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# Effect of solvent type and high pressure treatment on the extraction of *Gomphrena globosa* L. bioactive compounds

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# Effect of solvent type and high pressure treatment on the extraction of *Gomphrena globosa* L. bioactive compounds

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**Abstract**. The present study aimed to compare the influence of different extraction solvents (water, methanol, water:acetone (6:4, v/v)), methods (heating (37 °C, 30 min) or high pressure (HP) (300 or 500 MPa) and extraction time (7.5 or 15 min)) on flavonoids, hydrolysable tannins and antioxidant activity (Total Reducing Capacity (TRC), DPPH Free Radical Scavenging Activity and Reducing Power) of *Gomphrena globosa* L. flower extracts. The water:acetone extracts obtained by heating had the highest values of flavonoids, hydrolysable tannins and antioxidant activity. When applying HP, variable results were obtained. Still, the application of HP to water allowed to extract more hydrolysable tannins, as well as to obtain extracts with higher antioxidant activity than with heating, but no significant alterations were observed with methanol. In conclusion, both solvent and extraction method influence the content of bioactive compounds, being HP treatment a promising method to obtain enriched aqueous extracts in line with the principles of green-chemistry.

## 1. Introduction

*Gomphrena* spp. are edible, ornamental and medicinal plants commonly known as Globe Amaranth or Bachelor Button. The flowers of *Gomphrena globosa* L. have medicinal potential because they are rich in bioactive compounds such as betacyanins, betalaines and flavonoids. This flower has been studied by some researchers, who had identified phenolic compounds such as hydroxycinnamic acids and flavonoids [1], being kaempferol-3-*O*-(6-rhamnosyl) hexoside and kaempferol-3-*O*-hexoside the main compounds determined in decoction extracts [2].

In recent years the search for cheap and abundant sources of natural antioxidants has been increasing. There are many reports about different extraction methods for edible flowers such as Soxhlet [3-4], ultrasonics [4], supercritical fluid extraction [5] and solid phase microextraction [6]. These methods are based on an appropriate selection of solvent and energy input to increase chemical solubility and mass transfer rate [7]; however, high energy consumption, long extraction times and relatively low extraction yields can be observed [8]. High pressures (HP) have been recently applied to extract bioactive ingredients from plant materials [8, 9], taking advantage of time saving, higher

extraction yields, fewer impurities in the extraction solution, minimal heat and thermal degradation of the activity and structure of bioactive components.

Few studies have been performed on the antioxidant activity and bioactive compounds of *Gomphrena globosa* L. specie [1, 10, 11]; however none of these studies had applied HP.

In order to increase the knowledge on this subject, the present study aimed to perform a physicochemical characterization of dry *Gomphrena globosa* L. flowers obtained in a Portuguese herbal shop, followed by a comparison on the content of flavonoids, hydrolysable tannins, and antioxidant activity (Total Reducing Capacity, DPPH free radical scavenging activity and Reducing Power) of flower extracts obtained with different extraction solvents (water, methanol, water:acetone (6:4; v/v)) and by different extractive methods (heating under agitation at 37 °C for 30 min or high pressures 300 or 500 MPa at different times 7.5 or 15 min).

## 2. Materials and methods

### *2.1. Plant material*

Dry Globe Amaranth flowers (*Gomphrena globosa* L.) were purchased from a local herbalist in Bragança city (Portugal) in bags of 40 grams (Figure 1).

**Figure 1.** *Gomphrena globosa* L. flowers.

### *2.2. Physicochemical characterization*

The following parameters were evaluated in the flowers: weight, width, length and protein content. The width and length of ten flowers were measured with a digital caliper (Powerfix, Leeds, UK) and the weight in a digital balance (Kern, Balingen, Germany).

Protein content was determined by the Kjeldahl method using the AOAC procedure [12] using one gram of sample. The content of nitrogen was multiplied by the coefficient 6.25 and expressed as crude protein in %.

### *2.3. Flowers extraction*

*2.3.1. Heating under agitation.* Extraction was based on the method described by Li et al. [13] with slight modifications. Approximately, 5 grams of flowers were crushed and mixed with 100 mL of solvent (water, methanol and water:acetone (6:4, v/v)), at 37 °C, for 30 min under agitation (IKA, RCT Model B, Staufen, Germany) at 900 rpm. Subsequently, the supernatant was collected and the process was repeated twice. Then, the combined extracts were filtered, frozen and placed in the lyophilizer (Scanvac, Coolsafe, Lynge, Denmark) for 2 days. In the case of the methanol extracts, these were placed on a rotary evaporator at 40 °C. The extracts obtained were weighed and redissolved with the different solvents to a concentration of 50 mg extract/mL, covered with aluminium foil and stored under freezing until further analysis.

