Iraqi Journal of Biotechnology, , 2022, Vol. 21, No. 1, 50-60

Qualitative and Quantitative Evaluation of the Extracted Flavonoids in Iraqi-Sumac (*Rhus Coriaria L.*)

Wissam S. Mahmod[1] , Essam Fadel Al-wan Al-Jumaili[2] and Niyazi Burhan Aldin Mohamad[1]

[1]Genetic Engineering and Biotechnology Institute for post graduate studies / University of Baghdad
[2]College of Pharmacy / Al-Kitab University

Received: March 13, 2022 / Accepted: June 19 2022

Abstract: Sumac (*Rhus coriaria L.*) is one of the popular spices used in different medical purposes, different studies show that sumac fruits, leaves and seeds contained different types of flavonoids, so the present work was aimed to investigate quantitatively and qualitatively the polyphenolic components of sumac fruits using HPLC and different chemical methods using different standards and modified chemical methods as Ciocalteu phenol reagent and other reagents to do this project of evaluate quantitatively the total phenolic contents TFC and total flavonoids contents TFC in different Sumac extracted solutions using different organic solvents (Ethanol, Methanol and Ethyl Acetate) of different aqueous dilutions, while the total flavonoids evaluated qualitatively using HPLC were about 8 types of flavonoids have been identified in different aqueous methanolic concentrations (50, 75 and 95%v/v) and the higher methanolic concentration extracts shows higher extracted quantities of polyphenolic compounds and flavonoids in compare with the other aqueous diluted methanolic extraction solvents.

Keywords: Sumac fruits, *Rhus Coriaria* L., Flavonoids, polyphenolic, HPLC.

Corresponding author: (Email: prof.dressamal-jumaily@ige.uobaghdad.edu.iq).

Introduction

Sumac (*Rhus coriaria L.*, family *Anacardiaceae*) is one of the most widely consumed fruits of the world. It is known and popular spice extensively used for medicinal and other purposes (1). phytochemical studies reported that sumac leaves and fruits contained phenolic acids and scavenging activity (2). Another type of sumac plant as Chinese Sumac, (*Rhus typhina L.*) is mainly used for station and gardening where its fruit used to prepare a kind of beverage called (Sumac-ade) used in treating of gastrointestinal disorder, (3). Further studies indicated that Syrian sumac contains higher concentrations of organic acids than of Chinese sumac, (4). Sumac is believed to have atheroprotective effect and has been consumed in some dishes (5). Previous studies indicated that the tannin (polyphenolic) and its derivatives are strong antioxidants and can inhibit mechanism leading to vascular smooth muscle cell migration (VSMC), where those studies found that pure tannin that extracted from sumac reduced (VSMC) migration by 62% (6). Sumac is an Iranian spice used vastly in Iranian cuisine and was shown to have antimicrobial activity (7). A study on stability of antimicrobial activity of sumac during autoclaving shows that sumac is heat stable but has interactions

with salt and proteins that reduce its activity against gram-positive bacteria (8). Other studies indicate the antioxidant activity of sumac extract resulted from its polyphenolic constituents (9, 10). Sumc used and recommended for adjustment of blood lipid of diabetic patients. It was indicated that its fruit has an in vitro antioxidant activity (11). Sumac has been used in traditional medicine to treat many various types of diseases such as wound healing, diarrhea, ulcer, hemorrhoids and eye inflammation (12). This plant is rich in many types of phytochemical compounds such as Flavonoids, polyphenolic compounds, tannins, organic acids, and others (1). Sumac extract possess a powerful antioxidant activity make it a good choice with their other therapeutic benefits to treat many common diseases such as cardiovascular disease, diabetes, and cancer (12).

Phenolic compounds mainly founded as either a soluble or as a bound form in the plant kingdom molecules. (13).

Phenolic compounds have been reported to be present in some vegetable oils, which are very important for the oxidative stability of the polyunsaturated fatty acids. They are secondary metabolites, which have several advantages effects on human health, such as antimicrobial, antimutagenic, anticancer and anti-inflammatory due to its bioactivity. Moreover, they can also scavenge radicals, chelate metals, quench oxygen atoms and can act as ion or hydrogen donors. Polyphenols are a challenging issue in nutrition and pharmacology, not just because of its diverse natural sources and its physiological action and health protection interest, but also because of all the technological potential development induced by polyphenol delivery objectives (14).

