Acta Sci. Pol., Technol. Aliment. 12(2) 2013, 169-173
pISSN 1644-0730 eISSN 1889-9594 www.food.actapol.net/

PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *ARISARUM VULGARE* SEEDS

Hadjer Kadri, Salah Eddine Djilani, Abdelouaheb Djilani✉

LSBO Department of Chemistry, Badji Mokhtar University
B.P. 12, Annaba, **Algeria**

ABSTRACT

Background. *Arisarum vulgare* is screened and its total phenolic compounds and total flavonoid contents were measured. In addition, the antioxidant capacity of the methanol-water (7:3) extract of this plant is evaluated by DPPH (2,2-diphenyl-1 picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic)) tests expressed by Vitamin C Equivalent Antioxidant Capacity (VCEAC). HPLC analyses are carried out to identify some polyphenols. The aim of this study is to identify, to quantify the phenolic compounds contained in the seeds of *A. vulgare*, and to evaluate their antioxidant capacity.
Material and methods. Methanol-water (7:3) extract and three fraction obtained from silica gel column chromatography of *A. vulgare* were assayed by using RP-HPLC, spectrophotometric analyses, DPPH and ABTS tests.
Results. Results obtained in the present study, revealed that total phenolic and flavonoids of methanol-water extract of *A. vulgare* seeds were respectively 1.2 g GAE and 0.34 g QE per 100 g of plant extract dry weight. The total antioxidant capacity expressed as vitamin C equivalent antioxidant capacity (VCEAC) per 100 g of plant extract, obtained by ABTS and DPPH tests were respectively 1.3 g and 0.99 g VCE per 100 g dry weight. On the other hand RP-HPLC analyses reveal that the main phenolic compounds identified in the methanol-water (7:3) extract are gallic acid, caffeic acid and rutin.
Conclusions. The results reveal that the methanol-water extract of *A. vulgare* seeds possesses strong antioxidative properties *in vitro*. Results confirmed by high polyphenols and flavonoids contents and corroborated by HPLC identifications.

Key words: medicinal plants, screening, extraction, ABTS, DPPH, RP-HPLC

INTRODUCTION

Interest in toxic plants is increasing because it is recognised that these plants contain bioactive compounds and then, have medicinal virtues. So, medicinal plants are of great importance to the health. Also, many papers have reported that the major pharmacological proprieties of medicinal plants are associated and attributed to their antioxidant activity, then; much attention is being paid to antioxidant compounds [Gramza-Michałowska et al. 2008].

In this work, we targeted *Arisarum vulgare* plant growing in El-Kala National Park. This park is situated in the extreme North-East of Algeria (36°55'--36°90'N; 08°16'-08°43'E) is a specific site recognized for its floristic wealth, it presents a remarkable

✉jilaniouaheb@yahoo.fr, djillani.abdelouheb@univ-annaba.org

originality [Djilani et al. 2007]. *Arisarum vulgare* is very toxic, but their tubers are eaten in times of scarcity after boiling in water. It is also known for its uses in traditional medicine to treat various diseases. For example and according to ethnobotanical investigations, it is observed that in some regions of Algeria, *A. vulgare* is used for the treatment of several diseases such as headaches, asthma, flu and it promotes healing of early wound skin lesions.

To our knowledge, there are still no phytochemical and biological studies or reports on *A. vulgare* seeds. The present study is aimed to identify, to quantify the phenolic compounds contained in the seeds of *A. vulgare*, and to evaluate their antioxidant capacity by DPPH and ABTS *in vitro* test. Identification of some flavonoids from different fractions of methanol-water extract seeds was carried out, using RP-HPLC.

MATERIAL AND METHODS

Plant materials

Plants are collected from the National Park of El Kala and they were identified by Dr. G. Debelair (Department of Biology, University of Badji Mokhtar-Annaba, Algeria). Seeds of *A. vulgare* were collected in October 2011. The seeds were air dried in shade at room temperature, ground on mill to obtain powder and stored in a glass flask to protect them from humidity and light.

