GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Determination of polyphenolic components by High Performance Liquid Chromatography (HPLC) and evaluation of the antioxidant activity of leaves of *Olea europaea* L. *var. sylvestris* (Miller) Lehr

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GSC Biological and Pharmaceutical Sciences, 2022, 20(01), 095–099

Publication history: Received on 02 June 2022; revised on 06 July 2022; accepted on 08 July 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.20.1.0281

Abstract

Olea europaea L. *var. sylvestris* (Miller) Lehr is an inseparable entity of the Mediterranean people, used in traditional medicine for its many therapeutic virtues.Its leaves 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 extract of its leaves. For this evaluation we followed the free radical reduction method DPPH (2, 2-diphenyle-1-picryl hydrazyl) by an antioxidant. The results show, the presence of polyphenolic components such ascaffeic acid, chlorogenic acid and rutin, and significant antioxidant activity. *Olea europaea* L. *var. sylvestris* (Miller) Lehr is could be placed as an alternative treatment for certain pathologies because it is of value for public health.

Keywords: *Olea europaea* L. *var. sylvestris*; Leaves; Methanolic extract; Polyphenolic components; Antioxidant activity

1. Introduction

Olea europaea var. *sylvestris* is used in traditional medicine for its therapeutic properties. It has been used in traditional medicine to treat hypertension and diabetes [1]. It has also been widely used as a remedy for the treatment of fever and other diseases such as malaria [2]. The activity of standardized extract has been demonstrated in numerous in vitro or in vivo experiments [3]. The leaves of *Olea europaea* var. *sylvestris* are rich in polyphenolic components, mainly oleuropein, hydroxytyrosol and other 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 of this plant. The method that we have recommended is that of the reduction of the free radical DPPH (2, 2-diphenyle-1-picryl hydrazyl) by an antioxidant (anti-free radical). The results show the presence of polyphenolic components such as caffeic acid, chlorogenic acid and rutin and significant antioxidant activity. *Olea europaea* var. *sylvestris*, whose pharmacological properties have given it a good place in traditional medicine, could be placed as an alternative treatment against 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 region of Collo, Wilaya of Skikda. The plant part used in this study are the leaves of *Olea europaea* var. *sylvestris*. The leaves were dried out of direct sunlight, at room temperature in a dry and ventilated place. 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 10 grams (gr) of leaves in 100 milliliters (ml) of methanol in an Erlenmeyer flask for 48 hours (h) at room temperature. The extract is then filtered and then evaporated to dryness under reduced pressure using a rotary evaporator. The dry residue is taken up in 10 ml of methanol and stored in amber bottles at + 4 ° C. We calculated the residue after operation to determine the initial concentration of the extract:

The concentration of methanolic extract of leaf: ME_{lea} = 316milligrams (mg) / ml.

2.3. HPLC protocol

Before starting the chromatographic analysis, the mobile phases, controls and extract are placed in an ultrasonic tank for degassing. The extract analyzed is at concentrations of 5 mg / ml for an injected volume of 10 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 at a concentration of 0.025 by dissolving 1,25mg of DPPH in 50 ml of methanol. Preparation should be done at least 2 hours in advance for good solubilization. In solution in methanol, DPPH is characterized by a dark violet color.

2.4.2. Preparation of dilutions of the methanolic extract

We have prepared a stock solution of our extract at 1mg/ml in MeOH. From this solution, nine dilutions are prepared in amber vials at concentrations of 0.04 mg/ml; 0.08 mg/ml; 0.12 mg/ml; 0.16 mg/ml; 0.2 mg/ml; 0.24 mg/ml; 0.28 mg/ml; 0.32 mg/ml; 0.36 mg/ml.

2.4.3. DPPH test protocol

After the preparation of the dilutions of the extract in methanol, we took 50µl of different concentrations of the extract which we put in a tank and added 1950µl of the DPPH solution at 0.025 mg/ml. 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 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:

$$I\% = [(A1-A2) / A1] \times 100$$

A1: Negative control "Absorbance in the absence of the extract (inhibitor).
A2: Absorbance in the presence of the extract

3. Results and discussion

3.1. Results of HPLC

The chromatographic profile of the methanolic extracts of leaves of *Olea europaea* var. *sylvestris* analyzed by HPLC are compared with those of the standards (Figure 1).

