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Extraction, Separation and Determination Total Content of Flavonoids in Dried and Fresh Hawthorn Leaves and Flowers

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ABSTRACT

This study was carried out to determine the total content of flavonoids of dried and fresh hawthorn flowers and leaves in water and hydroalcoholic extracts. The method of cold and hot maceration was used to prepare these extracts. The total content of flavonoids was determined using spectrophotometric methods by forming complex with aluminum chloride and measurement absorbance at 510nm; it has been shown that quantity of flavonoids in dried samples ranges from 0.16 to 1%, while it's quantity in fresh samples 0.026- 0.18%.

KEY WORDS: Hawthorn, Flavonoids, Extraction, Spectrophotometer, Solid Phase Extraction,

1. INTRODUCTION

The genus Crataegus belongs to the family Rosaceae comprises of a complex group of trees and shrubs, native to Northern temperate zones, mostly between latitudes 30° and 50° N. Hawthorn refers to the plant Crataegus and is widely distributed throughout the Northern temperate region of the world with approximately 280 species (Kumar, 2012).

Most are large spiny shrubs or small trees. The deep green leaves are alternate, simple or lobed some toothed. The white to pink flowers have 5 sepals and/or petals, depending on the species (Kumar, 2012).

Hawthorn is well known in phytotherapy for the treatment of many cardiovascular diseases; it regulates blood pressure, increases the strength of heart muscle, and is used against arteriosclerosis and angina pectoris. Besides, hawthorn has a soothing effect on the nervous system, and is also used as a mild diuretic (Kostic, 2012). This is the great benefit of hawthorn due to the presence of different bioactive compounds, such as epicatechin, hyperoside, rutin and chlorogenic acid (Nabavi, 2015). Hawthorn fruit is commonly used for preparation of many foodstuffs, such as jam, jelly, drink, and wine (Gurikova, 2012).

References studies show that hawthorn content of flavonoids ranges (0.1 to 2%) which includes rutin, hyperoside, procyanidin and vitexin (Taherovic, 2014). The researcher (Amel, 2014) identified the total content of flavonoids in flowers and leaves hawthorn by chloride aluminuim method by using methanol 85% as extraction solvent. Results showed that the flavonoids content 32.73mg (QE)/ g extract. In a study (Bedreag, 2014) on hawthorn leaves and flowers for quantification total flavonoids by using aqueous methanol as extraction solvent in room temperature. Results showed that flower extract had a higher flavonoids content than leaf extract (67.04, 57.08mg/g respectively. The amount of flavonoids identified (Yang, 2012) in samples of dried hawthorn leaves and flowers ranged between 7-21 g/kg.

The importance of research comes from the fact that hawthorn is ranked fourth in the world in terms of its usefulness, according to the Food and Drug Administration of the United States FDA, the German Agency for Medicinal Herbs and the European Commission for Food Safety and Medicine.

Because of its medical benefits at the heart level and the treatment of heart attacks by expanding the diameter of coronary blood vessels.

The research aims to identify the active substances in hawthorn leaves and flowers, Then: extraction flavonoids from hawthorn flowers and leaves with both dried and fresh varieties, estimate the amount of flavonoids found in them, as well as compare the amount of flavonoids in leaf and flower samples and compare the amount of flavonoids in cold and hot leaves and flowers, study effect of the solvent used (water, alcohol) on the amount of flavonoids extracted from flower and leaves hawthorn (dried and fresh).

2. METHODS AND MATERIALS

Ethanol (99.9, shamlab), hexane (panreac, 95%), soduim nitrit, aluminum chloride, sodium hydroxide, distilled water, rutin (India, titan), rotary evaporator, cyclo mixer, spectrophotometer (60uv-vis).

Identification of plant material:

Samples were collected from the local market, flowers and leaves were separated separately and dried in the oven to get rid any moisture. And then grinded with electric mixer to obtain powder. Keep in the dryer away from light, heat, and humidity. For fresh samples: fresh samples were collected from the village of Ain al-Sharqiya which is a part of the city of jiblah, Syrian Latakia governorate. It was divided into small pieces.

