ISSN : 0974 - 7435 Volume 11 Issue 12

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 11(12), 2015 [469-475]

To study the antioxidant extracts of plant foods *Malva sylvestris L. et Rumex palustris Sm.*

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ABSTRACT

Our work has focused on the study of the antioxidant activity of the extracts of two food plants, Malva sylvestris L. and Rumexpalustris Sm. The development of this activity was carried out using the test of 1,1-diphenyl-di-picrylhydrazyl (DPPH). The results allowed to state that the extracts of the two species studied have significant anti-radical activity. Subsequently, we proceeded to the characterization of families of molecules involved in antioxidant activity. The results of this work reveal the richness of the two plants in polyphenols which reaches 8.62 mg EAG / 1g of dry matter for Malva sylvestris L. and 8.57 mg EAG / dry matter 1g for Rumexpalustris Sm. The content of both extracts ascorbic acid is also important, it is of the order of 3.43 mg / 1g Malva sylvestris for MS and 2.46 mg / 1g MS for Rumexpalustris. Search of ascorbic acid by HPLC confirms that Malva sylvestris includes that amount.

KEYWORDS

Malva sylvestris L.;
RumexpalustrisSm.;
Dropoff window antioxidant activity;
DPPH;
Polyphenols;
Food plants;
UV spectrophotometry.

INTRODUCTION

Antioxidants are chemicals that have the property of preventing the oxidation chain reactions caused by free radicals, molecules or peroxide dioxygenated which accelerates the aging of tissues and damage the cells of our body.

The effect of antioxidants on human health has been subject of several studies. Scientific research confirms that a diet rich in antioxidants can reduce the severity and frequency of health problems[1].[11].[12].[13] [20].

There are several sources of antioxidants (those synthesized by the "endogenous

ntioxidants" organism and those provided by the "exogenous antioxidants" foods). The two types of antioxidants react the same way[2].

The diversity of climate and the variety of eco-

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systems Morocco, offers him a wealth of natural and wild products, herbs, aromatic plants, medicinal and spice[3], with about 4,500 species and subspecies of plants, allowing it to be ranked at the third place in the Mediterranean biodiversity. However, very few of these species are studied and exploited[4].

As part of the enhancement of food plants in Morocco, we conducted preliminary research on two species; Malva sylvestris L. and Rumexpalustris Sm.

Our study focused on the enhancement of antioxidant activity of the extracts of two plants using the test DPPH°. This study involved the characterization of several families of molecules involved in the antioxidant effect.

To this end, we looked for total polyphenols and ascorbic acid. The analysis was performed by spectrophotometry and HPLC.

MATERIALS AND METHODS

The substrates selected for this study are herbaceous wild food plants that grow wild in the fields, they are chosen because of their high consumption by the Moroccan population, are:

- Malva sylvestris L., of the family Malvaceae, is a biennial or pérennante plant, known in France by the name; mallow, and Morocco by name (or khobbeyzabaqüla), considered as an edible wild grass, as it is also used in traditional herbal medicine[5] [6]. The extract of the flowers and M. sylvestris L. leaves is used as a valuable remedy for cough and inflammatory diseases of the mucous membranes[7], treatment of skin diseases[22].
- RumexpalustrisSm, vegetable plant with edible leaves, the family Polygonaceae.. In Morocco, it is eaten as the (homida).

To put the results of antioxidant activity of both species chosen in this study, we conducted to compare our results with those of the literature on the antioxidant activity of other substrates reference standards, it is fruits and vegetables, as well as the equivalent of our plants studied by other researchers.

Preparation of biomass

The aerial parts (leaves and stems) Malva sylvestris L. and Rumexpalustris Sm. were collected in March in the Province of Taounate in untreated wild fields.

The leaves and stems of plants were washed with distilled water and allowed to air until complete dehydration, they are then ground to a fine powder used for preparing extracts.

Preparation of extracts

The extracts are obtained by mixing one gram of each plant homogenate with 50 ml of the solution of methanol-ammonium citrate pH = 7.4 (6:40 v/v). The solutions are kept stirring for 3 hours at 500 rpm in the dark at room temperature (22 ± 1°C). Subsequently, the mixtures were filtered through a millipore membrane diameter 0,45µm and the extracts obtained were diluted in methanol - ammonium citrate at pH = 7.4 (60:40 v/v).

Study of antioxidant by the DPPH test

To evaluate the antioxidant activity of plants, we used the method DPPH (1,1-diphenyl-dipicrylhydrazyl). The analysis was carried out by spectrophotometry.