Polyphenols can be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another, the main classes include phenolic acids, flavonoids, stilbenes and lignans (15).

The hydroxybenzoic acid (phenolic acids) content of edible plants is generally low, with the exception of certain red fruits, black radish and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight. The hydroxycinnamic acids are more common than hydroxybenzoic acids and consist chiefly of *p*-coumaric, caffeic, ferulic and sinapic acids (16).

Stilbenes contain two phenyl moieties connected by a two-carbon methylene bridge. Occurrence of stilbenes in the human diet is quite low. Most stilbenes in plants act as antifungal phytoalexins, compounds that are synthesized only in response to infection or injury. One of the best studied, naturally occurring polyphenol stilbene is resveratrol (3,4′,5-trihydroxystilbene), found largely in grapes. A product of grapes, red wine also contains significant amount of resveratrol (17).

Lignans are diphenolic compounds that contain a 2,3-dibenzylbutane structure that is formed by the dimerization of two cinnamic acid residues., Several lignans, such as secoisolariciresinol, are considered to be phytoestrogens. The richest dietary source is linseed, which contains secoisolariciresinol (up to 3.7 g/kg dry weight) and low quantities of matairesinol (18).

Flavonoids comprise the most studied group of polyphenols. This

group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms which form an oxygenated heterocycle as shown by figure (**1**). More than four thousand varieties of flavonoids have been identified; many of them are responsible for the attractive colors of the flowers, fruits and leaves. Based on the variation in the type of heterocycle involved, flavonoids can be divided into six subclasses: flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones (19).

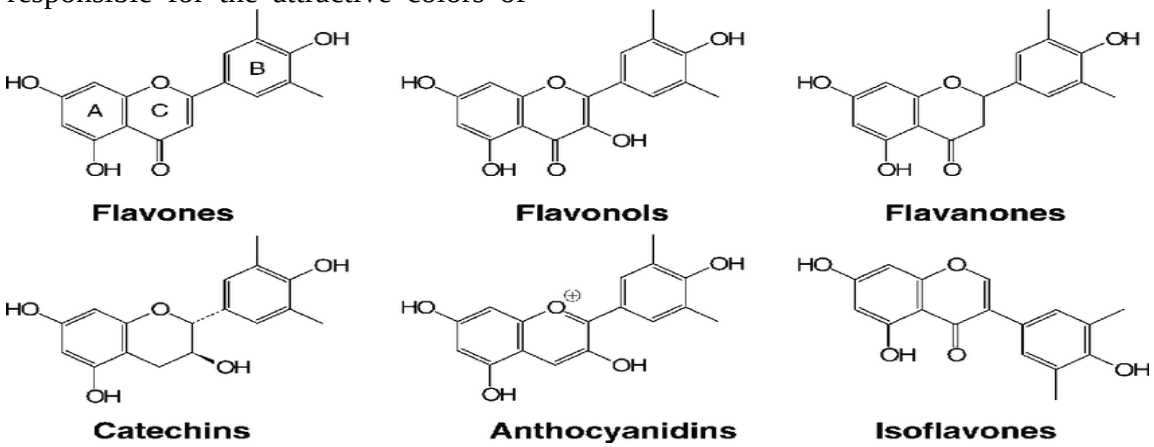

Figure (1) Chemical basic structures of the main six classes of Flavonoids

Materials and Methods

Materials

Iraqi Sumac (*Rhus coriaia L.*) collected from Iraqi market as dried fruits and seeds, all standards, chemicals, and solvents in analytical grade were purchased from Sigma and Merck companies (Darmstadt, Germany and USA) and Thomas Baker (India), absolute Methanol, absolute Ethanol and Ethyl acetate were purchased from Thomas Baker (India) while Folin-Ciocalteu Reagent (Merk Germany), Quercetin and Trisodium citrate dehydrate purchased from (Sigma-Aldrich company USA).

Methods

Sample Preparation

Iraqi Sumac Fruits about 250 gm of weight were brought from the local markets of north of Iraq, where these fruits are left to dry at dark place for 15 days. The dried fruits are grounded in household electric mill blender to get fine particles of uniform size, where these particles washed for about 15 minutes with petroleum ether two times to get rid fats impurities before starting of the extraction (20).