Extraction flovonoids: 1 gr of powdered plant material was macerated with 20 ml of hexane for 24h to get rid of chlorophyll and fat. The process was processed three times until the green color decreased. The aqueous and alcoholic extraction was carried out as follows:

A- Cold water extraction: 1 gr of powdered plant material (leaves, flowers) separately was soaked in 20 ml distilled water at room temperature for 24 h with stirring. Then percolated on mussel in cloth first then on filter paper. The process was repeated three times, the liquid percolators were then collected.

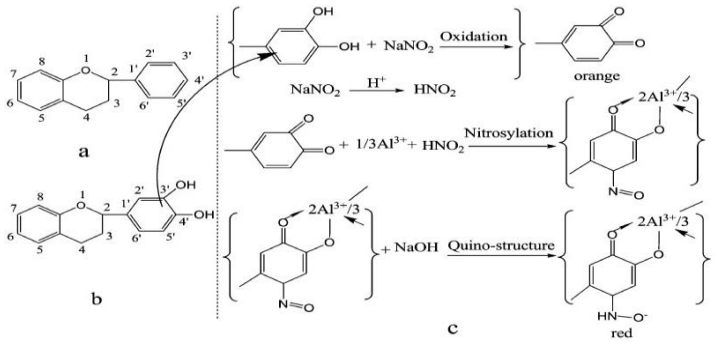
B-Hot water extraction: 1 gr of powdered hawthorn leaves and flowers separately were extracted with 20 ml of water at 100^0C for 15 min, after which the previous steps followed.

C-Cold alcohol extraction: 1gr of powdered hawthorn leaves and flowers separately were extracted with 20ml of ethanol 70% at room temperature for 24h with stirring. After which the previous steps followed.

D-Hot alcohol extraction: 1 gr of powdered hawthorn leaves and flowers separately extracted with 20ml of ethanol 70% on water bath at 60^0C. After which the previous steps followed.

Quantitative analysis of flavonoids: Total flavonoids content was determined using a spectrophotometric method based on formation of flavonoids complex with aluminum (Aggrey, 2012). A volume of 1ml of extract was transferred into 10ml volumetric flask then diluted to 10ml with distilled water.

1ml of diluted extract was transferred to another 10ml volumetric flask, 0.3ml of 5% solution of $NaNO_2$ added. After 6min 0.3ml of 10% solution of $AlCl_3$ was added. After another 6min 2ml of 4.3% solution of NaOH was added, and distilled water added to make the volume. The absorbance was measured in triplicate at 510nm with using ethanol 70% as blank. The following interaction are occurring:



Scheme.1. Chemical reaction to form triplicate

Preparation of standard solutions: Rutin was dissolved in ethanol 70% to obtain reference solution containing 0.08mg (rutin)/ml (ethanol). To 0.4- 0.8- 1.2- 1.6 and 2ml of reference solution in a 10ml volumetric flask, 0.3ml of 5% solution $NaNO_2$ was added. After 6min 0.3ml of 10% solution of $AlCl_3$ and allowed to stand for another 6 min after which 2ml of 4.3% solution of NaOH was added and then make up to the 10ml mark with distilled water. The absorbance was measured after 15 min at 510nm.using ethanol 70% as blank.

Preparation of fresh samples solutions: Sample solution for fresh samples prepared as dried samples, while standard solutions were prepared as follows:

Rutin was dissolved in ethanol 70% to obtain reference solution containing 0.008mg (rutin)/ml (ethanol). To 0.4- 0.8- 1.2- 1.6 and 2ml of reference solution in a 10ml volumetric flask, 0.3ml of 5% solution $NaNO_2$ was added. After 6min 0.3ml of 10% solution of $AlCl_3$ and allowed to stand for another 6 min after which 2ml of 4.3% solution of NaOH was added and then make up to the 10ml mark with distilled water. The absorbance was measured after 15 min at 510nm.using ethanol 70% as blank.

3. RESULTS AND DISCUSSION

Quantitative analysis: Standard curve of flavonoids (rutin) was studied to determine their concentrations in dried samples and this is illustrated by figure.1.

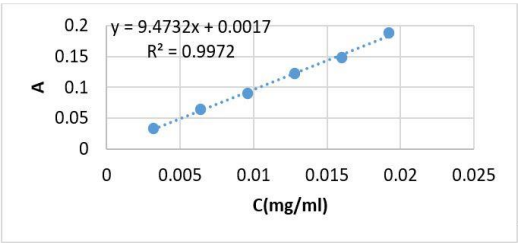


Figure.1. Standard curve of dried samples

From the equation whereas:

W: quantity of flavonoids calculated by mg/g.

