \mu\text{gg}^{-1}$ (*trans* only), also resveratrol has not been directly reported in Rose hip, Almond kernel, Apricot kernel, Sumac, cherry, Red beet, Lentil, Black pepper, Quince kernel, Garlic, Goat horn, Hackberry, Cinnamon, Date fruit seed, Donut as well as in Turmeric. In a research pistachio contained $0.09\text{--}1.67 \mu\text{gg}^{-1}$ ($\text{av} = 1.15 \mu\text{gg}^{-1}$) of resveratrol [84] where as in our finding this nut contains $30.35 \pm 0.79 \mu\text{gg}^{-1}$ also of *trans*-resveratrol. While other samples screened in this has no resveratrol content also has no reported resveratrol from literature, only pea nut was reported to have resveratrol in it, some of this literature work had it that pea nut level of resveratrol can be $5.138 \pm 2.849 \mu\text{gg}^{-1}$ [85] but our finding here contradicts this report as we cannot establish presence of resveratrol in pea nut.

4.2. Findings on Best mobile phase determination for resveratrol separation using TLC

The aim of this section of our research is to verify and/or establish a very good solvent systems that can best separate resveratrol from column chromatography, some previous work used dichloromethane on silica gel 60 coated TLC [86], others also try to find dissolution coefficient of resveratrol and other stilbenes using different mixers of solvents [87], running more than 40 TLC plate and alternating solvent composition and ratios we found some solvent system to give a better resolution and separation of resveratrol from crude ethanolic extract containing this compound, the best developed plates here as can be seen from TLC chromatogram pictures above are: plate number 16,17,18,19 and 20 (see figure 3.9.1 for various TLC chromatogram pictures). Although mixture of

Chloroform/methanol and n-hexane/ethyl acetate are both found to separate our crude extract but only chloroform/methanol elute our target molecule (resveratrol), the former solvent system removed many fractions without moving the target molecule, this is evident when a standard resveratrol spot is run together on TLC plate with crude extract sample. Also for HPLC different mobile phase and HPLC condition were tried to get the best separation (see figure 3.5a and figure 3.5b)

4.3. Findings on Best column filler (stationary phase) for separation of resveratrol from crude extract.

Several type of column fillers/stationary phase against mobile phase are used by analytical scientist to isolate natural products and organic compounds [88]. Several researchers for decades has been trying to isolate resveratrol form different plant's sources but yet the isolation and absolute purification of this compound is still a subject of researches, some of these early attempt can be seen in [86]. From our result and finding in this research four of the combination (stationary phase/mobile phase) used in this research prove to have an average reliability in separation of resveratrol from crude extract of plants source, but from our HPLC chromatogram; silica gel has the best separation power for this compound, this is because only silica gel HPLC chromatogram, shows relatively pure resveratrol presence although two other peak appear but suspected to be resveratrol glycoside and viniferin, (see figure 3.9.2) similar result is obtained from previous literature[64] Another column filler with much resveratrol resolution is Dowex (cation exchange) this according to [75] appear to hold resveratrol after eluting with water, it can then be recover by using aqueous ethanolic solution as mobile phase (see figure 3.9.3), anion exchange of Dowex also prove to separate resveratrol but the recovery is much less than that of the cation counterpart (see figure 3.9.4). Florisil although not been tried before by in isolation of resveratrol, according to our literature survey, but also we have found it to be good adsorbent to isolate resveratrol and its family, but as it was notice with eluents results of florisil column from HPLC chromatogram (see figure 3.9.5), has a rather tailing properties in separation, but can also resolute between *trans* and *cis* isomers of resveratrol, this is a very good quality noticed with florisil that other column filler did not demonstrate even the better silica gel (see figure 3.9.1). According to our finding here in this research, alumina column is totally negative as regard to isolation of resveratrol, this is because all the resveratrol has been almost permanently (maybe

covalently) adsorbed by the alumina as any attempt to alter the polarity of our mobile phase in order to recover resveratrol proved abortive. The confirmation of isolated and purified resveratrol is established using both HPLC and NMR spectra as can be seen (Figure 3.9.6) after hydrolysis and silica gel column chromatogram from black grape seed shows that the purification of resveratrol has been successful.