Optimize the Suitable Extracting Solvent for Sumac Flavonoids Extraction

Flavonoids extraction from Sumac Fruits dried powder by using different types of organic solvents (Methanol, Ethanol and Ethyl Acetate) where 40 gm of the dried fruits blended particles transferred into filter paper thimble then inserted into Soxhlet apparatus which fitted with 500 ml round bottom flask filled with 400 ml of the extraction solvent. The extraction was performed for about 10 hours where the temperature of extraction was kept at boiling point temperature depends on the type of solvent used. In this experiment five different organic solvents are used (Ethanol 95% v/v, Methanol 95% v/v, Ethyl Acetate,

Methanol 75% v/v and Methanol 50% v/v).

Determination of Total Phenolic Content

Take 0.2 ml of each of the extracted solution and mixed with 0.6 ml of distilled water and 0.2 ml of Folin-Ciocalteu phenol reagent. Then After 5 min, 1ml of saturated sodium carbonate solution of (8% w/v in water) added to the mixture and the volume was made up to 3 ml with distilled water. The solution was kept in the dark for 30 min.

The absorbance of blue color from different samples was measured at 765 nm spectrophotometrically and the measured absorbance values of samples were recorded and the total phenolic concentration in those test samples was calculated from the Gallic acid standard calibration curve (y=0.034x+0.014) where expressed as Gallic Acid Equivalent concentration (GAE) (21).

Determination of Total Flavonoids Content

Take 0.6 ml of each extracted solution and mixed separately with 0.6ml of 2% w/v aluminum chloride then incubate this solution for about 60 min at room temperature. The absorbance of the reaction mixtures was measured a against blank at 420 nm wavelength spectrophotometrically and the measured absorbance values of samples were recorded and the total flavonoids concentration in those test samples was calculated by using Quercetin standard calibration curve where (Y=0.016x+0.004*)* the calculated flavonoid concentration expressed as Quercetin Equivalent concentration (QE) (22).

Determination of Extracted Flavonoids Compounds using HPLC

The main Flavonoid compounds were separated of each of extracted sample using fast liquid chromatography (FLC) column under optimum condition. Column: Phenomenex C18, 3 µm particle size (50 x 2.0 mm I.D) column. Mobile Phase: linear gradient of solvent, A 0.1% phosphoric acid, solvent B was (6:3:1) v/v of acetonitrile: methanol: 0.1% phosphoric acid. Gradient program from 0% B to 100% B for 15minutes, Flow rate 1.2 ml/min, Detection UV at 280 nm, (23) where the concentration of each separated compound can be calculated by equation bellow:

$$\boldsymbol{Cs}\ \textbf{(µg/ml)} = \frac{\boldsymbol{Area\ of\ Sample}}{\boldsymbol{Area\ of\ Standard}} \times \boldsymbol{Cf} \times \boldsymbol{dilution\ factor}$$

Where: ***Cs*** is the concentration of the tested sample

C*f* is the concentration of the reference standard

Results and Discussions

Table (1): The Total Phenolic Content TPC and Total Flavonoids Content TFC of each Extract

Extract Code	Extraction Solvent	TPC (mg GAE/g)	TFC (mg QE/g)
E1	Ethanol 95%	152.7	12.86
E2	Ethyl Acetate	123.84	3.7
E3	Methanol 95%	181.7	13.2

Table (1) shows the total phenolic content in the extracts (E1, E2 and E3) which represents sumac extracts by using different solvents (ethanol 95%, ethyl acetate and methanol 95%) respectively, where the highest level of phenolic content was found in Methanol 95% (E3) (181.7 mg as GAE/g) as Gallic Acid Equivalent (GAE) per each gram (g) of the dried powder of sumac fruit, while in E1 and E2 was found (152.7 mg GAE/g and 123.84 mg GAE/g) respectively, the polarity of the extracting and the solubility of the chemical constituents in the extracting solvent are the major influencing factors effects on the extraction of TPC, where the sequence of polarity of the solvents used in our extraction experiment as (methanol > ethanol > ethyl acetate) for that reason the TPC was found in methanol higher than ethanol and ethanol higher than ethyl acetate; those results are supported by many researchers as shown by Neha Babbar *et al*. (24) studied the influence of different solvents in extraction of phenolic compounds from vegetable residues; which showed that among four solvents, methanolic extracts showed the highest total phenolic contents for all vegetable residues studied.

