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SHORT COMMUNICATION

Comparative phytochemical profiling, antioxidant activity, and subacute toxicity assessment of infusion and decoction extracts of pumpkin seeds

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Abstract

Pumpkin (*Cucurbita maxima*) seeds are rich in bioactive compounds, including phenolics and flavonoids, which contribute to their antioxidant properties. Traditional consumption often involves aqueous preparations, such as infusions and decoctions; however, comparative evaluations of their phytochemical content, antioxidant activity, and safety remain limited. This study compares the phytochemical profiles and antioxidant activities of pumpkin seed infusions and decoctions and evaluates their subacute oral toxicity over 14 days in rats. Pumpkin seed infusion and decoction extracts were prepared using traditional aqueous extraction methods. Total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride assays. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. Subacute oral toxicity was assessed in Wistar rats following daily oral administration of 200 mg/kg for 14 days. Phytochemical screening revealed that the infusion contained higher total phenolic content (7.5 ± 0.032 mg gallic acid equivalents/g dry extract) and total flavonoid content (2.72 ± 0.0018 mg quercetin equivalents/g dry extract) compared with the decoction (6.5 ± 0.026 mg gallic acid equivalents/g dry extract and 2.53 ± 0.0014 mg quercetin equivalents/g dry extract, respectively). Accordingly, the infusion exhibited higher antioxidant activity (IC_{50} = 3.66 ± 0.03 mg/mL) than the decoction (IC_{50} = 4.045 ± 0.02 mg/mL). No mortality or adverse clinical signs were observed in treated animals, indicating an absence of subacute toxicity at the tested dose. These findings support the traditional use of pumpkin seeds and demonstrate that their aqueous preparations, particularly infusion, exhibit antioxidant activity with a favorable safety profile. Overall, pumpkin seeds represent a promising source of bioactive compounds.

Keywords: Pumpkin seeds; Infusion; Decoction; Phytochemical profile; Antioxidant activity; Subacute toxicity

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1. Introduction

Pumpkin (*Cucurbita maxima*) seeds are increasingly recognized not only as a nutrientdense food but also as a source of biologically active compounds with potential health benefits.[1] Numerous studies have demonstrated that these seeds are rich in proteins, unsaturated fatty acids, vitamins, minerals, and secondary metabolites such as flavonoids and phenolic acids, which have been associated with cardioprotective, anti-inflammatory, and antioxidant properties.[2,3] Indeed, *C. maxima* seed extracts contain phenolic compounds—including p-hydroxybenzoic, caffeic, and p-coumaric acids—which contribute to their free radical scavenging capacity and functional properties in food and medicinal applications.[4,5] Both free and bound phenolic compounds have been found in pumpkin seeds, and their presence is closely correlated with antioxidant potential.[6]

However, the relationship between total phenolic content and antioxidant efficacy is not always linear and can vary depending on the extraction technique and seed processing.[7] In fact, the extraction method significantly influences the yield of phytochemicals and their associated biological activities.[8] In particular, traditional aqueous extraction methods, such as infusion and decoction, differ substantially in temperature and extraction time—factors known to affect the stability and release of phenolic and flavonoid compounds.[9] Decoctions involve prolonged boiling, which can enhance cell wall disintegration and the release of bound antioxidants, whereas infusions typically require shorter exposure to hot water and may better preserve thermolabile compounds.[10] As a result, significant variations in phytochemical profiles and functional characteristics, including antioxidant activity, may arise depending on the extraction method used.[11] Despite their widespread use in traditional medicine and as functional foods, direct and systematic comparisons of phytochemical content and antioxidant activity between pumpkin seed infusions and decoctions remain limited.

In addition to bioactivity, assessing the safety of bioactive extracts is essential for their potential application as nutraceuticals.[12] Previous studies on *C. maxima* seed extracts have generally reported a favorable safety profile in animal models; however, these investigations primarily focused on whole seeds, roasted powders, oils, or organic solvent extracts.[13–15] To our knowledge, the subacute (14-day) oral toxicity of aqueous infusion and decoction preparations of pumpkin seeds has not yet been reported, despite their widespread use in traditional medicine and functional foods.

