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Extraction and study of the phenolic compounds in the leaves and sticks of the Syrian sumac plant (*Rhus coriaria* L.)

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Abstract: The phenolic compounds located in leaves and sticks of Syrian sumac plant had determined. Samples were purified from fats and chlorophyll by petroleum ether, and then the phenolic compounds were extracted by methanol 70% then by ethyl acetate. Several methods of quantitative and qualitative have been used to determine extracted phenolic compounds such as Folin-ciocalteau and HPLC method; which showed that the phenolic compounds contain mainly Gallic acid, vanillic acid, 4- hydroxyl benzoic acid, synergic acid, and caffeic acid. The results indicated that the amounts of phenolic compounds were higher in leaves compared with sticks, and the amount of caffeic acid was lower than the rest of phenolic compounds in leaves, while it was absent in sticks.
The spectrophotometric method has been used in different pH to detect the anthocyanin compounds, and it was found that the quantity of it was higher in leaves compared with sticks.
Keywords: Sumac, phenolic, anthocyanin, HPLC, Folin.

1. Introduction

Sumac (*Rhus coriaria* L.), belonging to Anacardiaceae family, is a small tree or shrub. It's widely distributed in Al-qalamoone mountains, Syrian coasts and Swedaa conservatism in Syria. The leaves, sticks, and seeds, of sumac contains large amounts of several components with biological activities, and the most important of these components are the phenolic compounds, their amount ranges vary between 25-33% of the weight of the dried plant[1,2], which makes this plants have industrial and nutritional importance, in addition to its antibacterial properties, and the other medicinal uses such as antipyretic property[3]. The use of fruits and powder seeds of sumac was due to their rich content of different organic acids[4]. [2]Mavlyanov, S.M. et al reported that sumac's fruits contain flavonols, phenolic acids, hydrolysable tannins, anthocyanin and organic acids. Also pointed out that the fruits of sumac contain malic, citric and tartaric acids[1,5].

Syrian sumac is famously used in the Mediterranean region and Middle East as a spice, sauce and drink[6]. The fruits have been reported to possess antimicrobial and antioxidant activities[7-11] in addition, They are also used as remedy for reducing fever, diarrhea, dermatitis and stomach diseases[1].

Much more are the studies on the antioxidant effect of sumac and its derivatives, such as the extract[10].

leaves and sticks of sumac have been used in manufacturing process due to their high levels of phenolic compounds which consist of free phenolic compounds such as gallic acid, synergic acid, vanillic acid, and anthocyanins, while it contains tanninc phenolic polymers (gallotannins) resulting from condensed gallic acid and other phenolic acids with suger ring in the form β-pento galloyl-D glucose, which has large tanning capacity to animal leather. Which it was up to the middle of the last century one of the biggest industrial sources in Syria due to their ability to form insoluble complexes between tannins and animal leathers collagen[10,12,13].

The research which was published by[14] in his study on Turkish sumac, and the researcher Kossa[4] in her work on two types of Syrian and Chinese sumac explain that there are quantities and qualitative differences in the ionic contents between these types which makes it important to expand the study of their different types to determine their extract chemical composition. The best conditions for extraction phenolic compounds from sumac plant was by using polar solvents especially aqueous solution of methanol in the concentration between (50-75%) and in extraction time about (1-4 hours) and in the temperature of 40^{o}C.

The growth of sumac in the mountains of Al-Qalamone in large quantities, made it an important topic of study to identify the ability of use different parts in different applications and in daily life on the level of nutrition.

The aim of this study was to quantify and identify the types of phenolic acids resulting from hydrolysis of phenolic compounds in leaves and sticks of the sumac plant, and determine the most appropriate solvent for extraction and purification of these compounds to achieve: a. the most suitable method of extraction. b. use of spectrophotomrtric and chemical methods to calibrate the extracted compounds. c. use the chromatographic method to determine the extracted compounds.

2. Methods

2.1. Sampling

The leaves and sticks of sumac plant were collected from the Halpon area of Qalamoon mountains during September and August. Sample has been dried in room temperature then with air flow away from direct light.