LITERATURE

- [1] **Ness AR., Fowles JW.,** 1997. Fruit and vegetables and cardiovascular disease: a review. *Int J Epidemiol.* 26:1-13.
- [2] **Rui Hai L.,** 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am J Clin Nutr* 78:517S-20S.
- [3] **Bhardwaj R.L., Pandey S.,** 2011. Juice blends – A way of utilization of underutilized fruits, vegetables, and spices. A review, *Crit. Rev. Food Sci.* 51:563-570.
- [4] **Van Duyn MA., Pivonka E.,** 2000. Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *J Am Diet Assoc.* 100:1511–21.
- [5] **Counet C., Callemien D., Collin, S.,** 2006. Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. *Food Chem.* 98:649-657.
- [6] **Wang Y., Catana, F., Yang, Y., Roderick., R, Breemen, R.,** 2002. An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and wine. *J. Agric. Food Chem.* 50:431- 435.
- [7] **Careri M., Corradini C., Elviri L., Nicoletti I., Zagnoni I.,** 2003. Direct HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts. *J. Agric. Food Chem.* 51:5226-5231.
- [8] **Yilmaz Y., Toledo R.T.,** 2004. Major Flavonoids in Grape Seeds and Skins: Antioxidant Capacity of Catechin, Epicatechin, and Gallic Acid. *J. Agric. Food Chem.* 52:255–260.
- [9] **Lucie F.,** 2000. Biological Effects of Resveratrol. *Life Sciences.* 66 (8):663–673
Retrieved 6 June 2014
- [10] **Renaud S., Lorgeil M. W.,** 1992. alcohol, platelets, and the French paradox for coronary heart disease. *Lancet.* 339(8808):1523-1526.
- [11] **Schroder G., Brown J.W., Schroder J.,** 1988. Molecular analysis of resveratrol synthase. cDNA, genomic clones and relationship with chalcone synthase. *Eur J Biochem,* 172 (1):161–169.
- [12] Resveratrol MSDS on *Fisher Scientific* website.
- [13] **Camont L., Cottart C.H., Rhayem Y., Nivet-Antoine V., Djelidi R., Collin F., Beaudeux J.L., Bonnefont-Rousselot D.C., Rhayem N.D., Collin B.B.,** 2009. Simple spectrophotometric assessment of the *trans*-/*cis*-resveratrol ratio in aqueous solutions. *Anal. Chim. Acta.* 634 (1): 121-8.