The total flavonoids content (TFC) of each extract (E1-3) investigated by using aluminum chloride ($AlCl_3$) as colorimetric investigation method where the TFC are calculated as Quercetin Equivelent value (QE) per each gram of sumac fruit powder as shown by the table (1), where E3 methanolic extract shows higher flavonoids content in compare with (E1 and E2) as ethanolic extract and ethyl acetate extract respectively, the reason behind those different content values of the flavonoids is mainly related to their solubility by the extraction solvent and the extraction solvent polarity, which is typically the same reason of extraction of phenolic compounds where the flavonoids are subclass of phenolic compound which mostly show the same physical and chemical properties, the same of those extraction results have been also shown by Do *et al.* (25), so Methanol selected as the optimized organic solvent and used in different dilutions (95%, 75% and 50%) for further methanol concentrations optimization as methanol concentration and their effect on the extraction of total flavonoide from sumac fruits powder as shown by the next section.

Table (2): The Total Phenolic Content (TPC) and Total Flavonoids Content (TFC) of each Extract.

Extract Code	Extraction Solvent	TPC (mg GAE/g)	TFC (mg QE/g)
E3	Methanol 95%	181.7	13.2
E4	Methanol 75%	192.36	6.12
E5	Methanol 50%	193	3.1

As shown by results (table 1) methanol was selected as the optimized extracting solvent in compare with ethanol and ethyl acetate solvents regarding to the yielded TPC and TFC of each extract, we can also start further and deep optimization on the same extracting solvent (methanol) by using different water dilution of methanol (95, 75 and 50) as v/v % to select the

appropriate methanol concentration have the optimized yielded values of TPC and TFC as shown by table (2), where those methanolic extracts (E3, E4 and E5) shows near values (181.7, 192.36 and 193 mg as GAE/g) respectively of TPC as mg of GAE /g of sumac fruit powder while those same extracts shows wide range variations in TFC; methanol 95% extract (E3) shows the higher total flavonoids concentration (13.2 mg as QE/g of sumac fruit powder) while E4 and E5 extracts shows (6.12 and 3.1mg QE/g) respectively, this mean that the water dilution of methanol have less flavonoids extract in compare with the higher methanolic extraction solvent (ie: methanol 95% > methanol 75% > methanol 50% for flavonoids extraction) even have nearly the same values for extraction of TPC which depends on the polarity and the solubility of the extracted phenolic compounds by different polarity and solubilizing property of the used organic solvent (methanol) with different water dilutions, this finding of decrease TFC concentration as the methanol dilution increased (95% > 75% > 50%) while the TPC increased or nearly the same concentration with the same dilution sequence (95% < 75% ≤ 50%) with the same agreement by Jakopic *et al.* (26) of extraction flavonoids and polyphynolic compounds from different plants. So E3 selected as the optimized methanolic extract with higher TFC and acceptable TPC and passed through further evaluations and identifications by HPLC.

Table (3): Quantitative and Qualitative Evaluation of Flavonoids Content in E3 Extract

Seq.	Subject	Retention Time (min.)	Area µVolt (Standard)	Area µVolt (Sample)	Conc. µg / ml (Sample)
1	Myrecetin	1.7	331075	226945	257.05
2	Isoquercetin	2.62	398606	464985	437.45
3	Chrysanthemin	4.05	446032	396652	333.50
4	Kampferol	5.18	467562	628808	504.32
5	Rutin	5.95	414180	478098	432.88
6	Myrtillin	7.10	342749	311481	340.79
7	Caryophelline	7.81	486447	301284	232.26
8	Lemonine	8.89	368347	159837	162.72