Chemicals

Folin-Ciocalteu's phenol reagent, aluminum chloride, gallic acid, rutin, protocatechic acid, epicatechic acid, para-coumaric acid, quercetin, chlorogenic acid, caffeic acid and vitamin C were purchased from Across Organics. Potassium persulphate, ammonium phosphate, sodium carbonate, sodium nitrite, hydrochloric acid, ammonium hydroxide, sodium hydroxide, acetonitrile, methanol, dichlorethane, ethyl acetate, phosphoric acid, ABTS and DPPH were obtained from Sigma and Roth (France). The chemicals used were of HPLC and analytical grade.

Apparatus. The RP-HPLC analyses were performed with a Waters 600E pump coupled to a Waters 486 UV visible tunable detector and equipped with a 20 µL injection loop and an Alltech Intertsil ODS column (RP C_{18} column size 4.6 mm × 150 mm; particle size, 5 µm).

Phytochemical screening

Preliminary tests for determination of major chemical groups were carried out according to the Harborne technique [Harborne 1984].

Extraction procedure

The dried powdered seeds were extracted three times (24 h for each time) with methanol/water (7:3). Then the green organic phase was filtered through Whatmann paper N°1, concentrated under reduced pressure to dryness. The residue was dissolved in methanol and the solution was fractionated on silica gel column chromatography using dichloromethane-ethyl acetate-methanol gradient. Three major fractions obtained are (F1), (F2) and (F3).

DOSAGE OF PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

Determination of total phenols

Total phenolic contents were evaluated with Folin-Ciocalteu's phenol reagent [Amarowicz et al. 2008, Kim et al. 2003] using the spectrophotometric analyses (Cary 50 Scan UV-Visible apparatus). Briefly, an aliquot (1 ml) of standard solutions of gallic acid at different concentrations or appropriately diluted extract was added to a 25 ml volumetric flask containing 9 ml of ddH_2O. A reagent blank using ddH_2O was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 ml) with ddH_2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance versus prepared blank was read at 750 nm. Total phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g dry weight (dw). Samples were analysed in three replications.

Total flavonoid compounds

Total flavonoid content was measured according to a colorimetric assay [Zhishen et al. 1999]. A 1 ml aliquot of standard solutions of quercetin at different concentrations or appropriately diluted samples was added to a 10 ml volumetric flask containing 4 ml ddH_2O. At zero time, 0.3 ml 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 ml 10% $AlCl_3$ was added.

At 6 min, 2 ml of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to volume (10 ml) with ddH_2O and mixed thoroughly. Absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared blank. Total flavonoid content was expressed as mg quercetin equivalents (QE)/100 g dry weight (dw). Samples were analyzed in three replications.

Antioxidant activities

Two tests have been used to determine the total antioxidant capacity, the ABTS and the DPPH tests.

ABTS radical scavenging test

Total antioxidant activity was determined by scavenging blue-green ABTS radicals and was expressed as mg vitamin C equivalent (VCEAC)/100 g dry weight [Chun et al. 2003, Re et al. 1999]. ABTS radical cation ($ABTS^{\cdot+}$) was generated according to the experiment using an improved method. It is produced by reacting of ABTS solution (7 mM in water) with 2.5 mM potassium persulfate (final concentration) for 16 h at ambient temperature in the dark (stock solution). Then the $ABTS^{\cdot+}$ stock solution was diluted with methanol to an absorbance of 0.7 ±0.2 at 734 nm. A quantity of 1 mg of the extract was dissolved in 5 ml of 70% aqueous methanol. A quantity of 50 µl of extract solution, was added to 2.0 ml of diluted $ABTS^{\cdot+}$ solution (A = 0.7 ±0.2). The decrease of absorbance was measured after 5 min of incubation at room temperature in the dark and plotted as function of concentration of antioxidants. All determinations were carried out in triplicate on each occasion and at each separate concentration of the standard. Methanol and L-ascorbic acid were used as negative and positive control, respectively. All radical stock solutions were prepared fresh daily.