- [14] **Tome-Carneiro J., Larrosa M., Gonzalez-Sarrias A., Tomas-Barberan F.A., Garcia Conesa M.T., Espin J.C.,** 2013. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. *Curr Pharm Des.* 19(34):6064-6093.
- [15] **En-Qin Xia, Gui-Fang Deng, Ya-Jun Guo and Hua-Bin Li.** 2010. A review of Biological Activities of Polyphenols from Grapes. *Int. J. Mol. Sci.* 11(2), 622-646.
- [16] **Fremont L.,** 2000. Biological effects of resveratrol. *Life Sci.* 66:663–673.
- [17] **Silvia Bradamante.,** 2004. Cardiovascular Protective Effects of Resveratrol. *Cardiovascular Drug Reviews.* 22(3): 169–188
- [18] **Young-Joon S.,** 1999. Resveratrol, an antioxidant present in red wine, induces apoptos in human promyelocytic leukemia (HL-60) cells. *Cancer Letters.* 140(1–2):1 10.
- [19] **Soo-Jin L., Moon-Moo K.,** 2011. Resveratrol with antioxidant activity inhibits matrix metalloproteinase via modulation of SIRT1 in human fibrosarcoma cells. *Life Sciences,* 88(11–12): 465–472.
- [20] **Wang H., Yang Y.J., Qian H.Y., Zhang Q., Xu H., Li J.J.,** 2012. Resveratrol in cardiovascular disease: what is known from current research? *Heart Fail Rev.* 17(3):437-448.
- [21] **de Lorgeril M., Salen P., Paillard F., Laporte F., Boucher F., de Leiris J.,** 2002. Mediterranean diet and the French paradox: Two distinct biogeographic concepts for one consolidated scientific theory on the role of nutrition in coronary heart disease. *Cardiovasc Res* 54:503–515.
- [22] **Kris-Etherton P.M., Hecker K.D., Bonanome N.,** 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med* 113:71S-88S.
- [23] **Pace-Asciak C.R., Hahn S., Diamandis E.P., Soleas G., Goldberg D.M.,** 1995. The red wine phenolics *trans*-resveratrol and quercetin block human platelet aggregation and eicosanoid synthesis: implications for protection against coronary heart disease. *Clin Chim Acta.* 235(2):207-219.
- [24] **Wang Z., Huang Y., Zou J., Cao K., Xu Y., Wu J.M.,** 2002. Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro. *Int J Mol Med.* 9(1):77-79
- [25] **Kirk R.I., Deitch J.A., Wu J.M., Lerea K.M.,** 2000. Resveratrol decreases early signaling events in washed platelets but has little effect on platelet in whole blood. *Blood Cells Mol Dis.* 26(2):144-150.
- [26] **Raj P., Louis X.L., Thandapilly S.J., Movahed A., Zieroth., Netticadan T.,** 2014. Minireview; Potential of resveratrol in the treatment of heart failure. *Life Sciences.* 95(29): 63–71.
- [27] **Brisdelli F., D’Andrea G., Bozzi A.,** 2009. Resveratrol: a natural polyphenol with multiple chemopreventive properties. *Curr. Drug Metab.,* 10: 530–546.

- [28] **Aggarwal B.B., Bhardwaj A., Aggarwal R.S., Seeram N.P., Shishodia S., Takada Y.,** 2004. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res.* 24(5A):2783-2840.
- [29] **Siegel R.L., Miller K.D., Jemal A.,** 2016. Cancer statistics. *CA. a Cancer Journal for Clinicians*, 66: 7–30.
- [30] **Tomé-Carneiro J., Larrosa M., González-Sarriás A., Tomás-Barberán F.A., García Conesa M.T., Espín J.C.,** 2013. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. *Curr. Pharm. Des.* 19: 6064–6093.
- [31] **Sutton J.M.,** 2014. Neoadjuvant therapy for pancreas cancer: *Past lessons and future therapies.* 20: 15564.
- [32] **Tsubura A.,** 2012. Resveratrol induces apoptosis via ROS-triggered autophagy in human-colon cancer cells. *Int J Oncol*, 40: 1020.
- [33] **Conroy T., Desseigne F., Ychou M., Bouché O., Guimbaud R., Becouarn Y.,** 2011. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med*, 364: 1817–1825.
- [34] **Shankar S., Nall D., Tang S. N., Meeker D., Passarini J., Sharma J.,** 2011. Resveratrol Inhibits Pancreatic Cancer Stem Cell Characteristics in Human and KrasG12D Transgenic Mice by Inhibiting Pluripotency Maintaining Factors and Epithelial-Mesenchymal Transition. *PLoS ONE*, 6:16530.
- [35] **Bishayee A.,** 2009. Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials. *Cancer Prev Res (Phila)*. 2(5):409-418.
- [36] **Bishayee A., Darvesh A.S., Politis T., McGory R.,** 2010. Resveratrol and liver disease: from bench to bedside and community. *Liver Int.* 30(8):1103-1114.
- [37] **Chacona M.R., Ceperuelo M., Maymo-Masipa V., Mateo-Sanzb., Arolac J.M., Guitierrez L., Fernandez-Reald C., Ardevolc J.M., Simona A., Vendrella I. J.,** 2009. Grape-seed procyanidins modulate inflammation on human differentiated adipocytes in vitro. *Cytokine* 47, 137–142.
- [38] **Panico A.M., Cardile V., Avondo S., Garufi F., Gentile B., Puglia C., Bonina F., Santagati N.A., Ronsisvalle G.,** 2006. The in vitro effect of a lyophilized extract of wine obtained from Jacquez grapes on human chondrocytes. *Phytomedicine* 13: 522–526.
- [39] **Serra D., Almeida L.M., Dinis T.C.,** 2016. Anti-inflammatory protection afforded by cyanidin-3-glucoside and resveratrol in human intestinal cells via Nrf2 and PPAR- γ : Comparison with 5-aminosalicylic acid. *Chemico-Biological Interactions.* 260(25): 102–109.
- [40] **Jeong S.I., Shin J.A., Cho S., Kim H.W., Lee J.Y., Kang J.L., Park E.M.,** 2016. Resveratrol attenuates peripheral and brain inflammation and reduces ischemic brain injury in aged female mice. *Neurobiology of Aging.* 44: 74–84.