C: sample concentration of flavonoids calculated from standard curve equation in mg/ml.

N: dilution factor; N= 10. V: extract volume by ml.

F: dry sample weight by g.

Concentration of flavonoids were determinate as follows:

Table.1. Absorption values, concentrations, weight and percentage of flavonoids in each of dried hawthorn leaves and flower samples

Extract	A	C (mg/ml)	W (mg/g)	w%	sd	Rsd
Extract 1	0.027	0.002670	1.602	0.16	0.00346	2.13
Extract 2	0.045	0.00457	2.7424	0.27	0.00346	1.255
Extract 3	0.101	0.01	6.289	0.62	0.0363	0.5764
Extract 4	0.119	0.0123	7.429	0.74	0.004	0.545
Extract 5	0.031	0.00309	1.855	0.18	0.006	3.32
Extract 6	0.058	0.005943	3.565	0.35	0.003	0.978
Extract 7	0.144	0.0156	9.3928	0.93	0.0264	2.93
Extract 8	0.16	0.0167	10.0261	1	0.0346	3.392

Whereas: Extract 1: dried hawthorn leave sample/ cold water. Extract 2: dried hawthorn leave sample/ hot water. Extract 3: dried hawthorn leave sample/ cold alcohol. Extract 4: dried hawthorn leave sample/ hot alcohol. Extract 5: dried hawthorn flower sample/ cold water. Extract 6: dried hawthorn flower sample/ hot water. Extract 7: dried hawthorn flower sample/ cold alcohol. Extract 8: dried hawthorn flower sample/ hot alcohol.

Our results show that dried hawthorn flowers contain a greater amount of flavonoids than the amount contained in their leaves. And that 70% ethanol resulted in more flavonoids extraction. Hot extraction whether alcoholic or water gave a higher amount of flavonoids. For flavonoids in fresh samples, examine the standard curve as shown in Figure.2.

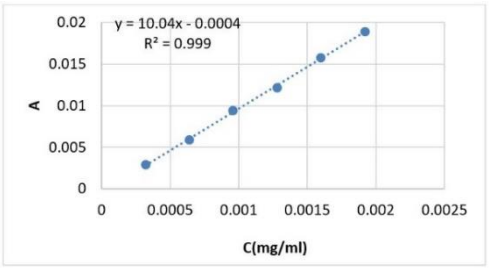


Figure.2. Standard curve of rutin

From Equivalent Equation Y = 10.043x-0.0004 Calculate the concentration of flavonoids in samples and in value compensation in relation to: Whereas: W: quantity of flavonoids calculated by mg/g, C: sample concentration of flavonoids calculated from standard curve equation in mg/ml, N: dilution factor; N=10, V: extract volume by ml, F: dry sample weight by g.

We obtain the amount of flavonoids in the samples estimated in mg /g and then calculate the percentage of flavonoids in all samples according to table.2.

Table.2. The percentage of flavonoids in all samples according

Extract	A	C (mg/ml)	W(mg/g)	W%	Sd	RSd
E1	0.004	0.0004381	0.2628	0.026	0.00346	14.4
E 2	0.009	0.000935	0.5615	0.56	0.00346	6.41
E 3	0.021	0.002130	1.2785	0.12	0.01	8.5
E 4	0.023	0.002329	1.3979	0.13	0.0065	4.95
E 5	0.008	0.0008364	0.5018	0.05	0.006	12
E 6	0.012	0.001234	0.7408	0.07	0.00346	4.81
E 7	0.025	0.002529	1.5174	0.15	0.00346	2.26
E 8	0.03	0.003026	1.8161	0.18	0.00583	3.31

Extract 1: fresh hawthorn leave sample/ cold water.
Extract 2: fresh hawthorn leave sample/ hot water.
Extract 3: fresh hawthorn leave sample/ cold alcohol.
Extract 4: fresh hawthorn leave sample/ hot alcohol.
Extract 5: fresh hawthorn flower sample/ cold water.
Extract 6: fresh hawthorn flower sample/ hot water.
Extract 7: fresh hawthorn flower sample/ cold alcohol.
Extract8: fresh hawthorn flower sample/ hot alcohol.