The method is based on the conversion of DPPH form (oxidized) in the form DPPH reduced according to the following mechanism[8].[15]: A-H + → DPPH° A° + DPPH-H

The antioxidants in the sample the decrease resulting in a color change from purple to yellow-green, measured spectrophotometrically at 515 nm.

The radical scavenging activity was calculated as a percentage of DPPH° remaining end kinetics, according to the following equation: (Eq. 1):

% DPPH ° r = [(A sample × 100)/A blank] (1)

DPPH ° remaining:

- A sample: sample absorbance contacted with the solution of DPPH° measured at the end of the reaction.
- White A: absorbance of the DPPH° initial solution.

Inhibition percentages were calculated using the formula:

I% = 100 × (A witness - A test) / A witness

- A witness absorbance of control (containing all reagents without the test product)

- A test: the test absorbance.

The graph of the variation of the percentage of inhibition against the sample concentration has allowed to determine the IC50 (concentration of the extract provides 50% inhibition, which is the antioxidant activity of the sample).

Preparation of the solution DPPH°

We used different concentrations of equal volumes of plant extracts and solution of 1,1-diphenyl-di-picrylhydrazyl in oxidized form or DPPH ° to 71μM, dissolved in a methanol-citrate buffer pH = 7.4 (60 : 40 v/v). After homogenization and incubation of the mixture in the dark at room temperature (22±1°C) for 2 hours, measuring the variation of absorbance was made by spectrophotometry at a wavelength of 515 nm. The concentrations of DPPH ° in the middle were estimated using the calibration curve of DPPH °.

Determination of polyphenols by the folin ciocalteu

The assay of the content of total polyphenols in samples was determined by the method described by Folin-Ciocalteumethod[10]. The latter is constituted by a mixture of phosphotungstic acid and phosphomolybdic acid which is reduced during the oxidation of phenols mixture of the blue oxides of tungsten and molybdenum (RibereauGayon et al., 1968). The blue coloration produced has an absorption read at 765 nm.

The reference compound used for making the calibration curve is gallic acid.

1 ml of the extract of each plant diluted in methanol - ammonium citrate pH=7.4 (60:40 v/v) is mixed with 4 ml of a sodium carbonate solution (Na2CO3) 7.5 (v / v) and 5 ml of Folin-Ciocalteu reagent diluted 1:10 in distilled water. The mixture was incubated for 2 hours in the dark and at room temperature with agitation. At the end of the reaction, a blue color is observed. The absorbance is measured by spectrometry at 765 nm and the results are reported to the standard calibration curve and expressed as gallic acid equivalent per gram of dry matter.

Determination of ascorbic acid

Vitamin C is involved in redox reactions through the ene-diol functional group[9], L-ascorbic acid is oxidized to dehydro-ascorbic acid, by reacting with O-phenylenediamine for give a compound having a maximum absorbance at 323nm.

Quantitative determination of the content of ascorbic acid in samples was performed by spectrophotometry and HPLC.

Dosage spectrophotometry

1 ml of the extract of each plant was placed in contact with the same volume of the solution of O-phenylenediamine and 1 ml of buffer + NH3 / NH4 + at pH 9.5 was added. Then the mixture was homogenized and incubated for 30 min protected from light and at room temperature (22 ± 1°C). Absorbance was read in a spectrophotometer at 323 nm against a blank. The quantities of ascorbic acid were calculated on the basis of the calibration curve and the results were expressed in mg of ascorbic acid / 1 g of dry matter.

HPLC Assay

Vitamin C is extracted from the samples in water. A reducing solution is used to transform the L (+) - dehydroascorbic acid L (+) - ascorbic acid. HPLC analysis was performed according to the requirements of the NF 03-135 standard.

The system used consists of aisochratique pump, autosampler, a refractometric detector UV set at 265nm, and a data acquisition system, and integration calculation.

The column used is type RP18 (250mm, 5 .mu.m, 0.4 mm) eluted with an acetonitrile buffer solution - 10 mM ammonium citrate pH 6.8 (70:30, V / V) and a temperature 40°C at a rate of 1ml/min. Spectrophotometricdetectionwasperformedat a wavelength of 265 nm. The injected volume was 20 .mu.l.

HPLC analysis required optimization chromatographic parameters used.

Therefore, we used as mobile phase:

- Solution A: Dissolve 13.6 g of potassium dihydrogen phosphate in 900 ml of water.
  Filter through a filter of 0.45 .mu.m.
- Solution B: Dissolve 1.82 g of n-Cétyli-N, N, N-trimethylammonium chloride in 100 ml of methanol.