Therefore, the present study aims to compare the phytochemical profiles and antioxidant activities of pumpkin seed extracts prepared as infusions and decoctions and to evaluate the subacute oral toxicity of these aqueous preparations over 14 days in rats. We hypothesize that differences in extraction temperature and duration between infusion and decoction influence the phytochemical content and antioxidant capacity of the resulting extracts. Accordingly, this study provides an integrated evaluation of both efficacy and safety of water-based pumpkin seed extracts in alignment with these objectives.

2. Materials and methods

2.1. Plant preparation

Cucurbita maxima seeds were purchased from a local market and botanically authenticated by Pr. Fethia Harzallah Skhiri (Institute of Biotechnology of Monastir, Tunisia). They were washed, air-dried in the dark at room temperature, ground into a fine powder using a grinder, and stored in a tightly closed container.

2.2. Preparation of infusion

The *C. maxima* infusion was prepared to simulate the traditional method of consumption. Briefly, 10 g of dried powdered *C. maxima* seeds was placed in a beaker, and 100 mL of boiling distilled water was added. The mixture was covered and allowed to infuse for 15 min. After cooling to room temperature, the infusion was filtered through Whatman No. 1 filter paper, and the filtrate was stored at −20 °C until further analysis.

2.3. Preparation of decoction

For the decoction, 10 g of dried *C. maxima* powder was boiled in 100 mL of distilled water for 20 min. The mixture was then cooled to room temperature and filtered to remove plant residues. The decoction was prepared to facilitate the extraction of water-soluble compounds requiring prolonged heating. The extract was stored in airtight amber containers at −20 °C until further analysis.

2.4. Determination of total phenolic contents

Total phenolic content in the extracts was determined using the Folin–Ciocalteu method. Briefly, the dry extract obtained from infusion and decoction was dissolved in distilled water or methanol to prepare a stock solution (1 mg/mL). Then, 0.25 mL of the stock solution was mixed with 1.25 mL of 10% (v/v) Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 1 mL of 7.5% (m/v) sodium carbonate (Sigma-Aldrich, St. Louis, MO, USA). The mixture was vortexed for 10 s and incubated in the dark for 30 min.

The total phenolic content of the extracts was calculated

using a calibration curve constructed with gallic acid as the standard, prepared at different concentrations under the same conditions as the samples. Absorbance was measured using an ultraviolet (UV)–visible spectrophotometer (Cole-Parmer, SP-200-UV, USA) at 765 nm. All determinations were performed in triplicate.

The results are expressed as mg gallic acid equivalents (GAE)/g of dry extract, calculated using the following formula:

$$TPC = \frac{C_g \times F \times V}{m} \quad (1)$$

where:

(i) TPC is the total phenolic content (mg GAE/g of dry extract).
(ii) C_g is the gallic acid concentration (mg/mL).
(iii) F is the dilution factor (F = 50).
(iv) V is the extract volume (mL).
(v) m is the dry extract weight (g).

A blank solution was prepared by mixing the corresponding solvent (distilled water) with Folin–Ciocalteu reagent and sodium carbonate.

2.5. Determination of flavonoid content

The flavonoid content of the extracts was determined using a colorimetric aluminum chloride method. Briefly, 1 mL of each extract solution at different concentrations (0.5, 1.0, and 2.0 mg/mL) was mixed with 1 mL of 2% aluminum chloride solution (Merck, Darmstadt, Germany) and incubated at room temperature for 10 min. Absorbance was measured using a UV–visible spectrophotometer at 430 nm. All determinations were performed in triplicate.

The results are expressed as mg quercetin equivalents (QE)/g of dry extract, calculated from the quercetin calibration curve using the following formula:

$$CF = \frac{C_q \times F \times V}{m} \quad (2)$$

where:

(i) CF is the flavonoid content (mg QE/g of dry extract).
(ii) C_q is the quercetin concentration (mg/mL).
(iii) F is the dilution factor (F = 2).
(iv) V is the extract volume (mL).
(v) m is the dry extract weight (g).

A blank solution was prepared by mixing ethanol with the aluminum chloride solution.