2.2. Materials

The gallic acid, vanillic acid, 4- hydroxybenzoic acid, synergic acid, caffeic acid were obtained from (Merk & Co. Merk Produ), the Folin-ciocalteau obtained from (BDH company), distilled water prepared by laboratory distillation device, all other chemical used were obtained from analytical grand (BHD company).

2.3. Apparatus

Spectrophotometer UV-VIS (Thuramed T60 style, Germany), HPLC jasco Japanese style of 1998, equipped with C18 column with UV-VIS Detector at 280 nm.

2.4. Extraction phenolic compounds:

In two compared samples of leaves and sticks of sumac plant, several extraction steps by non-polar then polar solvents was used to extract phenolic compounds according to method proposed by researchers[9,15].

Stage one: petroleum ether have been used to extract fatty compounds from studied samples without prejudicing the phenolic compounds. (10) g of each studied sample have been taken and dried in air away from direct light before milling to fine powder. Then in a conical flask we had added to it (100)ml of petroleum ether, and put on an electromagnet blend for 24 hours, after filtration the precipitate was treated with a new amount of petroleum ether, and the process was repeated until the color of ether solution remained unchanged. The resulted precipitate was dried by air to proceed in the extraction process.

Second stage: to (3)g of each dried precipitate samples, a (50)ml of methanol 70% was added and incubated in 40^{o}C, and then blended for (30)minutes on a magnetic blender. After filtration the extraction was (3) times repeated. Then the extraction mixtures were collected, and the solvent was evaporated by rotary evaporator in 40^{o}C. The presented solid extract contained water soluble compounds according to[16], the

precipitate continent was solved by 75 ml distilled water, then an equal amount of ethyl acetate was added to extract the phenolic compounds by using separatory funnel, the process was repeated three times.

The resulted ethyl acetate layer was collected and evaporated to obtain phenolic compounds, where a yellow precipitate was produced.

2.5. Total phenolic compounds

To calibrate phenolic compounds the[17] method was used, which must start with hydrolyzed the phenolic compounds in the studied extract. (150) ml of methanol 50% was added to (5)g of dried extract, and the hydrolysis process was done by using aqueous solution of HCl 1.2 mol/litter at 80^{O}C on a water bath for (60) min using reflected evaporator. The solution was cooled and filtered, then the methanol evaporated by rotary evaporator while the aqueous phase remained, and was treated by ethyl acetate in separatory funnel to extract the hydrolysis resulting compounds, then ethyl acetate was evaporated and a yellowish precipitate was produced.

The total phenolic compounds was calibrated by Folin reagent according to[18] method 1999, which depend on the blue complex presented between phenolic compounds and Folin reagent, which quantity measured by spectrophotometer at 750 nm according to the following steps: 1- addition of 2.5 ml of Folin reagent to 0.5 ml of studied sample (solved with methanol), 2- addition of (5)ml of sodium carbonate (20%), to react with bleed of Folin reagent, 3- the absorption measures after 60 min at 20^{o}C on spectrophotometer, compared to blank of distilled water.

To draw calibration curve, a standard solution of Gallic acid (1000 mg/L) was prepared, then a serial of standards was prepared according to concentrations (50-100-150-200) mg/l by using methanol 50% solution. Then these solutions were calibrated by Folin reagent, and blank of distilled water.

Table (1) illustrate absorbance of standard serial solutions of Gallic acid, and the Figure (1) showed the calibration curve of Gallic acid's standard using Folin-Ciocalteu method.

Table (1) : Standard Series for Gallic acid

Conc. Gallic acid mg/l	**50**	**100**	**150**	**200**
Absorbance	0.145	0.275	0.413	0.557