- [41] **Szkudelski T., Szkudelska K.**, 2015. Resveratrol and diabetes: from animal to human studies. *Biochim Biophys Acta.*; 1852(6):1145-1154.
- [42] **Xia T., Yanlin L., Guangwei R., Rufeng A.**, 2016. Resveratrol limits diabetes-associated cognitive decline in rats by preventing oxidative stress and inflammation and modulating hippocampal structural synaptic plasticity. *Brain Research*. 1650: 1–9.
- [43] **Yufeng B., Ruihua A.**, 2015. Resveratrol and sildenafil synergistically improve diabetes-associated erectile dysfunction in streptozotocin-induced diabetic rats. *Life Sciences*. 135: 43–48.
- [44] **Waite J.G., Daeschel M.A.**, 2007. Contribution of wine components to inactivation of food-borne pathogens. *Journal of Food Science*. 72: M286–M291.
- [45] **Selma M.V., Larrosa M., Beltrán D., Lucas R., Morales J.C. and Tomás-Barberán F.**, 2012. Resveratrol and some glucosyl, glucosylacyl, and glucuronide derivatives reduce Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes Scott A adhesion to colonic epithelial cell lines. *Journal of Agricultural Food Chemistry*. 60: 7367–7374.
- [46] **Xiu-Zhuang L., Xia W., Chun-Jiang Z., Xiao-Ling J., Jiang-Jiang., Gui-Juan F., Bo Z.**, 2012. Hypohalous acid-mediated halogenation of resveratrol and its role in antioxidant and antimicrobial activities. *Food Chemistry*. 135(3): 1239–1244.
- [47] **Mi O., Ji-Hye L., Seon Y. B., Jong H. S., Sella K., Yeon B. C., Kang R. H., Kyung H. K., Mi S.C.**, 2015. Protective effects of red wine and resveratrol for foodborne virus surrogates. *Food Control*. 47: 502–509.
- [48] **Faith S.A., Sweet T.J., Bailey E., Booth T., Docherty J.J.**, 2006. Resveratrol suppresses nuclear factor-kappa B in herpes simplex virus infected cells. *Antiviral Research*. 72: 242–251.
- [49] **Chung S., Yao H., Caito S., Hwang J.W., Arunachalam G., Rahman I.**, 2010. Regulation of SIRT1 in cellular functions: role of polyphenols. *Arch Biochem Biophys*. 501: 79–90.
- [50] **Whitlock N.C., Bahn J.H., Lee S.H., Eling T.E., Baek S.J.**, 2011. Resveratrol-induced apoptosis is mediated by early growth response-1 Kruppel-like factor 4, and activating transcription factor 3. *Cancer Prev Res (Phila)*. 4: 116–127.
- [51] **Berna A., Cháfer A., Montón J.**, 2001. High-pressure solubility data of the system resveratrol (3)+ethanol (2)+CO₂ (1). *J. Supercrit. Fluids*. 19: 133–139.
- [52] **Larronde F., Gaudillère J.P., Krisa S., Decendit A., Deffieux G., Mérillon J.M.**, 2014. Airborne methyl jasmonate induces stilbene accumulation in leaves and berries of grapevine plants, *Am. J. Enol Vitic*. 9:54-59.
- [53] **Landrault N., Larronde F., Mérillon J.M., and Teissedre P.L.**, 1999. Etude de stilbenes-*trans*. (astringine, resvératrol, picéïde) par CLHP-fluorimétrie au cours de la transformation du raisin en vin, *Ann. Des Falsif. l'expertise Chim. Toxicol*. 92: 443-453.