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Mix and filter through a filter 0,45µm (solution B). A mixed solution of 900ml with 100ml of solution B.

The mobile phase was filtered is degassed by sparging with helium prior to use.

The vitamin C content in the samples is expressed in mg / l, is calculated from the peak area on the chromatogram using the following formula:

C = Fd. Cs. Se/Ss

- Se: the peak area for L-ascorbic acid, obtained with the sample solution.
- Ss: the peak area for L-ascorbic acid, obtained with the calibration solution.
- Cs: the concentration of L-ascorbic acid of the calibration solution (mg / l)
- Fd: the sample dilution factor.

RESULTS AND DISCUSSION

Test the DPPH °

The reaction between the DPPH ° and plant extracts was studied in methanol - ammonium citrate (pH = 7.4) (60: 40 v / v), the analysis was carried out by spectrophotometry at 515nm corresponding to the maximum absorbance of DPPH ° in the visible.

From the values obtained, we calculated the percentages of remaining DPPH° using different concentrations performed according to the formula given earlier.

By analyzing the percentages of remaining DPPH ° after contacting the plant extract with diluted ° DPPH solution (71µM), it is found that the higher the concentration of the extract is small, the higher the percentage of DPPH ° remaining is high. The anti-radical activityis dose-dependent.

M/5: Extract Malva diluted 5 times; M/10: Extract Malva diluted 10 times; M/20: Extract Malva diluted 20 times; M / 50: Extract Malva diluted 50 times; R / 20: Rumex extract diluted 20 times; R / 50: Rumex extract diluted 50 times

From Figure 2 and Figure 1, note that the extract is diluted further, the percentage of inhibition of the free radical DPPH decreases, indicating that the antioxidant activity is dependent on the dose of the sample.

In the case of two samples, a 20-fold dilution resulted in inhibiting approximately 50% of the oxidation of the radical (EC50), 54.49% for Malva sylvestris L. and 51.89% for Rumexpalustris Sm., as shown in Figure 2. Extracts Malvasylvestris and Rumexpalustris inhibit oxidation of DPPH° significantly and dose-dependent.

The anti oxidant that have studied extracts have caused the decrease in the absorbance of DPPH ° 50% with only 0.00086 mg / ml Malva sylvestris L. Compared to the results of the study by[16] (Lilian Barros et al in 2010), on the leaves of Malva sylvestris harvested Portugal (EC50 of the order of 0.43±0.05 mg/ml), reveal our samples with antioxidant activity much greater. Other Italian authors indicated that entrapment of 24% of DPPH required 0.02mg / ml of the extract[17] (Della Greca et al, 2009).Ethanolic Extracts Portuguese samples analyzed by[18] Ferreira et al. (2006), are not active against DPPH radicals, thereby extracting methanol

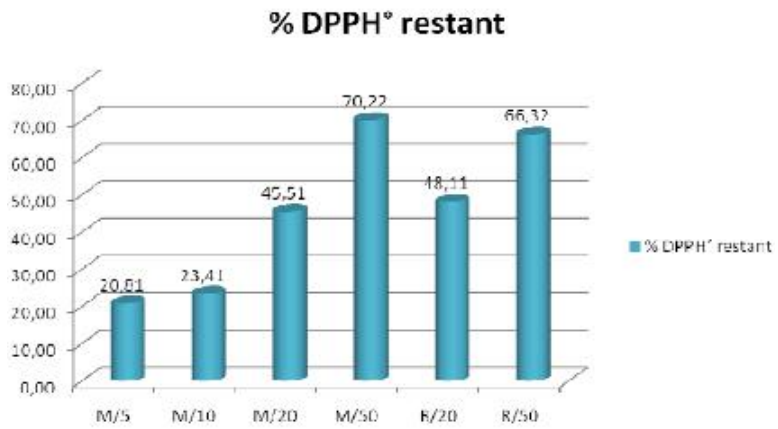

Figure 1 : Percentage of DPPH ° remaining at the end of the reaction to the different concentrations used

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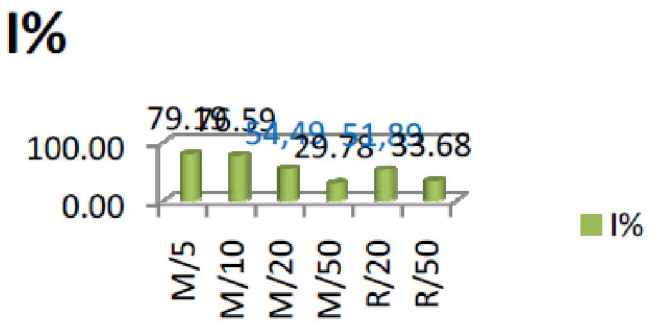

Figure 2 : Percentage of inhibition of oxidation of DPPH ° for the different concentrations used

spectively.