From the above we conclude that fresh and dried hawthorn flowers contain more flavonoids than those contained in their leaves, and that hot-water extraction gave a higher amount of flavonoids than cold- extraction. Compared dried samples with fresh we note that dried samples (leaves and flowers) contain flavonoids more than fresh samples. This is illustrated by the figure.3.

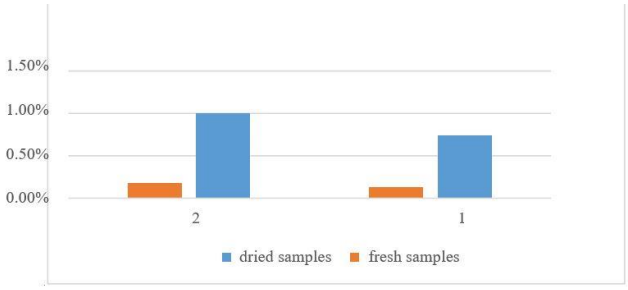


Figure.3. Comparison of percentage of flavonoids in fresh and dried hawthorn leaves and flowers

Solid phase extraction: Solid phase extraction was done by using C_{18} cartridges according to the following conditions:

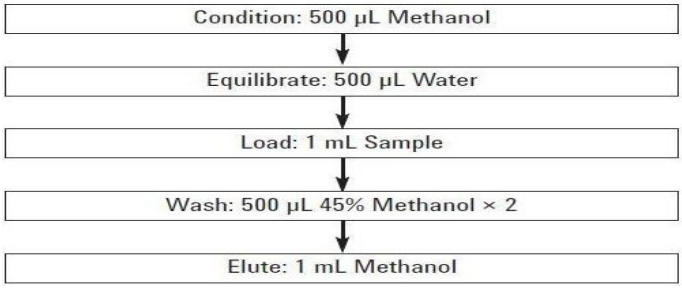


Figure.4. SPE procedure for cleaning flavonoids

Thin layer chromatography: Separation was done on thin layer of silica gel. First we dried the layers in oven at 100C for one hour, then putted a drop of alcoholic extract of hawthorn leaves, (extraction on hot), and another drop of rutin solution**,** at that time mobile phase chamber was prepared, whereas we prepared three mobiles:

1- Ethyl acetate: formic acid: glagial acetic acid: water (26:11:11:100)
2- Ethyl acetate: aceton: water (10:50:40)
3- Ethyl acetate: formic acid: water (10:10:60)

We note that the mobile phase: ethyl acetate: formic acid: glacial acetic acid: water (26: 11: 11: 100) led to the best separation.

After spraying the layer with the aluminum chloride detector, the sample is separated into two spots. The sample and the standard solution spot are colored in yellow as a result of spraying with the aluminum chloride detector.

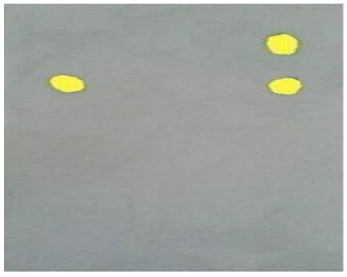


Figure.5. TLC of standard solution (rutin) and ethanolic extract of hawthow

Calculation Rf:

Rf= distance traveled by spot/ distance traveled by mobile phase.
Rf (rutin)= 3.6/10=0.36
Rf $_2$= 5.6/10=0.56

We then sampled the sample and dissolved it by the ethanol and measured the absorbance at 510nm on the spectrophotometer. Absorption was 0.114 and dropping it on the standard curve of the rutin: we found that: c= 0.011mg/ml, then we calculate quantity of flavonoids from relationship: F= 0.011* 10*60/1=6.6mg/g.

4. CONCLUSIONS

- The concentration of flavonoids in hawthorn (leaves and flowers) are grater in ethanolic extract than in the aqueous extracts**.**
- The amount of flavonoids extracted at hot extraction is increased whether water or alcohol extraction.
- The amount of flavonoids in hawthorn flower samples, whether dried or fresh, is greater than in leaf samples.
- The amount of flavonoids found in dried hawthorn leaves (flowers) is larger than in fresh ones.
- We conclude that the desired health benefit of drinking boiled leaves and hawthorn flowers occur when using dried species, i.e., those found in the rapists.

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