*2.3.2. High pressure (HP).* Five-gram of flowers powder was mixed with 100 ml of each solvent, as reported above, in polyethylene bags. The HP treatments were carried in an Hiperbaric (Burgos, Spain) equipment with 55 L of vessel volume. After the selected high hydrostatic pressure treatments (300 and 500 MPa and holding times of 7.5 and 15 min) at room temperature, the mixtures were

filtered through filter paper. Assays were done in duplicate. The water and water:acetone extracts were preserved as indicated above.

*2.4. Flavonoids and hydrolysable tannins*

Total flavonoid and hydrolysable tannins contents were determined by the method described by Viuda-Martos et al. [14] and Elfalleh et al. [15] respectively.

*2.5. Antioxidant activity*

Antioxidant activity of the flower extracts was determined by the following methods: total reducing capacity (TRC), DPPH free radical scavenging activity and Reducing Power, by the procedure described by Delgado et al. [16] with some modifications.

*2.6. Statistical analysis*

The Statistic SPSS software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data.

## 3. Results and discussion

*3.1. Physicochemical characterization*

The dry *Gomphrena globosa* flowers had a mean width of 1.47 cm and length of 1.55 cm (Table 1), with a low average weight of 0.26 g each. The flower heads were colourful with purple shades (Figure 1).

**Table 1.** Physicochemical characterization of *Gomphrena globosa* flowers, n=10

| Parameter | Values | Min | Max |
|---|---|---|---|
| Weight (g) | 0.26±0.07 | 0.16 | 0.35 |
| Width (cm) | 1.47±0.36 | 1.40 | 1.70 |
| Length (cm) | 1.55±0.14 | 0.90 | 1.90 |
| Crude protein (%, dry weight) | 9.41±0.12 | --- | ---- |

*Gomphrena globosa* flowers had a crude protein content of 94.1 g/kg (dry weight) that was within the range of the three edible flowers studied by Navarro-González et al. [17] (79-186 g/kg dry weight for *Tagetes erecta* and *Tropaeolum majus*, respectively).

*3.2. Flavonoids and hydrolysable tannins*

The extracted flavonoids and hydrolysable tannins showed significant differences among the five extraction conditions, as well as between solvents (Figure 2).

The water:acetone extracts obtained by heating had the highest amounts of flavonoids and hydrolysable tannins (Figure 2A, B). So, the highest molecular weight compounds such as tannins and flavonoids are better extracted by using aqueous organic solvent solutions.

Among HP treatments, the highest value of flavonoids was also obtained with water:acetone (P300/15 min with a value of 7.4 mg of QE/g dry flower). Concerning methanol, the extracts obtained after classical heating were not significant different to those obtained with HP. For water the highest amounts were extracted with HP treatments, except for the binomial P500/15 min that gave the lowest flavonoid content; however, the HP results were not statistically different to that obtained with the heating treatment. Thus, the solvent effect is more relevant than the treatment applied, showing that different solvents extract distinct compounds. With the exception of water:acetone solvent, similar concentrations of flavonoids were obtained between HP treatment and the traditional method with water (green solvent) or methanol, suggesting similar extraction efficiencies. On contrary, when using water:acetone solvent the heating treatment gave the best results.

For hydrolysable tannins, when performing methanolic extractions, the highest value was obtained with the heating treatment. Within the HP treatments, the highest values were observed for longer

times, with the binominals P300/15 and P500/15. For water, the highest value was obtained with the P300/15 HP treatment. Nevertheless, the extracts obtained with the other HP treatments were not significantly different to that prepared by heating under agitation.