The antioxidant activity of polyphenols gives our samples selected a relatively important role in the fight against oxidation.

Compared to the works cited in the bibliography, we find that both species contain large amounts of polyphenols compared to other foods;[14].

Determination of ascorbic acid (vitamin C)

In order to highlight the presence of ascorbic acid in the two plants tested, the assay was performed

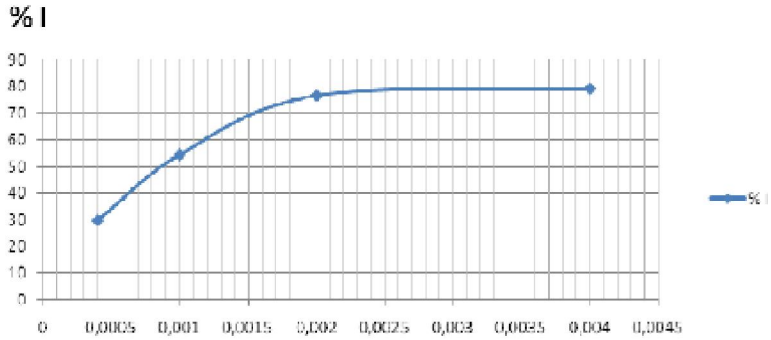

Chart 1 : Variation of the percentage of inhibition of oxidation of DPPH ° depending on the different concentrations used Malva sylvestris L.

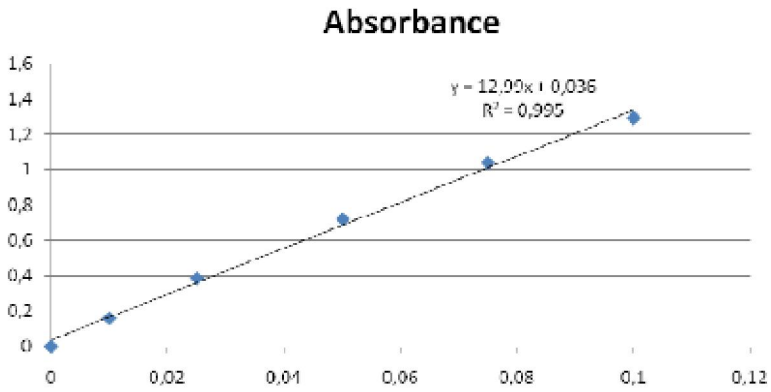

Figure 3 : Calibration curve of the absorbance of the gallic acid

is more effective.

Content of total polyphenols

By reference to the reference range, shown in Figure 3, obtained with a phenolic acid marker; gallic acid; Absorbance allowed to determine the amount of total polyphenols present in each extract. The polyphenol contents are expressed in milligrams of gallic acid equivalents per gram of dry matter.

Several concentrations of the extracts were tested, the results obtained have shown that the polyphenol content of Malvasylvestris and Rumexpalustris is 8.62 mg / g and 8.57 mg / g re-

according to two techniques: spectrophotometry and HPLC.

Spectrophotometricassay

From the calibration curve, we calculated the concentrations of ascorbic acid. The ascorbic acid content is of the order of 3.43 mg/g dry for Rumexpalustris and 2.46 mg/g dry matter Malva sylvestris.

HPLC analysis

The chromatogram obtained for the standard of ascorbic acid is shown in Figure 5. In Figure 6, is

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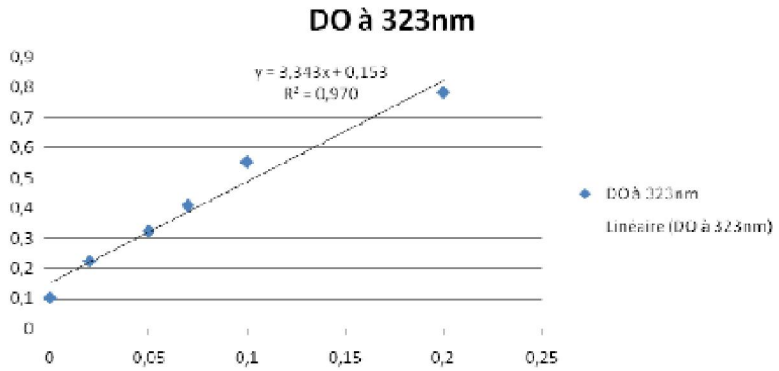