- [54] **Kübra I.E., Öz S., Atakol A., Abdülkadir M.A., Atakol O.,** 2013. Liquid-liquid. Extraction based simple *trans*-resveratrol analysis in wine samples, *Suleyman. Demirel Univ. J. Sci.* 8: 114.
- [55] **Medina-Bolivar F., Condori J., Rimando A.M., Hubstenberger J., Shelton K., O’Keefe S.F.,** 2013. Production and secretion of resveratrol in hairy root cultures of peanut. *Phytochemistry.* 68:1992–2003.
- [56] **Wang D.G., Liu W.Y., Chen G.T.,** 2007. A simple method for the isolation and purification of resveratrol from *Polygonum cuspidatum*, *J. Pharm. Anal.* 3: 241–247.
- [57] **Xiang H.Y., Zhou C.S., and Lei Q.F.,** 2005. Studies on separation and purification of piceid from *Polygonum cuspidatum* by macroporous adsorption resin, *Chin. Pharm. J.* 40:96- 98 (in Chinese).
- [58] **Benova B., Adam M., Pavli P.,** 2010. Supercritical fluid Extraction of piceid, resveratrol and emodin from Japanese knot weed, *J. Supercrit. Fluids.* 51: 325–330.
- [59] **Yu W., Shu B., Zhao Y.,** 2005. Supercritical CO₂ extraction of resveratrol and its glycoside piceid from Chinese traditional medicinal herb *Polygonum cuspidatum*, *J. Sci. Food Agric.* 85: 489–492.
- [60] **Dong-Geng W., Wen-Ying L., Guang-Tong C.,** 2013. A simple method for the isolation and purification of Resveratrol from *Polygonum cuspidatum*. *Journal of Pharmaceutical Analysis.* 3(4): 241–247.
- [61] **Dalei Z., Xiunan L., Dongxia H., Guisheng L., Benming X., Guanghui M., Zhiguo S.,** 2009. Systematic purification of polydatin, resveratrol and anthraglycoside B from *Polygonum cuspidatum* Sieb. Zucc. *Journal of Separation and Purification Technology.* 66: 329–339.
- [62] **Fuquan Y., Tianyou Z., Yoichiro I.,** 2001. Large-scale separation of resveratrol, anthraglycoside A. And anthraglycoside B from *Polygonum cuspidatum* Sieb. et Zucc by high-speed counter-current Chromatography. *Journal of Chromatography.* 919: 443–448.
- [63] **Altıok E., Bayc D., Bayraktar O., Ülküa S.,** 2008. *Sep. Purif. Technol.* 62: 342–348.
- [64] **Tang Z., Qin J., Xu X., Shi G., Yang H., Liang Y.,** 2011. Applying silica gel column chromatography purify resveratrol from extracts of *Morus alba* L. Leaf. *Journal of Medicinal Plants Research.* 5(14): 020-3027.
- [65] **Qian L., Qin Z., Qiong-Wei Y., Yu-Qi F.,** 2015. Use of Pollen Solid-Phase Extraction for the Determination of *trans*-Resveratrol in Peanut Oils. *J. Agric. Food Chem.* 63: 4771–4776.
- [66] **Juan M.E., Maijó M., Planas J.M.,** 2010. Quantification of *trans*-resveratrol and its metabolites in rat plasma and tissues by HPLC, *J. Pharm. Biomed. Anal.* 51: 391–398.
- [67] **Burkon A., Somoza V.,** 2008. Quantification of free and protein-bound *trans*-resveratrol metabolites and identification of *trans*-resveratrol-C/O-conjugated