DPPH radical scavenging activity

The antioxidant activity of plant extract was estimated using a slight modification of the DPPH radical scavenging protocol reported by [Chun et al. 2003]. Two and nine tenths ml of 100 mM DPPH solution in methanol was mixed with 0.1 ml of plant extract. The reaction mixture was incubated in the dark for 30 min and thereafter the optical density was monitored at 517 nm against the blank. Vitamin C equivalent antioxidant capacity (VCEAC) was calculated by using ascorbic acid as a reference compound to prepare the standard curve and was expressed as mg/100 g of dry matter of VCEAC. For the control, 2.9 ml of DPPH solution in methanol (100 mM) was mixed with 0.1 ml of methanol. The radical solution was prepared daily.

Phenolic compounds extraction for RP-HPLC analysis. A sample of different fraction obtained by column chromatography was dissolved by ultrasound bath in minimum of methanol. The sample was centrifuged at 1536 × g for 15 minutes at ambient temperature. The supernatant was taken into a 10 ml volumetric flask. All solutions were prepared daily and stored at 4°C.

Statistical analysis

All analyses were carried out in triplicates. The results of scavenger activity, total phenolic and total flavonoid contents were performed from the averages of all samples reading mean ±standard deviation using Excel 2007 and STATISTICA software on student one Test. Observed differences were statistically considered significant at the level of $P < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of *Arisarum vulgare* seeds revealed the presence of flavonoids, tannins, lipids, anthocyanins, sterols and terpenes.

Determination of total phenolic, total flavonoids and total antioxidant capacity (VCEAC assay)

The results obtained in the present study and shown in Figure 1, revealed that total phenolic and flavonoids of methanol-water extract of *A. vulgare* seeds were respectively 1233.68 ±51.54 mg GAE and 347.77 ±33.14 mg QE per 100 g of plant extract dry weight. The total antioxidant capacity expressed as vitamin C equivalent antioxidant capacity (VCEAC) per 100 g of plant extract, obtained by ABTS and DPPH tests were respectively 1300.8 ±66.21 mg and 991.2 ±55.45 mg VCE per 100 g dry weight. A correlation between recorded antioxidant activity and total phenolic compounds is found ($R^2 = 0.95$) but there is no correlation between total flavonoid compounds and antioxidant activity ($R^2 = 0.70$).

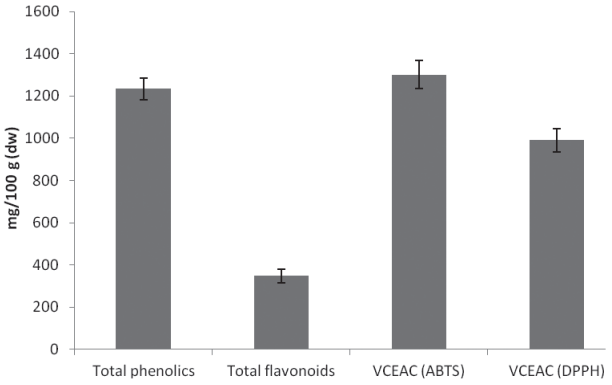

Fig. 1. Total phenol and flavonoid content in methanol-water (7:3) extract of *A. vulgare* (seeds) and quantification of antioxidant activity by VCEAC assay

RP-HPLC analysis

The samples (F1), (F2) and (F3) were filtered through a 0.45 µm PTFE syringe tip filter and were analysed using an RP-HPLC system equipped with a 20 µL injection loop, a waters UV-Visible tunable detector on a reverse phase (RP C_{18}) column Alltech Interstsil ODS-5 µm × 4.6 mm × 150 mm. The flow rate was set at 1 ml/minute at room temperature. To perform this study, a gradient of three mobile phases was used; solvent A: 50 mM ammonium phosphate ($NH_4H_2PO_4$) pH 2.6 (adjusted with phosphoric acid), solvent B: (80:20 (v/v)) acetonitrile/solvent A, and solvent C: 200 mM of phosphoric acid pH 1.5 (pH adjusted with ammonium hydroxide). The solvents were filtered through a Whatman paper N°1 and placed in an ultrasonic