2.6. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed with minor modifications to improve reproducibility. A fresh 0.1 mM DPPH solution in methanol was prepared and protected from light. Then, 180 µL of test sample (10, 20, 40, 60, and 80 mg/mL) or gallic acid (standard) was mixed with 1.62 mL of DPPH solution and incubated in the dark at room temperature for 30 min. A solvent control (180 µL methanol + 1.62 mL DPPH) was included.

Absorbance was measured at 517 nm using a UV–visible spectrophotometer. All determinations were performed in triplicate. Percent radical scavenging activity was calculated using the following formula:

$$Percentage\ inhibition(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of DPPH in the presence of the sample.

The concentration required to produce 50% inhibition (IC_{50}, mg/mL) was determined by nonlinear regression analysis of the dose–response curve (percent inhibition versus log concentration). Results for each extract are expressed as IC_{50} (mean ± standard deviation, n = 3).

2.7. *In vivo* toxicity study

Preliminary subacute oral toxicity of *C. maxima* infusion and decoction was evaluated in healthy adult Wistar rats (n = 12) following daily oral administration for 14 consecutive days. Female Wistar rats (180–200 g) were obtained from the Pasteur Institute of Tunisia.

Rats were housed under standard laboratory conditions (22 °C, 12 h light/dark cycle) with free access to water and a standard diet and were acclimatized for one week prior to experimentation.

Animals were randomly divided into three groups (n = 4 per group): the treated groups received 200 mg/kg body weight of the infusion and decoction extracts (freshly prepared in distilled water), whereas the control group received an equivalent volume of distilled water. All animals were fasted overnight prior to the first administration. Clinical signs, body weight, and food and water intake were monitored daily throughout the study.

All animal experiments were conducted in accordance with the European Community guidelines for the care and use of laboratory animals (EEC Directive 86/609/EEC) and were approved by the Research Ethics Committee for Life and Health Sciences at the Higher Institute of Biotechnology of Monastir (cer-svs10/2020).

3. Results

3.1. Phytochemical screening

In this study, the total phenolic and flavonoid contents of *C. maxima* infusion and decoction were determined using

the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. Calibration curves for total phenolic and flavonoid contents were constructed using gallic acid and quercetin standards, respectively (Figure 1). The correlation coefficients were $R^2 = 0.993$ for phenolics and $R^2 = 0.9509$ for flavonoids, confirming good linearity of the assays.

As shown in Table 1, the infusion exhibited a higher total phenolic content (7.5 ± 0.032 mg GAE/g dry extract) compared with the decoction (6.5 ± 0.026 mg GAE/g dry extract). Total flavonoid content was comparable between the extracts, with values of 2.72 ± 0.0018 mg QE/g dry extract for the infusion and 2.53 ± 0.0014 mg QE/g dry extract for the decoction.

Table 1. Phytochemical composition of aqueous pumpkin seed extracts

Parameter	Infusion	Decoction
Total phenolic content (mg GAE/g dry extract)	7.5 ± 0.032	6.5 ± 0.026
Total flavonoid content (mg QE/g dry extract)	2.72 ± 0.0018	2.53 ± 0.0014

Abbreviations: GAE: Gallic acid equivalents; QE: Quercetin equivalents.

3.2. Antioxidant activity

The antioxidant activity of the aqueous extracts was evaluated using the DPPH radical scavenging assay, and IC_{50} values were determined and compared with that of gallic acid (reference standard) (Table 2). The infusion (IC_{50} = 3.66 ± 0.03 mg/mL) exhibited higher antioxidant activity than the decoction (IC_{50} = 4.045 ± 0.02 mg/mL). As expected, gallic acid (standard) showed the strongest antioxidant activity (IC_{50} = 1.79 ± 0.08 mg/mL).

These results indicate that the infusion retains a higher level of antioxidant constituents than the decoction, possibly due to differences in extraction temperature and duration, as well as the thermal sensitivity of phenolic compounds.

Table 2. Antioxidant activity of aqueous pumpkin seed extracts using DPPH assay

Sample	IC_{50}
Infusion (mg/mL)	3.66 ± 0.03
Decoction (mg/mL)	4.045 ± 0.02
Gallic acid (mg/mL)	1.79 ± 0.08

Abbreviation: IC_{50} = Half-maximal inhibitory concentration.