diglucuronides –two novel resveratrol metabolites in human plasma, *Mol. Nutr. Food Res.* 52: 549–557,

- [68] **Zhang J., Zhou L., Zhang P., Liu T., Yang G., Lin R., Zhou J.,** 2015. Extraction of polydatin and resveratrol from *Polygonum cuspidatum* root: Kinetics and modeling. *Journal of Food and Bioproducts Processing.* 94:518-524
- [69] **Zhang Q., Bian Y., Shi Y., Zheng S., Gao X., Zhang D., Zhu X., Wang X., Jiang Di., Xiong Q.,** 2015. An economical and efficient technology for the extraction of resveratrol from peanut (*Arachis hypogaea*) sprouts by multi-stage countercurrent extraction. *Journal of Food chemistry.* 179:15-25
- [70] **Soural I., Vrchotová N., Tříska J., Balík J., Horník Š., Cuřínová P., Sýkora J.,** 2015. Various Extraction Methods for Obtaining Stilbenes from Grape Cane of *Vitis vinifera* L. *Molecules* 20: 6093-6112.
- [71] **Márcia L.M., Marcela B., Milena K.B., Carmen M. D., Marcelo S. K., Lucia R.C., Diógenes G. C., Campo M.V.,** 2016. *Trans*-resveratrol and antioxidant activity of grape (*Vitis vinifera* sp) byproducts, *Brazilian Journal of Food Research.* 7(1): 66-81.
- [72] **Lotz A., Spangenberg B.,** 2016. New and sensitive TLC method to measure *trans*-resveratrol in *Physalis peruviana*, *Journal of Liquid Chromatography & Related Technologies* 39(5-6): Special Issue: Thin Layer Chromatography.
- [73] **Glavnik V., Simonovska B., Albrecht A., Vovk I.,** 2012. TLC and HPLC screening of *p*-coumaric acid, *trans*-resveratrol, and pterostilbene in bacterial cultures, food supplements, and wine, *Journal of Planar Chromatography* 25:2012-3
- [74] **Nakanishi K., Minakuchi H., Soga N.,** 1998. Structure design of doublepore silica and its application to HPLC. *J. Sol-Gel Sci. Technol.*, 13(1-3): 163-169.
- [75] **Kamel A., Élodie R., Waffo-Tégou P., Jean-Michel M., Tristan R., Alain D.,** 2017. Identification and quantification of stilbenes in some Tunisian red wines using UPLC-MS and HPLC-DAD. *OENO One*, 51(3): 231-236.
- [76] **Zhao R.Z., Liu S., Zhou L.L.,** 2005. Rapid quantitative HPTLC analysis, on one plate, of emodin, resveratrol, and polydatin in the Chinese herb *Polygonum cuspidatum*. *Chromatographia.* 61(5-6): 311-314.
- [77] **Jelena M. C., Sanja V. D., Aleksandar V. P., Milica T. A., Slobodan M. J., Ilija D. B., Ljiljana C. G.,** 2010. Determination of *trans*- and *cis*-Resveratrol in Serbian Commercial Wines. *Journal of Chromatographic Science.* 48(3):229-34.
- [78] **Amr S. R., Jennifer V. F., Boris J., Joon-Hyun P., Steven C. B.,** 2006. Detection and Quantitation of Resveratrol in Tomato Fruit (*Lycopersicon esculentum* Mill.). *J. Agric. Food Chem.* 54: 7175-7179.
- [79] **Mary M. L., Chongwoo-Yu, R. B., Sool Y. C., Wendy R., Jacqueline L., Richard B., Van B.,** 2003. Resveratrol in Raw and Baked Blueberries and Bilberries. *J. Agric. Food Chem.* 51: 5867-5870.