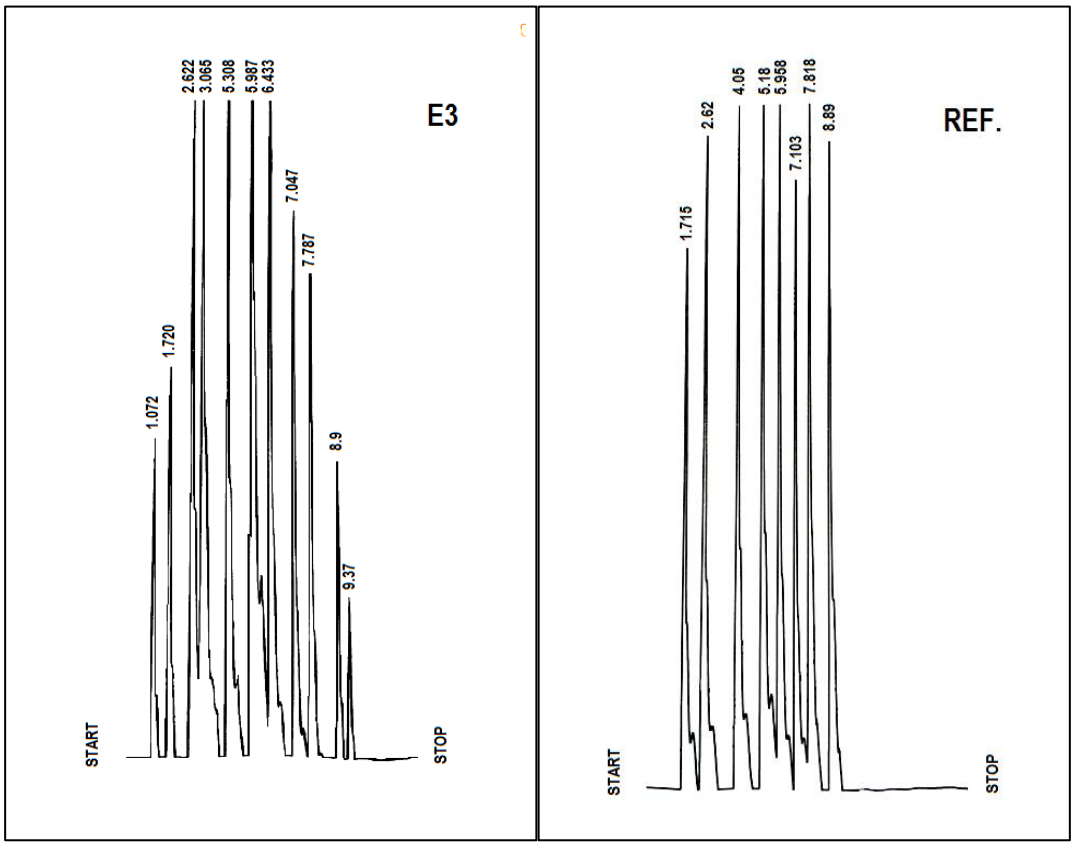

Figure (2): HPLC Chromatograph of Extracted Flavonoids (E3) Against the Standard References

Table (4): Quantitative and Qualitative Evaluation of Flavonoids Content in E4 Extract

Seq.	Subject	Retention Time (min.)	Area µVolt (Standard)	Area µVolt (Sample)	Conc. µg / ml (Sample)
1	Myrecetin	1.7	331075	99289	112.46
2	Isoquercetin	2.62	398606	144903	136.32
3	Chrysanthemin	4.05	446032	311786	262.13
4	Kampferol	5.18	467562	232803	186.71
5	Rutin	5.95	414180	200628	181.65
6	Myrtillin	7.10	342749	177291	193.97
7	Caryophelline	7.81	486447	176936	136.40
8	Lemonine	8.89	368347	47116	47.96