3.3. Subacute oral toxicity in rats

The findings revealed that repeated daily oral administration of pumpkin seed infusion and decoction at 200 mg/kg body weight for 14 days was well tolerated in rats. No mortality or significant clinical signs of toxicity were observed throughout the experiment. Body weight gain, food intake, and water consumption were comparable between the treated and control groups.

These preliminary findings indicate that repeated oral administration of *C. maxima* infusion and decoction at 200 mg/kg body weight does not induce observable toxicity under the experimental conditions and is considered safe within the scope of this study.

4. Discussion

In this study, the phytochemical composition, antioxidant activity, and subacute toxicity of pumpkin seed aqueous extracts prepared as infusion and decoction were evaluated.

Phytochemical screening revealed that the infusion contained higher total phenolic (7.5 ± 0.032 mg GAE/g dry

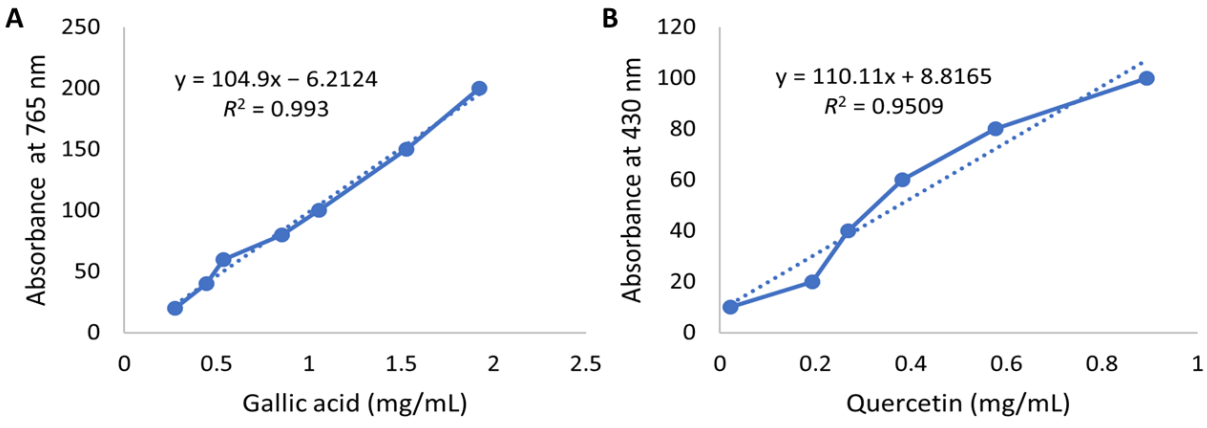


Figure 1. Calibration curves of (A) gallic acid for total phenolic content determination and (B) quercetin for total flavonoid content determination

extract) and flavonoid contents (2.72 ± 0.0018 mg QE/g dry extract) compared with the decoction (6.5 ± 0.026 mg GAE/g dry extract and 2.53 ± 0.0014 mg QE/g dry extract, respectively). These findings are consistent with previous reports on aqueous extracts of pumpkin seeds (*Cucurbita pepo*), where total phenolic contents typically range from 0.3 to 15 mg GAE/g extract and total flavonoid contents from 0.1 to 3 mg QE/g extract, depending on the extraction method, solvent system, and expression basis [16–18]. Another study reported that total phenolic contents of pumpkin seed flour and extracts ranged from 0.95 to 9.82 mg GAE/g extract, with lower values generally observed in crude aqueous extracts and higher values obtained following optimized processing or solvent extraction conditions.[7,19] Total flavonoid contents have also been reported at approximately 3 mg QE/g extract, although values vary widely across studies.[17]

These variations in reported values are primarily attributed to differences in seed preparation, solvent polarity, extraction conditions, and analytical expression units. The higher phytochemical content observed in the infusion compared with the decoction suggests that extraction conditions may promote degradation and reduced recovery of phenolic compounds, particularly under prolonged heating. This trend has also been reported in studies on thermal processing of pumpkin seeds, where heat treatment affects the stability and release of phenolic compounds. In this context, roasting pumpkin seeds at temperatures ranging from 90 °C to 200 °C induced changes in phenolic composition, with a marked decline observed above 130 °C, highlighting the thermal sensitivity of these bioactive compounds. These findings suggest that infusion, which involves milder thermal conditions, generally preserves a higher phenolic profile than decoction, where prolonged heating may contribute to degradation of thermolabile phenolics.[20]