- [80] **Shiow Y. W., Chi-Tsun C., Chien Y. W., Pei C.,** 2007. Resveratrol Content in Strawberry Fruit Is Affected by Preharvest Conditions. *J. Agric. Food Chem.* 55: 8269–8274.
- [81] **Gao L., Chu Q., Jiannong Y.,** 2002. Determination of *trans*-Resveratrol in wines, herbs and health food by capillary electrophoresis with electrochemical detection. *Food Chemistry.* 78: 255–260.
- [82] **Agnes M. R., Wilhelmina K., James B. M., Jim D., James R. B.,** 2004. Resveratrol, Pterostilbene, and Piceatannol in Vaccinium Berries. *J. Agric. Food Chem.* 52: 4713-4719.
- [83] **Sebastià N., Montoro A., Mañes J., Soriano J.M.,** 2012. A preliminary study of presence of resveratrol in skins and pulps of European and Japanese plum cultivars. *J Sci Food Agric.*;92(15): 3091-4.
- [84] **Tokuşoğlu Ö., Ünal M.K., Yemiş F.,** 2005. Determination of the Phytoalexin Resveratrol (3,5,4'-Trihydroxystilbene) in Peanuts and Pistachios by High-Performance Liquid Chromatographic Diode Array (HPLC-DAD) and Gas Chromatography-Mass Spectrometry (GC-MS). *J. Agric. Food Chem.* 53 (12): 5003-5009.
- [85] **Victor S. S. Richard J. C.,** 1999. *trans*-Resveratrol Content in Commercial Peanuts and Peanut Products. *J. Agric. Food Chem.* 47: 1435-1439.
- [86] **Richard G. Powell M.R., Tepaske R.D., Plattner J. F., Stephen L. C.,** 1993. Isolation of Resveratrol from *Festuca Versuta* and Evidence for The Widespread Occurrence of this Stilbene n the Poaceae, *Phyrochemistry*, 35(2): 335-338. *Elsevier Science.* 31:9422/94 S6
- [87] **Yangji W., Pi L., Liyan M., Jingming L.P.,** 2014. Separation and Purification of Four Stilbenes from *Vitis vinifera* L. cv. Cabernet Sauvignon Roots Through High-speed Countercurrent Chromatography. *S. Afr. J. EnoL Vitic.*, 35(2): 226-231
- [88] **Satyajit D. S., Zahid L. A., Gray I.,** 2006. Natural Products Isolation, *Methods In biotechnology* Humana Press Inc. 999 Riverview Drive, Suite 208 Totowa, New Jersey 07512.

CURRICULUM VITAE

Personal Information

Name: Yusuf Ibrahim

Surname: IBRAHIM

Date of Birth: 02/04/1981

Place of Birth: Warure Quarters Kano city

Marital Status: Married

Permanent Address: Number 128 Warure quarters Gwale Local Government Area, Kano State.

Phone Number: +2348130043527

Education and Academic Status

Primary School: Ungogo Special Primary, Kano Nigeria (1987-1992)

Government Junior Technical Collage Ungogo (1992-1995)

Government Secondary School Gorondutse Kano (2000-2003)

Bachelor of Science BSc., Biochemistry. Bayero University Kano (2004-2008)

Masters Degree MSc., Biochemistry. Firat University Elazig Turkey (2015-2018)

Language Speaking: English (Official) Arabic and Hausa (Native)

Experience

Teacher Pinnacle School Kano 2008 – 2013

Lecturar Kano State Polytechnics 2013 – Date