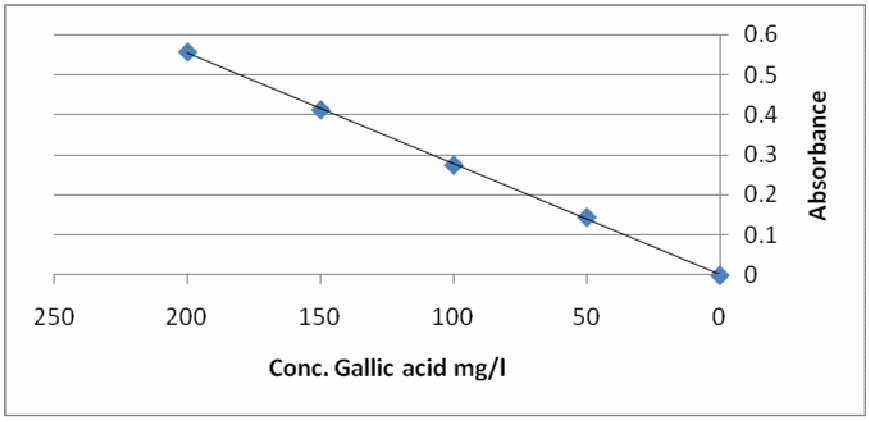

Figure (1): The calibration curve of Gallic acid's standard

Equation (1) used to calculate the concentration of phenolic compounds. (equaling to mg of standard Gallic acid/ g) in the study plant.

$$Y = \frac{C \times V}{W} \times 10^{-1}$$ equation (1)

Where C: is the concentration of phenolic compounds in the taken sample for analysis (mg).

V: the volume of total extract (ml).
W: weight of plant sample taken for study (g).

2.6. Total content of anthocyanins

Total anthocyanins were estimated by a pH differential method [19] using spectrophotometer. Absorbance was measured at 510 nm and 700 nm in buffers at pH =1 and pH = 4.5 using a molar extinction coefficient of 29.600. Results were expressed as mg cyanidin-3-glucoside equivalent per g dry extract equation (2).

$$A = (A_{510} - A_{700})_{pH=1} - (A_{510} - A_{700})_{pH=4.5}$$ equation (2)

Where A_{510} extract absorbance in (λ=510 nm) in acidic media shown in equation (2).
Where A_{700} extract absorbance in (λ=700 nm) in acidic media shown in equation (2).

To calculated the actual quantity of anthocyanin as (mg cynidin 3-glycoside/1g dried extract) the Equation (3) was used, which bind between A value and molecular factor of anthocyanin[19].

Total anthocyanin Conc. = A × 29.600 equation (3)

2.7. HPLC methods

Samples were analyzed using HPLC system equipped with UV detectors at 280 nm. Samples were separated on a reverse-phase C18 (NUCLEODUR® C_{18} Pyramid, 5µm, 250 × 4 mm ID) column using 0,25 ml/min flow rate. Solvent A, 0.5% formic acid in water; and solvent B, 0.5% formic acid in methanol were used as the mobile phase for the analysis of phenolic acids. Linear gradients from 15% to 30% B in 15 min, from 30% to 50% B in 5 min and hold at 50% B for 5 min and increase from 50% to 80% B in 5 min, and from 80% to 90% B in 5 min were applied, followed by a return to the initial conditions in 5 min and re-equilibration of the column, the method time was (29) min.

The concentration of phenolic compounds was measured comparing with phenolic acid's standards in concentration 1 mg/ml with methanol 50%.

3. Results and discussion

3.1. Folin method

The total phenolic compounds in the leaves and sticks of sumac plant were determined using Folin reagent, and the table (2,3) illustrate the results in consecutive time periods, that's befor exposuring it do hydrolysis and after hydrolysis by HCl 1.2 mol/L:

Table (2): The quantity of phenolic compounds in plant's leaves

Harvest time	Conc. Phenolic compounds in sumac's leaves(mg/l)	
	Before hydrolysis	After hydrolysis
August	181.25 ± 1.15	193.15 ± 1.20
September	171.18 ± 1.12	184.35 ± 1.17

Table (3): The quantity of phenolic compounds in plant's sticks

Harvest time	Conc. Phenolic compounds in sumac's sticks (mg/l)	
	Before hydrolysis	After hydrolysis
August	109.25 ± 1.16	111.18 ± 1.08
September	101.81 ± 1.19	107.17 ± 1.05

With regard to the changes in the amounts of these phenolic compounds during the growth period, the tables above demonstrated the amounts of them have decreased in all of the leaves and sticks in the late period of growth during the month of September then it was in the month of August.