The antioxidant potential of the extracts, evaluated using the DPPH radical scavenging assay, was higher for the infusion (IC_{50} = 3.66 ± 0.03 mg/mL) than for the decoction (IC_{50} = 4.045 ± 0.02 mg/mL), in agreement with their respective phenolic contents. Previous studies have reported similar trends in pumpkin seed extracts (*C. pepo*), where DPPH radical scavenging activity was higher at moderate thermal treatments (90–110 °C) and decreased at higher temperatures.[20] For example, heat treatment (boiling or roasting) has been shown to enhance DPPH scavenging capacity through the release of bound phenolic compounds.[21] For aqueous or heat-processed extracts, IC_{50} values have been reported to range between 2 and 6 mg/mL.[17,22] The influence of extraction conditions on phenolic profiles and functional properties is further highlighted by the limited availability of direct comparisons between infusion and decoction preparations of pumpkin seeds, as most reported phenolic data are derived from organic solvent or oil-based extracts rather than hot-water preparations.

The subacute toxicity assessment represents an important contribution of this study, as it provides initial safety data for the oral consumption of pumpkin seed extracts. In the present study, repeated oral administration of pumpkin seed aqueous extracts at 200 mg/kg body weight for 14 days resulted in no mortality, no observable clinical signs of toxicity, and no changes in body weight, food intake, or water consumption, indicating a favorable safety profile. These results are consistent with earlier studies reporting that pumpkin seeds and their constituents are generally non-toxic. For example, studies in rodents have demonstrated that *C. pepo* seed aqueous and ethanolic extracts exhibit favorable safety profiles, with no adverse effects on hematological, biochemical, or histopathological parameters.[23,24]

Pumpkin seeds have also been proposed as functional food ingredients or nutraceuticals, as long-term dietary supplementation has not been associated with toxicity or organ damage in animal models.[13,14,25] Future studies should include a comprehensive toxicological evaluation following internationally recognized guidelines to determine the median lethal dose (LD_{50}) and establish a complete safety profile. Additionally, the assessment of biochemical parameters (e.g., alanine aminotransferase, aspartate aminotransferase, and creatinine) and histopathological examinations would further strengthen the toxicological characterization of the extract and support its potential nutraceutical or therapeutic applications.

Taken together, these findings suggest that a 14-day oral regimen of pumpkin seed extract at 200 mg/kg is well tolerated *in vivo*. Before progressing to long-term toxicity studies or clinical evaluation, establishing such a safety profile is essential. It also contributes to the growing body of evidence that pumpkin seeds are a safe functional food ingredient and potential nutraceutical candidate.

5. Conclusion

The findings demonstrate that *C. maxima* seeds are rich in bioactive compounds, with notable levels of total phenolic and flavonoid contents observed in both infusion and decoction extracts. Antioxidant activity varied according to extraction method, with the infusion yielding higher phenolic and flavonoid contents under milder thermal conditions.

These findings support the potential use of pumpkin

seed extracts as safe nutraceuticals and functional food ingredients with antioxidant properties. Further studies are warranted to explore bioavailability, underlying mechanistic pathways, and long-term *in vivo* safety, as well as their potential applications in mitigating oxidative stress-related disorders.

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Conflict of interest

The authors declare that they have no competing interests.

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Project administration: Sahbi el Mtaoua
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Supervision: Sahbi el Mtaoua
Writing–original draft: Hana Nasrallah
Writing–review & editing: Sahbi el Mtaoua

Ethics approval and consent to participate

All animal experiments were conducted in accordance with the European Community guidelines for the care and use of laboratory animals (EEC Directive 86/609/EEC) and were approved by the Research Ethics Committee for Life and Health Sciences at the Higher Institute of Biotechnology of Monastir (cer-svs10/2020).

Consent for publication

Not applicable.

Availability of data

The datasets used during the current study are available from the corresponding author on reasonable request.

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