GSC Biological and Pharmaceutical Sciences, 2020, 13(01), 251-256

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Determination of polyphenolic components by high performance liquid chromatography (HPLC) and evaluation of the antioxidant activity of leaves and fruits of *Crataegus mongyna* Jacq

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Publication history: Received on 16 October 2020; revised on 27 October 2020; accepted on 28 October 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.13.1.0344

Abstract

Crataegus mongyna Jacq is an endemic species of eastern Algeria, used in traditional medicine for its many therapeutic virtues. Its leaves and fruits are rich in polyphenols. In the present study we wanted to research its polyphenolic components in which it is rich. The method we used is high performance liquid chromatography (HPLC) and evaluation of the antioxidant activity in the methanolic extracts of its leaves and fruits. For this evaluation we followed the free radical reduction method DPPH (2, 2-diphenyl-1-picryl hydrazyl) by an antioxidant. The results show, the presence of polyphenolic components such as rutin, quercetin and isoquercetin and significant antioxidant activity. *Crataegus monogyna* Jacq is could be placed as an alternative treatment for certain pathologies because it is of value for public health.

Keywords: *Crataegus mongyna* Jacq; Leaves; Fruits; Methanolic extracts; Polyphenolic components; Antioxidant activity

1. Introduction

Crataegus monogyna Jacq is used in traditional medicine for its therapeutic properties. It has been used in traditional medicine for the treatment of cardiovascular disorders [1]. It is a species with antispasmodic and tranquilizing properties, allowing to regulate the tension and to suppress the tachycardia [2]. The activity of standardized extracts has been demonstrated in numerous in vitro or in vivo experiments [3]. The fruits and leaves of *Crataegus monogyna* Jacq are rich in polyphenolic components, mainly flavonoids [4]. The present study aims to determine these polyphenol components by high performance liquid chromatography (HPLC) and to evaluate the antioxidant activity of the leaves and fruits of this plant. The method that we have recommended is that of the reduction of the free radical DPPH (2, 2-diphenyl-1-picryl hydrazyl) by an antioxidant (anti-free radical). The results show the presence of polyphenolic components such as rutin, quercetin and isoquercetin and significant antioxidant activity. *Crataegus monogyna* Jacq whose pharmacological properties have given it a good place in traditional medicine, could be placed as an alternative treatment for certain pathologies because it is of value for public health.

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2. Material and methods

2.1. Plant material

The harvest of plant material is carried out in the North-East of Algeria. The plant parts used in this study are the leaves and fruits of *Crataegus monogyna* Jacq. The leaves were dried out of direct sunlight, at room temperature in a dry and ventilated place and the fruits were dried in an oven at 50 °C for 20 hours. The dried plant parts are crushed and sieved. The water content is checked (less than 10%). The recovered powder is stored in a glass container at room temperature and protected from light.

2.2. Preparation of methanolic extract

We deposited 2.5 grams (gr) of each part studied (leaves and fruits) in 20 milliliters (ml) of methanol in an Erlenmeyer flask for 24 hours (h) at room temperature. The extracts are then filtered and then evaporated to dryness under reduced pressure using a rotary evaporator. The dry residues are taken up in 10 ml of methanol and stored in amber bottles at + 4 ° C. We calculated the residue after each operation to determine the initial concentration of each extract:

- The concentration of methanolic extract of leaf: ME_{lea} = 26.4 milligrams (mg) / ml.
- The concentration of methanolic extract of fruit: ME_{frt} = 94.2 mg / ml.

2.3. HPLC protocol

Before starting the chromatographic analysis, the mobile phases, controls and extracts are placed in an ultrasonic tank for degassing. The extracts analyzed are at concentrations of 0.5 mg / ml for an injected volume of 10 microliter (µl) at 40 ° C. On the other hand, the solutions of the controls were prepared in methanol at a concentration of 1 mg / ml. After each injection the analytical system was rinsed for 30 minutes with the mobile phase to ensure that any products that might have remained on the column were dislodged. A baseline free of peaks was the prerequisite for any injection. For all the analyzes, the solvents used are of HPLC quality, the flow rate is set at 1 ml / min. The detection was carried out by a UV-Visible detector and the measurement wavelength set at 350 nanometers (nm). The identification of the products on the chromatograms was made by comparing the retention times with those of the standards.