The methanolic extract of leaves of *Olea europaea* var. *sylvestris,* appear to contain caffeic acid, chlorogenic acid, trihydroxyflavone, rutin, kaempferol and quercetin (Figure 1).

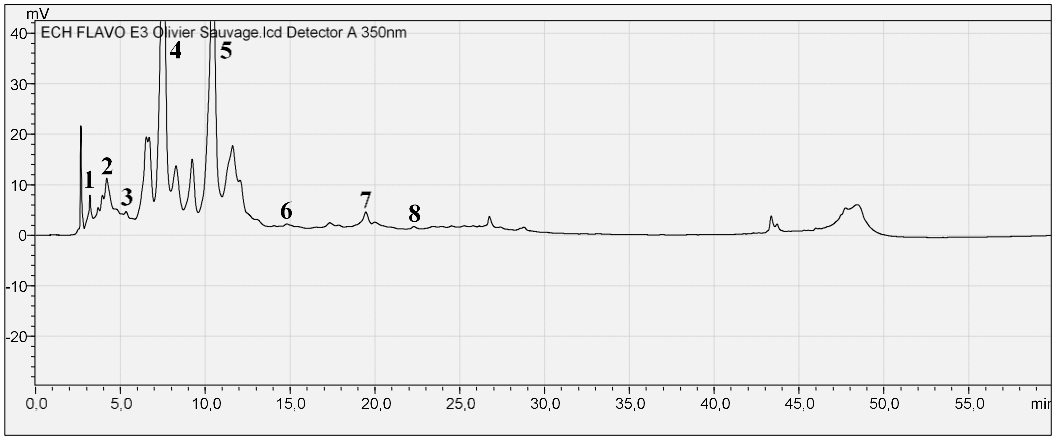

Figure 1 Chromatogram of the methanolic extract of *Olea europaea* var. *sylvestris* leaves

Table 1 Interpretation of HPLC results of wild olive leaf methanolic extract

Pic	Compound	tr (min)	Proportion (%)
1	Chlorogenic acid	3,205	1.505
2	Caffeic acid	4,200	3.355
3	Rutin	5,100	0.422
4	Composé majoritaire (NI)	7,528	20.597
5	Composé majoritaire (NI)	10,444	20.610
6	Quercetin	14,382	0.183
7	5,3',4'-trihydroxyflavone	19,996	0.636
8	Kaempferol	23,432	0.221

« The results of our work are similar to those obtained by Djenane [5] who showed the presence of caffeic acid and rutin in the hydromethanolic extract of wild olive leaves ». « Makowska-Wąs [6] also revealed the presence of rutin in the methanolic extract of Oleaster leaves, however the latter mentioned the absence of caffeic acid, chlorogenic acid, quercetin and kaempferol that were identified in our extract ».

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 extract. This reduction capacity is determined by a decrease in absorbance induced by anti-free radical substances.

- 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 extract tested.
- According to the results recorded in the previous figures :
- $CI_{50}$of ascorbic acid (reference oxidant) is 0,041mg/ ml= 41,5 µg/ml (Figure 2).