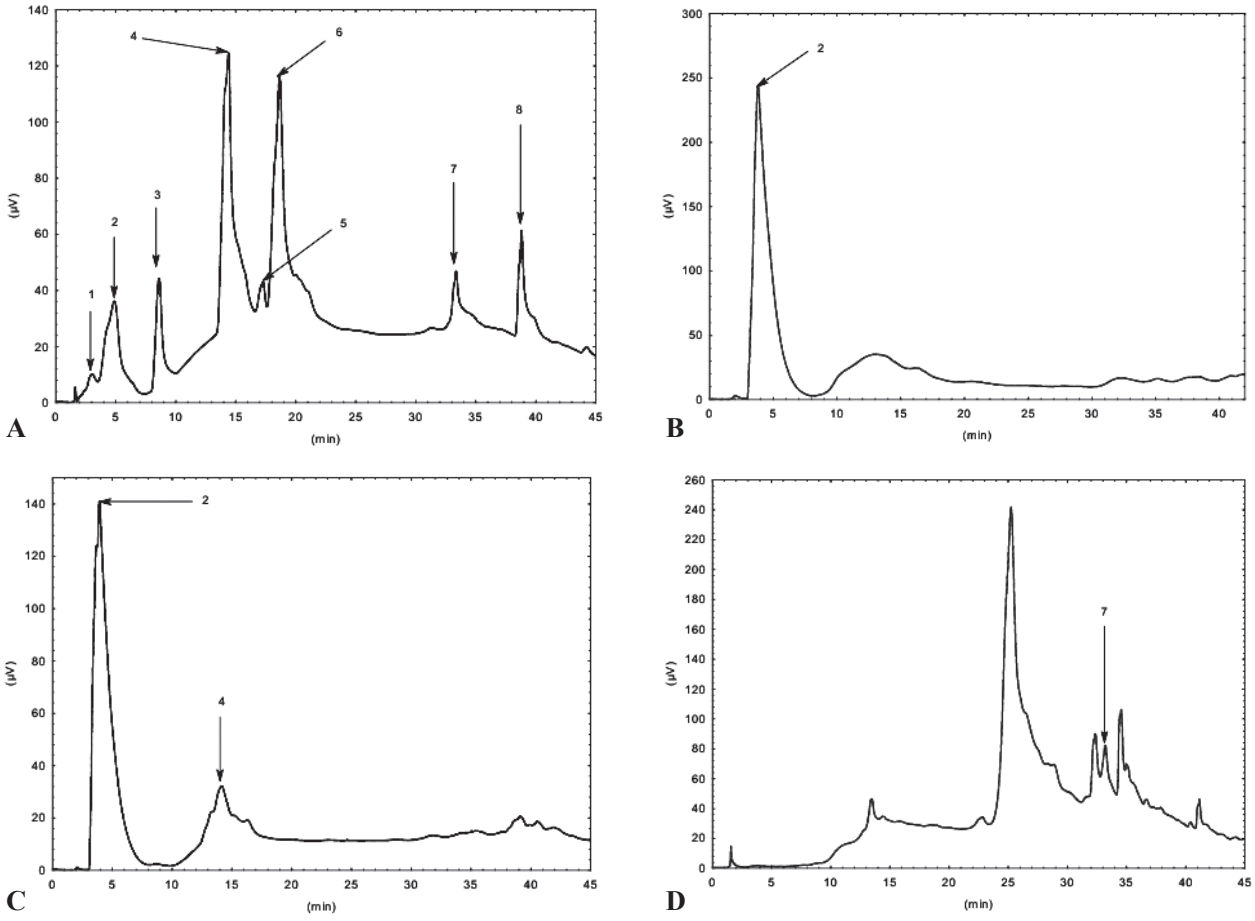

Fig. 2. A. HPLC chromatogram at 280 nm of polyphenols standards: 1 – chlorogenic acid, 2 – gallic acid, 3 – protocatechic acid, 4 – caffeic acid, 5 – epicatechic acid, 6 – para coumaric acid, 7 – rutin, 8 – quercitine. **B.** HPLC chromatogram at 280 nm of fraction F1. **C.** HPLC chromatogram at 280 nm of fraction F2. **D.** HPLC chromatogram at 280 nm of fraction F3

bath for 20 minutes. The gradient profile was linearly changed as follows (total 60 minutes): 100% solvent A at zero minutes, 92% A/8% B at 4 minutes, 14% B/86% C at 10 minutes, 16% B/84% C at 22.5 minutes, 25% B/75% C at 27.5 minutes, 80% B/20% C at 50 minutes, 100% solvent A at 55 minutes, and 100% A at 60 minutes. After each run, the system was reconditioned for 10 minutes before analysis of the next sample. Under these conditions, each sample analysis was done in triplicate. Polyphenolic external standards were prepared by dissolving 2 mg/ml and used as reference. In each sample, polyphenol was identified by comparing its retention time with that of the corresponding external standard. Detection was done at 280 nm.

CONCLUSION

The results indicate that the methanol-water extract of *A. vulgare* seeds possesses strong antioxidative properties *in vitro.* They are confirmed by high polyphenols and flavonoids contents and corroborated by HPLC identifications. Moreover, HPLC analysis reveal the presence of gallic acid, caffeic acid and rutin in the different fractions and then corroborate the richness of *A. vulgare* seeds in polyphenols.

ACKNOWLEDGMENT

The authors wish to thank Professor A. Dicko from 'Université de Lorraine' (France) for his assistance in HPLC analyses.