2.4. DPPH protocol

2.4.1. Preparation of the DPPH solution

We prepared the DPPH solution by dissolving 2 mg of DPPH in 50 ml of methanol. The preparation must be carried out at least two hours in advance for good solubilization. DPPH is characterized by a dark purple color when placed in a methanolic solution.

2.4.2. Preparation of the dilution of DPPH

We prepared the dilutions of our methanolic extracts of leaves and fruits at different concentrations from the methanolic extract stock solutions of leaves (Mes_{lea}) and methanolic extract stock solution of fruits (Mes_{frt}):

- Concentrations from the MES_{lea} (Table 1).

Table 1 Dilutions of methanolic leaf extract

Solution	Concentration in mg / ml	Preparation of the dilution
Mes_{lea}	26.4 mg/ml	
DS 1	0.264	10 µl Mes_{lea} + 990 µl methanol
DS 2	0.132	1ml Dls 1 + 1 ml methanol
DS 3	0.066	1 ml Dls 2 + 1 ml methanol
DS 4	0.033	1 ml Dls 3 + 1 ml methanol
DS 5	0.016	1 ml Dls 4 + 1 ml methanol

Concentrations from the Mes_{frt} (Table 2).

Table 2 Dilutions of methanolic fruit extract

Solution	Concentration in mg / ml	Preparation of the dilution
Mes_{frt}	94.2 mg/ml	
DS 1	0.942	10 µl Mes_{frt} + 990 µl methanol
DS 2	0.471	1 ml Dfs 1 + 1 ml methanol
DS 3	0.235	1 ml Dfs 2 + 1 ml methanol.
DS 4	0.117	1 ml Dfs 3 + 1 ml methanol
DS 5	0.058	1 ml Dfs 4 + 1 ml methanol

Dfs: dilute fruit solution; Dls: dilute leaves solution

2.4.3. DPPH test protocol

After the preparation of the dilutions of the extracts in methanol, we took 1 ml of each extract which we put in a tank and added 1 ml of the DPPH solution. The reaction mixture is stirred before being placed for 60 minutes in the dark and at room temperature in the laboratory. The absorbance of the reaction medium was measured at 515 nanometer (nm) using a spectrophotometer against a negative control (containing methanol instead of the extract). The percentage inhibition (I %) of the DPPH radical by our extract is calculated as follows:

$$I\ \% = [(A\ 1\text{-}A\ 2) / A\ 1] \times 100$$

A 1: Negative control "Absorbance in the absence of the extract (inhibitor).

A 2: Absorbance in the presence of the extract.

3. Results and discussion

3.1. Results of HPLC

The chromatographic profiles of the methanolic extracts of leaves and fruits of *Crataegus monogyna* Jacq analyzed by HPLC are compared with those of the standards.

The methanolic extracts of leaves and fruits of *Crataegus monogyna,* appear to contain chlorogenic acid, rutin, isoquercetin, hesperidin and catechin (Figure 1).

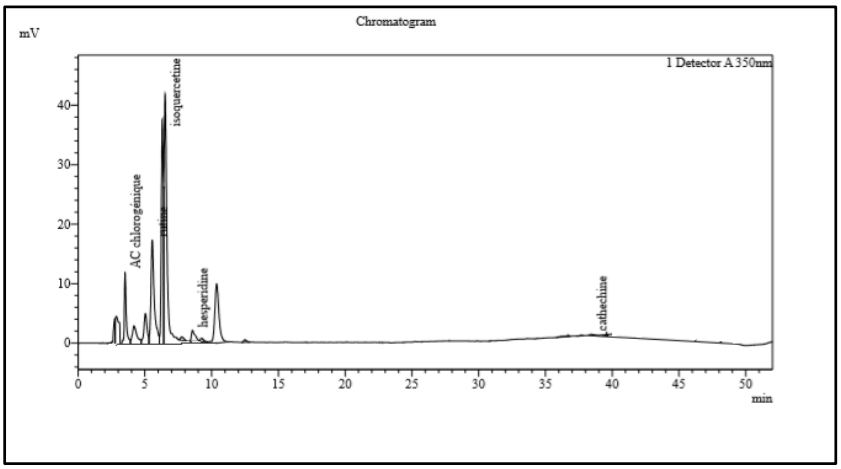

Figure 2 Chromatogram of the methanolic extract of Crataegus monogyna Jacq leaves

The methanolic fruit extract appears to contain additional caffeic acid and quercetin (Figure 3).