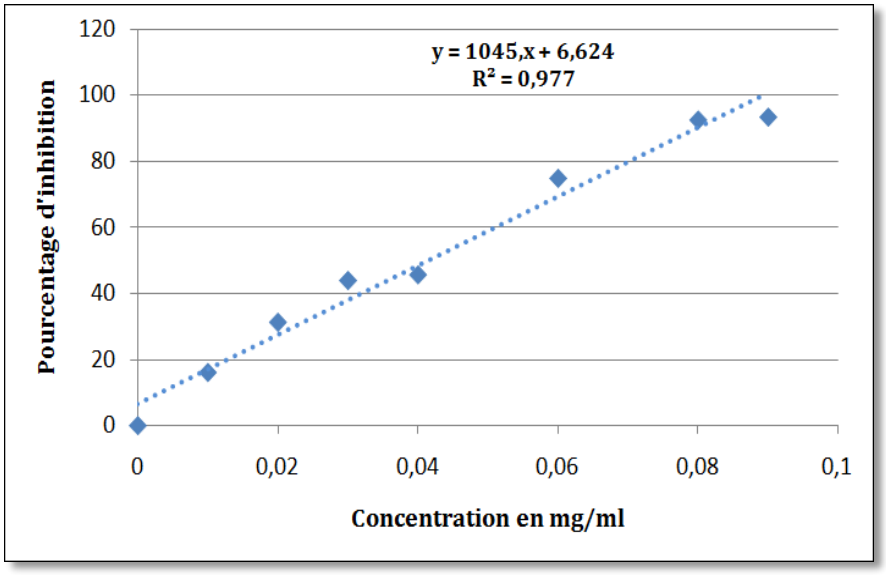

Figure 2 Curve of ascorbic acid activity

- IC_{50} of the methanolic extract of *Olea europaea* var. *sylvestris* leaves is 0,1061 mg/ml=106,10 µg/ml(Figure 3).

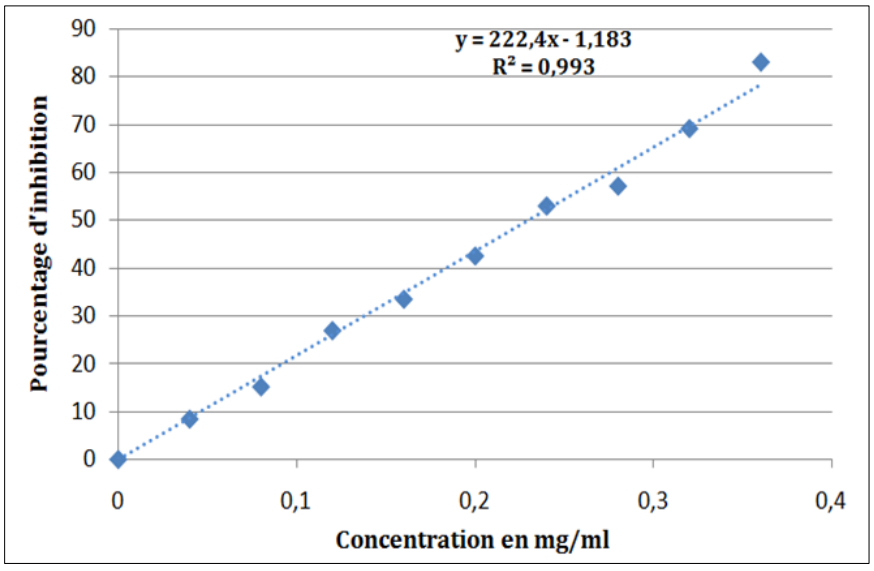

Figure 3 Curve representing the activity of the extract of *Olea europaea* var. *sylvestris* leaves

Our test results show that the methanolic extract of Oleaster leaves has an anti-radical activity with an IC50 of the order of 106.10µg / ml. In comparison with ascorbic acid which demonstrates an IC50 of 41.5 µg / ml, we find that our extract has lower antioxidant activity than that ofthe reference antioxidant.

« These results are in agreement with those of Addab [7] who showed a relatively high anti-radical power of the hydroethanolic extract of Oleaster leaves vis-à-vis DPPH close to that of vitamin C with CI50 of 84.74±1.16 mg/ ml, and 32.60±0.98 respectively ». « According to studies conducted by Mezouar [8] and Djenane [5], hydromethanolic extracts of wild olive leaves exhibit significant antioxidant activity with CI 50 of 13.14±0.16µg/ml and 19.2±1.6µg/ml respectively ». « Similarly Makowska-Wąsshowed a high free radical scavenging capacity with IC50 of 62.48±3.7 µg/ml for Oleaster leaf methanolic extract. This confirms the results recorded in our study [6] ».

The antioxidant activity of wild olive leaves could be attributed to their richness in phenolic compounds, which was previously proven by the results of the chromatographic study.

4. Conclusion

Analysis of methanolic extract of *Olea europaea* var. *sylvestris* by high performance liquid chromatography revealed the presence of phenolic components in the leaves, such as caffeic acid, chlorogenic acid and rutin. The study of the antioxidant activity of methanolic extract of leaves of *Olea europaea* var. *sylvestris* found that the leaf extract has antioxidant activity. This activity could be due to its richness in phenolic compounds.

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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