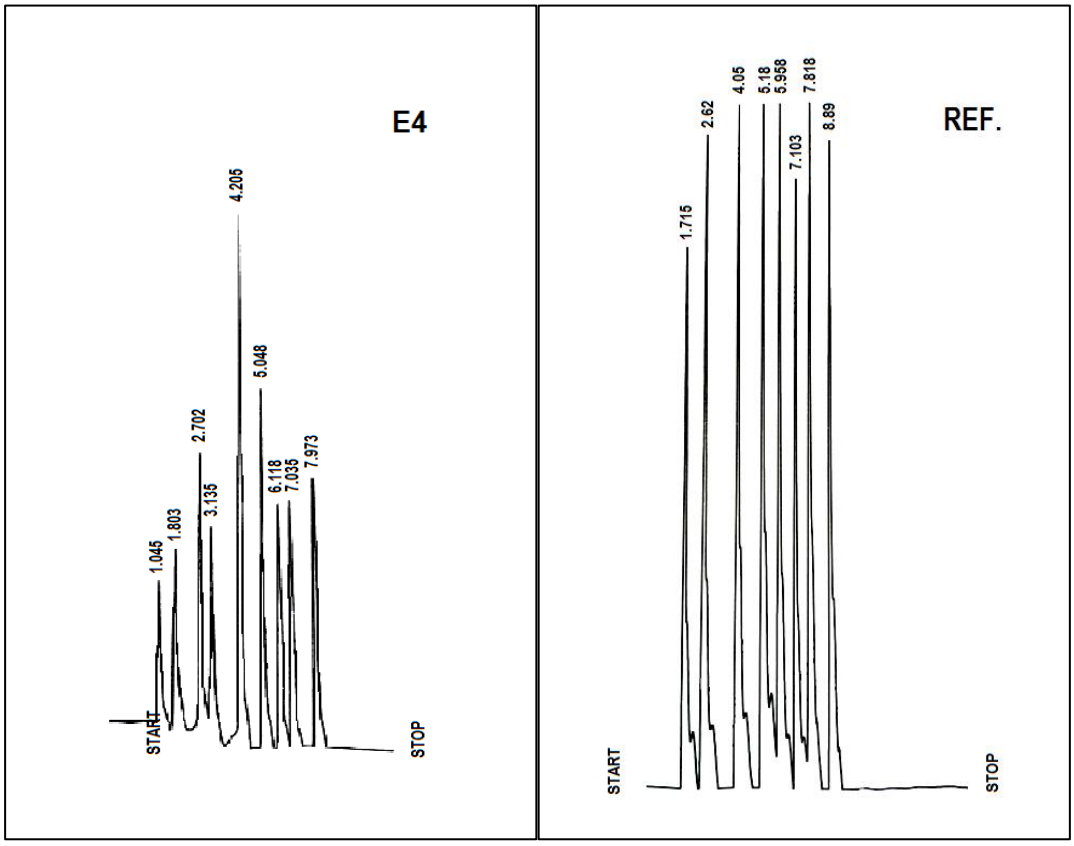

Figure (3): HPLC Chromatograph of Extracted Flavonoids (E4) Against the Standard

Table (5): Quantitative and Qualitative Evaluation of Flavonoids Content in E5 Extract

Seq.	Subject	Retention Time (min.)	Area µVolt (Standard)	Area µVolt (Sample)	Conc. µg / ml (Sample)
1	Myrecetin	1.7	331075	60565	68.60
2	Isoquercetin	2.62	398606	138555	130.34
3	Chrysanthemin	4.05	446032	86325	72.57
4	Kampferol	5.18	467562	183091	146.84
5	Rutin	5.95	414180	125424	113.56
6	Myrtillin	7.10	342749	74216	81.19
7	Caryophelline	7.81	486447	73195	56.42
8	Lemonine	8.89	368347	36209	36.86