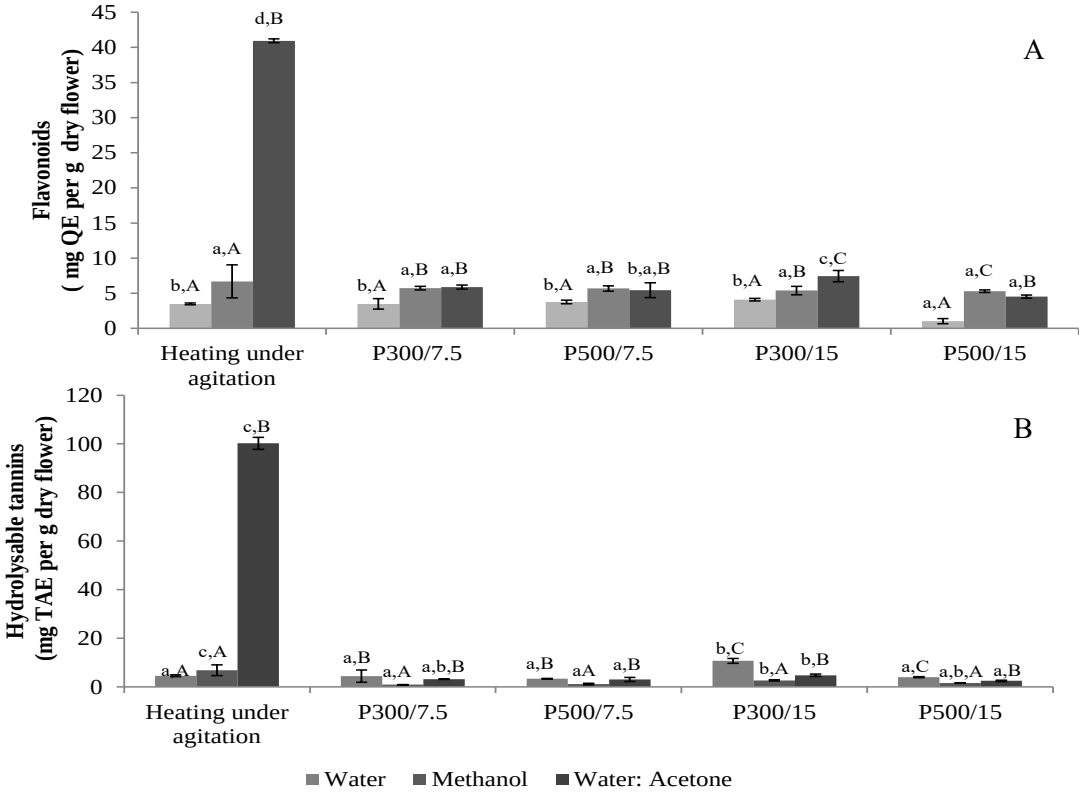


**Figure 2.** Flavonoids and hydrolysable tannins contents of flower extracts obtained after application of different extraction methods and solvents. Values with the same lowercase letter are not statistically different ($p>0.05$) in what concerns treatment into the same solvent. Values with same uppercase letter are not statistically different ($p>0.05$) in what concerns solvent into the same treatment.

*3.3. Antioxidant activity*

*3.3.1. Total Reducing Capacity (TRC).* The TRC of *Gomphrena globosa* L. flowers extracts varied between 1.50 to 48.60 mg GAE/g dry flower, obtained after HP treatment (P500/15) and heating treatment with water and water:acetone (6:4, v/v), respectively (Table 2). Among the solvents used, water:acetone (6:4, v/v) extracts showed the highest value of TRC, followed by methanol by heating. Within the HP treatments, the extracts with the highest TRC were again obtained with water:acetone at P300/15. These results suggested that a mixture with water and an organic solvent such as water:acetone, can be the best way to extract compounds with Reducing Capacity in this flower specie, such as phenols. This result is in agreement with Kuźma et al. [18], who reported that higher phenolic contents and antioxidant activity were obtained with aqueous organic solvents than with the respective absolute organic solvents, as methanol, ethanol, acetone and ethyl acetate. By adding water the polarity increases and so the aqueous organic solvents are able to extract both high and low polarity compounds [19]. For methanol the highest value was observed with the heating treatment. Regarding water, the HP and heating treatments were not statistically different.

**Table 2.** Total Reducing Capacity, $EC_{50}$ DPPH and $EC_{50}$ Reducing Power values of flower extracts obtained with different solvents and extraction methods.*