3.2. HPLC method in detecting phenolic compounds

The standard's mixture of phenolic acids was separated with HPLC and the retention time was determined for each phenolic acids table (4), and the figure (2) showed the chromatogram of the standard's mixture.

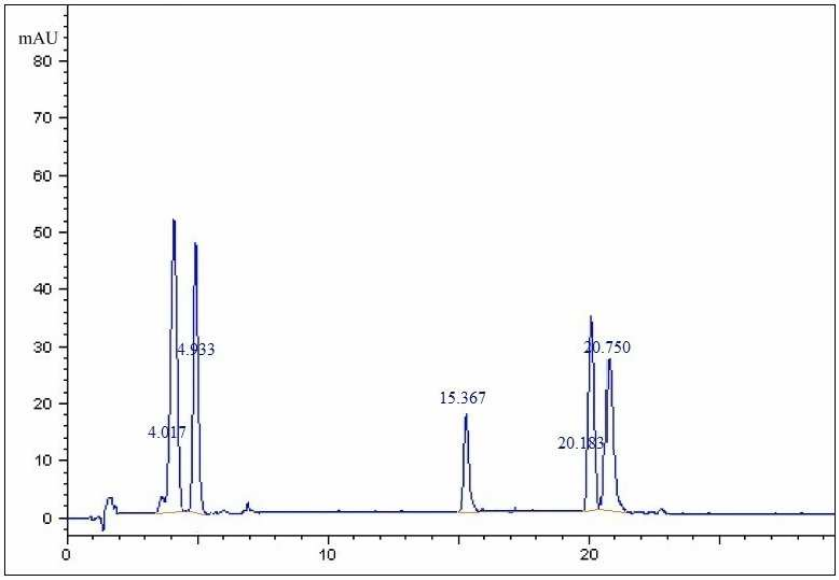

Figure (2): Chromatogram for standard mixture of phenolic acids

Table (4): The retention time of phenolic acid's standards

Retention time	Phenolic acids
4.017	Vanillic acid
4.933	Gallic acid
15.367	Caffeic acid
20.183	4-hydroxy benzoic acid
20.75	Synergic acid

The concentration of extracted phenolic compounds before exposure to hydrolysis was determined by HPLC system comparing with standards, and the figure (3), (4) showed the chromatogram for phenolic acids in leaves and sticks of sumac samples.