Figure 4 : the absorbance of the calibration curve of ascorbic acid based on the concentration

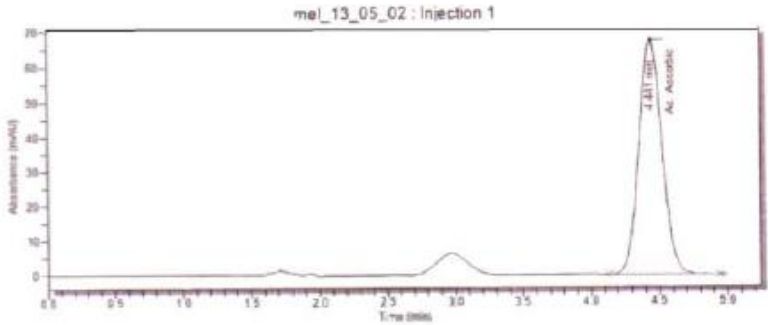

Figure 5 : Chromatogram obtained for the standard of ascorbic acid

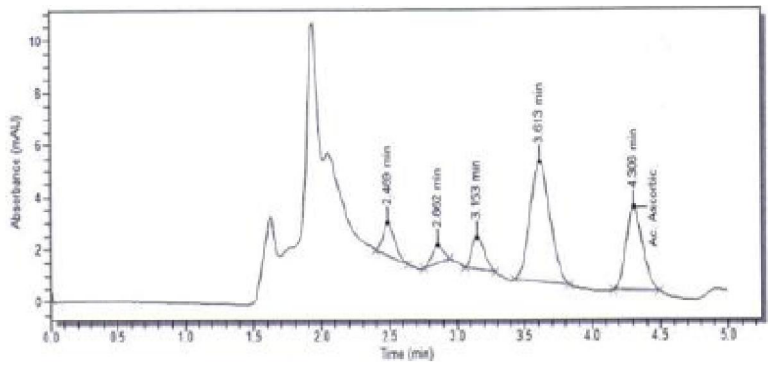

Figure 6 : Chromatogram corresponding to the analysis of the Malva sylvestris L. extract

shown in the chromatogram obtained for the sample extract Malva sylvestris L.

(The stallion area: 785.33; retention time: 4.44 min)

The chromatogram shown in Figure 6 corresponds to the analysis of the extract Malva shows a peak at retention time 4.31 min, which is close to that corresponding to the presented ascorbic acid on the chromatogram of the standard of ascorbic acid.

(The sample area: 26.657; retention time: 4.31 min)

Mobile phase:

- Solution A: 90% (K22 + HPO42 / H2O).
- Solution B: 10% (C19H42BrN / MeOH).

Column: RP18-type (250mm, 5 .mu.m, 0.4mm)

HPLC analysis revealed the presence of several peaks. The results obtained from the chromatograms showed that the content of Malva sylvestris ascorbic acid is about 2 mg / l.

For comparison, in indicated that the content of the leaves of Malva sylvestris ascorbic acid reached 0.17 ± 0.05 mg / 1g of dry matter, while the study by[19] on a Malva showed that the most rich in ascorbic acid part is the flower containing 1.11 mg / 1g MS.

CONCLUSION

The antioxidant activity of extracts from Malva sylvestris L. and Rumexpalustris Sm. has been dem-

onstrated using the test of DPPH° and it was followed by colorimetry. Both species have a major antioxidant power. In the case of two species studied, the inhibition of 50% of the oxidation is achieved using extracts diluted 20 times. Two families of molecules that may be involved in this activity were assessed, polyphenols and ascorbic acid. Recorded in the total polyphenol content is in the order of 8.62 mg / 1g MS Malva sylvestris L. and 8.57 mg / 1g MS for Rumexpalustris Sm. Determining the concentration of ascorbic acid was conducted, the results have shown that L. Malvasylvetris present an amount equivalent to 3.43 mg / 1g MS and Rumexpalustris Sm. contains 2.46 mg / 1g MS. HPLC analysis of the extract Malva, revealed the presence of a peak corresponding to a retention time similar to that of ascorbic acid, thus confirming the presence of this vitamin in our case.

Thanks to their high phenolic compounds and ascorbic acid, Malva sylvestris L. and Rumexpalustris Sm. have significant antioxidant potential. Which gives them an important role in various fields such as herbal medicine, the environment, the food industry and others. Thus, a phytochemical study and a study of the adsorption of heavy metals of both species will be of great interest.

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