REFERENCES

Amarowicz R., Estrella I., Hernandez T., Troszynska A., 2008. Antioxidant activity of extract of Adzuki bean and its fractions. J. Food Lipid. 15, 119-136.

Chun O.K., Kim D.O., Lee C.Y., 2003. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem. 51, 8067-8072.

Djilani A., Bayoud B., Legseir B., Soulimani R., Dicko A., Younos C., 2007. Antibacterial activity of some toxic plants of Algerian flora growing in El-Kala National Park. In: Recent progress in medicinal plants: natural products II. Eds J.N. Govil, V.K. Singh, N. Siddiqui. Studium Press, LLC, USA, 18, 35-39.

Gramza-Michałowska A., Abramowski Z., Jovel E., Hęś M., 2008. Antioxidant potential of herbs extracts and impact on HEPG2 cells viability. Acta Sci. Pol., Technol. Aliment. 7(4), 61-72.

Harborne J.B., 1984. Phytochemical methods. A guide to modern techniques of plants analysis. Chapman & Hall London.

Kim D.-O., Chun O.K., Kim Y.J., Moon H.-Y., Lee C.Y., 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem. 51, 6509-6515.

Re R., Pellegrini N., Proteggente A., Pamala A., Yang M., Rice-Evans C., 1999. Antioxidant activity applying an improved ABTS radicalcation decolorization assay. Free Rad. Biol. Med. 26, 1231-1237.

Zhishen J., Mengcheng T., Jianming W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.

Received – Przyjęto: 12.10.2012

Accepted for print – Zaakceptowano do druku: 20.02.2013

For citation – Do cytowania

Kadri H., Djilani S.E., Djilani A., 2013. Phytochemical constituents, antioxidant activity, total phenolic and flavonoid contents of *Arisarum vulgare* seeds. Acta Sci. Pol., Technol. Aliment. 12(2), 169-173.