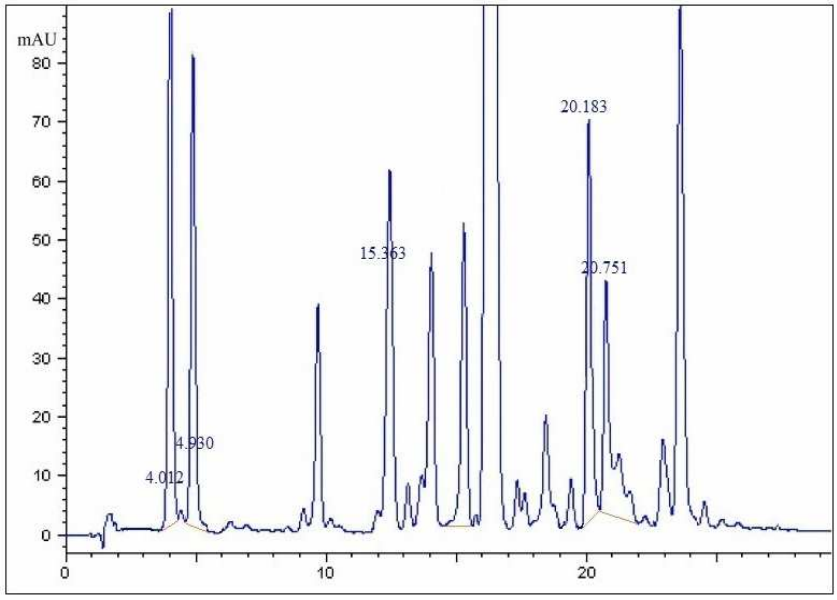

Figure (3): Chromatogram for phenolic acids in sumac leaves sample

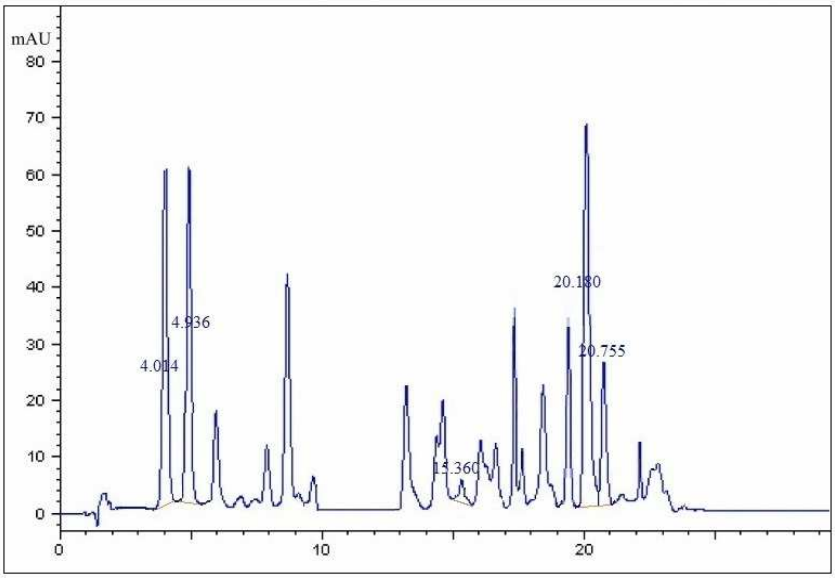

Figure (4): Chromatogram for phenolic acids in sumac sticks sample

The table (5) showed the concentration of phenolic acids in leaves and sticks of sumac mg/g of the weight of the studied sample.

Table (5) : Showed the concentration of phenolic acids in leaves and sticks

Phenolic acids	Concentration mg/g weight of sample	
	Leaves	Sticks
Vanillic acid	4.89 ± 0.13	2.91 ± 0.04
Gallic acid	4.78 ± 0.26	2.86 ± 0.06
Caffeic acid	3.2 ± 0.09	0.02 ± 0.01
4-hydroxy benzoic acid	4.81 ± 0.11	4.9 ± 0.12
Synergic acid	4.83 ± 0.07	1.02 ± 0.02

The concentration of each Gallic acid, vanilic acid, synergic acid, 4-hydroxy benzoic acid in leaves were similar 4.8 mg/g, while caffeic acid was 3.2 mg/g.
But the concentration of these acids in the sticks of sumac plant showed that the higher concentration was for the 4-hydroxy benzoic acid, it reached 4.9 mg/g of the weight of the studied sample, while The synergic acid value was low, it didn't exceed 1 mg/g, the gallic acid and vanilic acid there ratios were approximate to 2.9 mg/g in the sudied sample, the caffiec acid was found in trace amounts in the studied sample.

Results illustrated that the amounts of phenolic compounds in the leaves were higher than sticks in Syrian sumac plant.

As well as the analysis showed that the amounts of phenolic compounds in leaves exceeds 20% of leaves weight, while it didn't exceed 10% of the sticks mass.

3.3. Spectrophotometric calibration of anthcyanin compounds

The amounts of anthocyanin compounds were calculated in mg/g of studied sample weight equivelent to cyanidin 3-glycoside. Table (6) demonstrate the calibration results for each anthocyanin in leaves and sticks in sumac plant.

Table (6): The anthocyanin amount in sumac plant

Harvest time	Conc. anthocyanin compounds in sumac (mg/g)	
	Leaves	Sticks
August	4.21 ± 0.12	1.05 ± 0.13
September	3.92 ± 0.11	1.02 ± 0.11

4. Conclusion

Its obvious from the results that have been reached that the plant of sumac in its vital parts is an important and rich soure of phenolic compounds that could be exploited for food and industrial purposes, it doesn't contain health hazards. In the same degree that we use its fruit as nutritional importance as a flavoring agent in food for its known role as an effective antioxidant distinguished by phenolic compounds.

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