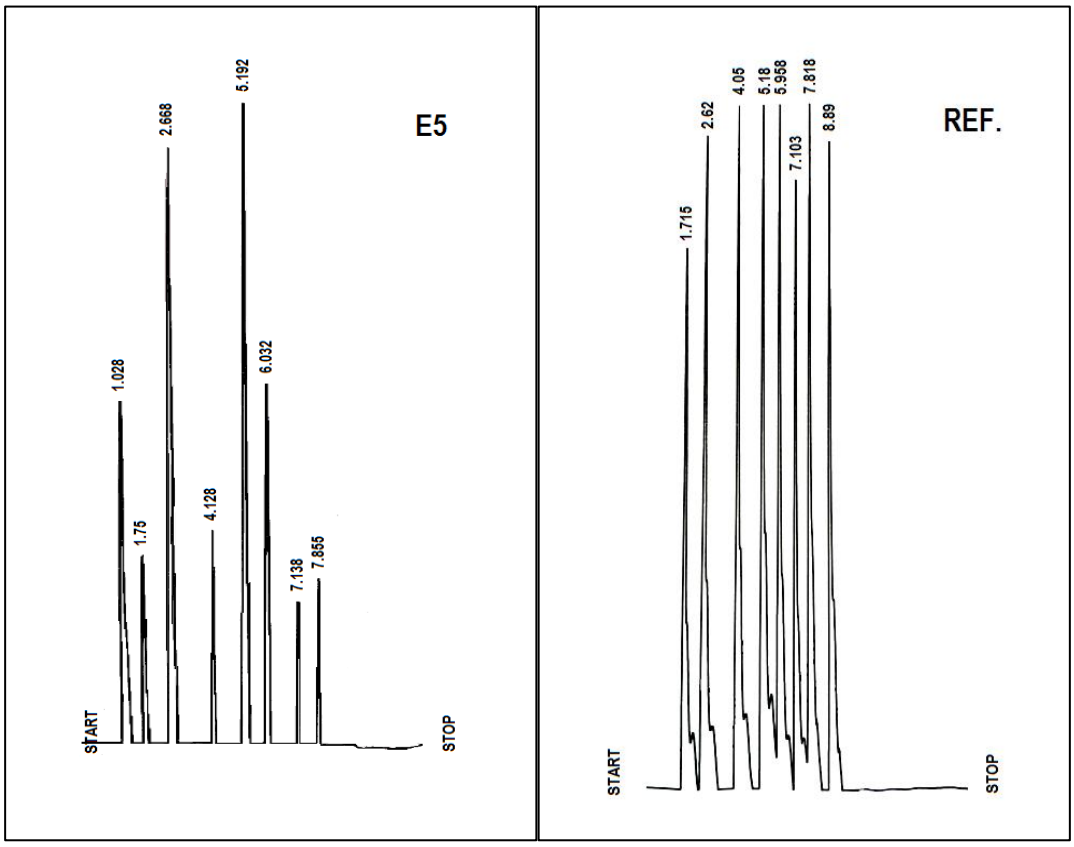

Figure (4): HPLC Chromatograph of Extracted Flavonoids (E5) Against the Standard References

Three methanolic extracts (E3, E4 and E5) have been enrolled for quantitative and qualitative evaluations of the flavonoids content of each extract using HPLC depending on the retention time of separation and area under the curve of each nominated peak of each extract sample against the standard samples as shown by figures (2, 3 and 4) and tables (3, 4 and 5); were they shows the HPLC raw data of the extracted flavonoids (E3, E4 & E5) respectively, those data shows mainly extracted (8) flavonoids (Myrecetin, Isoquercetn, Chrysanthemin, Kampferol, Rutin, Myrtillin, Caryophelline and Lemonine) those anthocyanic phnolic compounds are confirmed by AbouReidah *et al.* **(** 27) and Romeo *et al*. (28) as the main flavonoids content in Sumac (*Rhus Coriaria*) of different plant part source or origin over the world.

Those extracts show different flavonoids concentrations and quantities values of each methanolic extracted solvent (E3, E4 and E5) which confirm that the highest methanolic concentration (95% v/v E3) have the largest quantities of extracted flavonoids in compare with the lower methanolic concentrations (E4 and E5) as shown by tables (3, 4 and 5). So E3 selected as an optimized flavonoid methanolic extract with highest quantities of the isolated flavonoids according to (TPC, TFC and HPLC) evaluation values.

Conclusion

The sequence of polarity of the solvents used in extraction experiment (Methanol > Ethanol > Ethyl Acetate) according to that the TPC was found in Methanol higher than Ethanol and in Ethanol higher than Ethyl Acetate which mainly depend on the solubility

of these extracted compounds in the extracted solvents and also depends polarity of these constituents, where the flavonoids are subclass of the phenolic compound which mostly show the same or near physical and chemical properties.

References

1- Alsamri, H.; Athamneh, K.; Pintus, G.; Eid, A.H. and Iratni, R. (2021). Pharmacological and antioxidant activities of Rhus coriaria L.(Sumac). Antioxidants, 10(1): 1-27.

2- Elagbar, Z.A.; Shakya, A.K.; Barhoumi, L.M. and Al-Jaber, H.I. (2020). Phytochemical diversity and pharmacological properties of Rhus coriaria. Chemistry and Biodiversity, 17(4): 1-15.

3- Al-Muwaly, K.Y.; Al-Flayeh, K.A. and Ali, A. (2013). Antioxidant and free radical scavenging effects of Iraqi sumac (Rhus coriaria L). Baghdad Science Journal, 10(3): 921-33.

4- Kossah, R.; Nsabimana, C.; Zhang, H. and Chen, W. (2013). Evaluation of antimicrobial and antioxidant activities of Syrian Sumac fruit extract. Journal of Natural Products. 6(2013): 96-102.

5- Abdul-Jalil, T.Z. (2020). Rhus coriaria (Sumac): A Magical Spice. In Herbs and Spices, (p. 39). IntechOpen.

6- Zargham, H. and Zargham, R. (2008). Tannin extracted from Sumac inhibits vascular smooth muscle cell migration. McGill Journal of Medicine,11(2): 119-123.

7- Amin, G.; Fazeli, M.R. and Ashtiani, H. (2008). The effects of autoclaving, salt and protein on antimicrobial activities of Iranian sumac, 7(25): 49-53.

8- Al-Muwaly, K.Y.; Al-Flayeh, K.A. and Ali, A. (2013). Antioxidant and free radical scavenging effects of Iraqi sumac (Rhus coriaria L). Baghdad Science Journal, 10(3): 921-933.