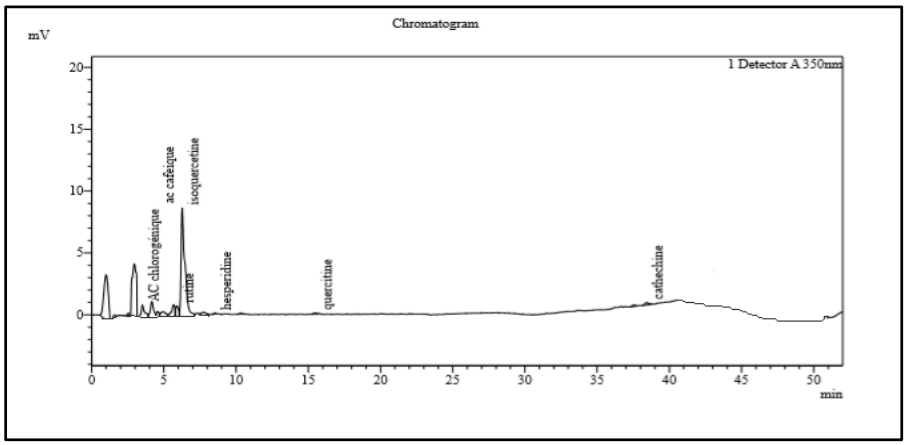

Figure 2 Chromatogram of the methanolic extract of *Crataegus monogyna* Jacq fruits

"Prinz [5] obtained similar results". "Sagaradze [6] also obtained the same results; it confirms the presence of rutin, quercetin and isoquercetin in extracts of leaves and flowers of *Crataegus monogyna* Jacq".

3.2. Results of antioxidant activity

The results are evaluated by spectrophotometer by following the reduction of this radical which is accompanied by the deviation of the violet color at yellow color at 515 nm:

In the first place, by the observation of color change (the purple color of the DPPH solution turns yellow). This color change indicates that the DPPH is reduced to 2.2 Diphenyl 1 picryl hydrazine in the presence of free radical scavengers in the extracts.

In the second place, CI_{50} being the concentration of the test sample necessary to reduce 50% of the DPPH radical. The results being expressed relative to those obtained for ascorbic acid, which represents the reference antioxidant and the IC50s are calculated graphically by percentages of inhibition according to the different concentrations of the extracts tested.

According to the results recorded in the previous figures:

- CI_{50} of ascorbic acid (reference oxidant) is 2.74 µg / ml (Figure 3).

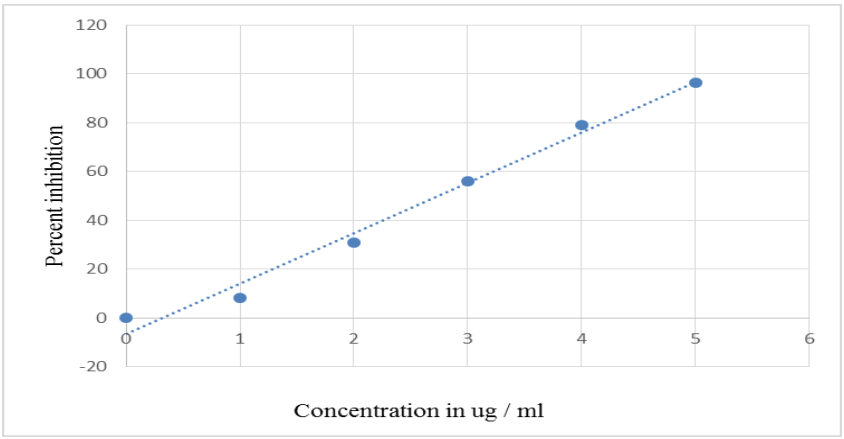

Figure 3 Curve of ascorbic acid activity

- IC_{50} of the methanolic extract of *Crataegus monogyna* leaves is 24.59 µg / ml (Figure 4).