| Assay | Methods | Water | Methanol | Water:acetone |
|---|---|---|---|---|
| TRC (mg GAE per g dry flower) | Heating | $1.73\pm0.07^{a,A}$ | $22.6\pm0.79^{c,B}$ | $48.60\pm1.87^{c,C}$ |
| | P300/7.5 | $1.69\pm0.26^{a,A}$ | $2.55\pm0.25^{b,B}$ | $7.58\pm0.31^{a,C}$ |
| | P500/7.5 | $1.57\pm0.12^{a,A}$ | $2.24\pm0.22^{a,b,B}$ | $7.25\pm0.94^{a,C}$ |
| | P300/15 | $1.67\pm0.11^{a,A}$ | $1.90\pm0.20^{a,A}$ | $9.99\pm0.70^{b,B}$ |
| | P500/15 | $1.50\pm0.05^{a,A}$ | $2.03\pm0.26^{a,b,B}$ | $6.31\pm0.47^{a,C}$ |
| $EC_{50}$ DPPH (mg extract per mL) | Heating | $3.74\pm0.20^{c,C}$ | $1.79\pm0.18^{a,B}$ | $0.95\pm0.04^{a,A}$ |
| | P300/7.5 | $2.44\pm0.49^{b,B}$ | $2.21\pm0.48^{a,B}$ | $1.10\pm0.02^{b,A}$ |
| | P500/7.5 | $1.74\pm0.28^{a,B}$ | $2.90\pm0.31^{b,C}$ | $0.97\pm0.12^{a,b,A}$ |
| | P300/15 | $2.09\pm0.24^{a,b,B}$ | $2.71\pm0.27^{b,C}$ | $0.97\pm0.05^{a,b,A}$ |
| | P500/15 | $1.58\pm0.03^{a,B}$ | $1.77\pm0.32^{a,C}$ | $0.87\pm0.08^{a,A}$ |
| $EC_{50}$ Reducing Power (mg extract per mL) | Heating | $3.01\pm0.01^{b,C}$ | $1.12\pm0.01^{a,B}$ | $0.08\pm0.01^{a,A}$ |
| | P300/7.5 | $2.34\pm0.08^{a,C}$ | $1.72\pm0.06^{b,A}$ | $1.90\pm0.11^{c,B}$ |
| | P500/7.5 | $2.50\pm0.13^{a,C}$ | $1.84\pm0.05^{c,A}$ | $2.26\pm0.04^{d,B}$ |
| | P300/15 | $2.35\pm0.13^{a,C}$ | $1.80\pm0.06^{b,c,B}$ | $1.61\pm0.05^{b,A}$ |
| | P500/15 | $2.45\pm0.09^{a,C}$ | $1.88\pm0.06^{c,A}$ | $2.29\pm0.03^{d,B}$ |

*Values are expressed as: Mean±Standard deviation. Values with the same lowercase letter are not statistically different ($p>0.05$) in what concerns treatment into the same solvent. Values with the same uppercase letter are not statistically different ($p>0.05$) in what concerns solvent into the same treatment.

*3.3.2. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity.* The water:acetone extracts were again those that presented the lowest $EC_{50}$ values of DPPH free radical scavenging activity**,** suggesting higher antioxidant activity than with other solvents (Table 2). For water, the lowest values of $EC_{50}$ were obtained with all conditions of HP treatment, while for methanol the lowest values were obtained with the heating treatment and the two HP treatments of P300/7.5 and P500/15. Our results of $EC_{50}$ for the methanolic extracts were lower than the values obtained by Roriz et al. [20] (4.87 mg/mL) after using methanol at 25 °C for 1 hour, but higher than the value reported by Hamiduzzaman and Azam [11] of 0.020 mg/mL for the whole plant after an extraction at room temperature but during 15 days, what might explain the difference obtained.

Regarding HP treatments, again different results were obtained with different time and pressure combinations, as well as solvents. Nevertheless, the lowest $EC_{50}$ value of DPPH was obtained at P500/15 for all solvents. So, this binomial time/pressure showed the highest antioxidant potential.

When comparing the two extraction methods (heating *versus* HP) applied to water (green solvent), the HP treatments always originated lower $EC_{50}$ values than the conventional one. Moreover, when the pressure increased from 300 to 500 MPa in water, the $EC_{50}$ also decreased. This can be explained because when pressure is applied, the permeability of the cells increases, enabling increased solvent permeation in the cells and therefore the extraction will be more efficient [9]. Thus, HP can have higher extraction efficiency and can greatly shorten the extraction time. Other advantage of HP treatment is to operate at room temperature without any heating process, so there is a lower energy expense, while the bioactivity of the extracted compounds is preserved.

*3.3.3. Reducing Power.* The $EC_{50}$ values of reducing power showed again that antioxidant activity of the extracts is strongly dependent on the extraction solvent. The water:acetone extract obtained by heating had the lowest $EC_{50}$ value, followed by the HP treatment (P300/15). With methanol, the lowest

value of $EC_{50}$ was obtained with the heating treatment, while for water the lowest values were obtained after any of the HP treatments.

## 4. Conclusion

In summary, the results showed that *Gomphrena globosa* L. flowers are a promising source of natural antioxidants, with the solvent and conditions used in the extraction having direct influence on the content of bioactive compounds. Among the solvents tested, water:acetone was found to be the most efficient solvent to extract bioactive compounds, originating extracts with higher antioxidant activity, followed by methanol and water, for both extractive methods. Our results also showed that the application of HP can be a promising method to extract more natural antioxidants with green solvents, like water, from natural sources such as *Gomphrena globosa* L.. However, in the future more studies must be performed to identify the individual compounds extracted after application of different time/pressure binomials to better understand their extractability.

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