9- Hosseini, S.; Ramezan, Y. and Arab, S. (2020). A comparative study on physicochemical characteristics and antioxidant activity of sumac (Rhus coriaria L.), cumin (*Cuminum cyminum*), and caraway (Carum carvil) oils. Journal of Food Measurement and Characterization, 14(6): 3175-3183.

10- Alawsy, T.T.J. and Al-Jumaili, E.F. (2020). Antioxidant Activity of tannic acid purified from Sumac seeds (Rhus Chinensis): Its Scavenging effect on free radical and active oxygen. Plant Archives, 20 (1): 2901-2906.

11- Shidfar, F.; Rahideh, S.T.; Rajab, A.; Khandozi, N.; Hosseini, S.; Shidfar, S., et al. (2014). The effect of sumac (Rhus coriaria L.) Powder on Serum Glycemic Status, ApoB, ApoA-I and total antioxidant capacity in type 2 diabetic patients. Iranian Journal of Pharmaceutical Research, 13(4): 1249-55.

12- Shabbir A. (2012) Rhus coriaria linn, a plant of medicinal, nutritional and industrial importance: a review. Journal of Plant Sciences. 22(2):505-12.

13- Agati, G.; Azzarello, E.; Pollastri, S. and Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. Plant Science, 196(2012): 67-76.

14- Siger, A.; Nogala kalucka, M. and Lampart Szczapa, E. (2008). The content and antioxidant activity of phenolic compounds in cold pressed plant oils. Journal of Food Lipids, 15(2): 137-49.

15- González Sarrías, A.; Tomás Barberán, F.A. and García Villalba, R. (2020). Structural diversity of polyphenols and distribution in foods. Dietary Polyphenols: Their Metabolism and Health Effects. Chapter 1, 10: 1-29.

16- Mattila, P. and Kumpulainen, J. (2002). Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. Journal of Agricultural and Food Chemistry, 50(13): 3660-3667.

17- Mushtaq, M.E. and Wani, S.M. (2013). Polyphenols and human health: A review. International Journal of Pharma and Bio Sciences, 4(2): 338-360.

18- Pandey, K.B. and Rizvi, S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2(5): 270-278.

19- Singla, R.K.; Dubey, A.K.; Garg, A.; Sharma, R.K.; Fiorino, M.; Ameen, S.M., et al. (2019). Natural polyphenols: Chemical classification, definition of classes, subcategories, and structures. Journal of AOAC International, 102(5): 1397-1400.

20- Issa, N.K. (2021). In Vitro antioxidant characterization, optimization and

antibacterial activity evaluation of Iraqi sumac (Rhus coriaria) fruits extracts. Iranian Journal of Ichthyology, 8(2021): 211-221.

21- Atanassova, M.; Georgieva, S. and Ivancheva, K. (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of the University of Chemical Technology and Metallurgy, 46(1):81:88.

22- Al-Saeedi, A.H. and Hossain, M.A. (2015). Evaluation of total phenols, total flavonoids and antioxidant activity of the leaves crude extracts of locally grown pigeon pea traditionally used in Sultanate of Oman for the treatment of jaundice and diabetes. Journal of Coastal Life Medicine, 3(4): 317-321.

23- Farhan, M.M.; Hassawi, D.S. and Ibraheem, N.K. (2018). Polyphenols compounds investigation of leaves and callus artichokes (Cynara scolymus). Plant Archives, 18(2): 2629-2635.

24- Babbar, N.; Oberoi, H.S.; Sandhu, S.K. and Bhargav, V.K. (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. Journal of Food Science and Technology, 51(10): 2568-2575.

25- Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S., et al (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3): 296-302.

26- Jakopic, J. and Veberic, R. (2009). Extraction of phenolic compounds from green walnut fruits in different solvents. Acta Agriculturae Slovenica, 93(1): 11.

27- AbouReidah, I.; Jamous, R. and Shtayeh, M. (2014). Phytochemistry, pharmacological properties and industrial applications of Rhus coriaria L. Jordan Journal of Biological Sciences, 7(4): 233-244.

28- Romeo, F.V.; Ballistreri, G.; Fabroni, S.; Pangallo, S.; Nicosia, M.G.; Schena, L., et al. (2015). Chemical characterization of different sumac and pomegranate extracts effective against Botrytis cinerea rots. Molecules, 20(7): 11941-11958.