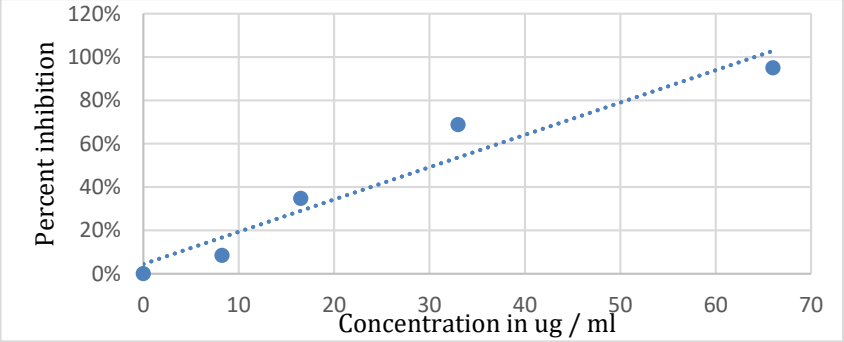

Figure 4 Curve representing the activity of the extract of *Crataegus monogyna* Jacq leaves

- IC_{50} of the methanolic extract of *Crataegus monogyna* fruits is 323.87 µg / ml (Figure 5).

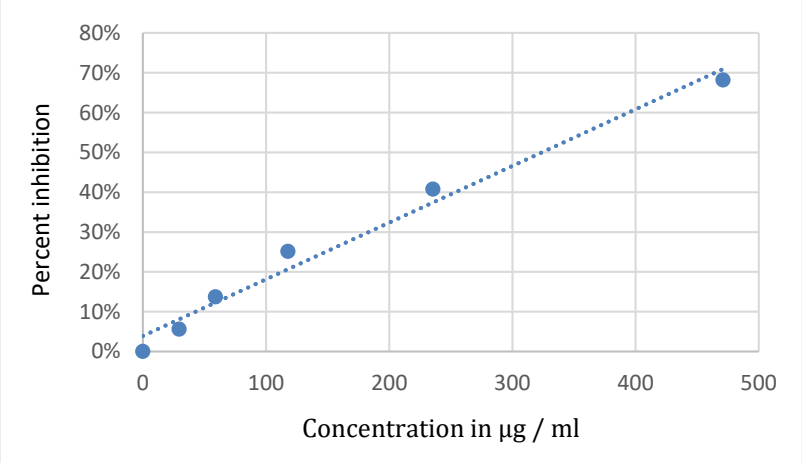

Figure 5 Curve representing the activity of the extract of *Crataegus monogyna* Jacq fruits

Our test results show that the methanolic extract of *Crataegus monogyna* Jacq leaves has greater antioxidant activity than that of the fruit extract (the CI50 of which are 24.52 µg / ml and 323.87 µg / ml, respectively). This can be explained by the richness of the leaves in phenolic compounds compared to the fruits. However, the CI50 of ascorbic acid is 2.74 µg / ml, it is lower than that of fruits. While for the leaves, the result shows an average antioxidant activity when compared with that of the reference antioxidant. "Mraihi [7] obtained results of the antioxidant activity of the fruits of *Crataegus monogyna* superior to those which we obtained". However, "Barros [8] obtained lower results than we obtained for the leaf extract".

4. Conclusion

Analysis of methanolic extracts of *Crataegus monogyna* Jacq by high performance liquid chromatography revealed the presence of phenolic components in the leaves, such as chlorogenic acid, rutin and catechin. While in the methanolic extract of the fruit, we additionally note the presence of quercetin and caffeic acid. The study of the antioxidant activity of methanolic extracts of leaves and fruits of *Crataegus monogyna* Jacq found that the leaf extract has medium antioxidant activity, unlike the fruit extract which has low antioxidant power. It is therefore that the leaves are richer in phenolic compounds than the fruits.

Compliance with ethical standards

Acknowledgments

We thank the members of the Pharmacognosy laboratory and those of the Pharmaceutical Development and Control Laboratory of the Faculty of Medicine for the help they gave us in carrying out this work.

Disclosure of conflict of interest

I declare and all co-authors that we participated in the design, execution and analysis of the document and that I approve the final version. In addition, there is no conflict of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

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