 Phenolic compounds and biological activities of different organs from aerial part of *Nitraria retusa* (Forssk.) Asch.: effects of solvents

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**ABSTRACT**

*Nitraria retusa* is an edible halophyte shrub that is known for its high importance in folk medicine and ecology. The fruits are tasty, eaten by humans and birds and are used to prepare a refreshing juice. The fleshy and bitter leaves of *N. retusa*, mixed with water, were used to make poultices, which are applied to reduce swellings. The dry leaves are used, in decoction, as a substitute for tea. Many phytochemical studies on this plant showed its diversity in active compounds such as phenolic compounds. In this work, the biochemical analysis and biological effects were investigated for water-ethanol/methanol/acetone extracted from leaves, flowers, stems, and fruits using two different extraction methods. Results revealed that polyphenol amounts were highest in leaves extracted by hydroethanolic decoction method with 46.97 mg GAE/g DW. Ethanol 70% leaves extracts has the highest contents of condensed tannins with 26.06 mg Cat/g DW by decoction, followed by methanol 70% water. Furthermore, four flavonoids were identified by HPLC-DAD: Luteolin 7-O-glucoside, Rutin, Kaempferol 3-O-rutinoside, and Isorhamnetin 3-O-rutinoside. For antioxidant activity, ethanol fraction was renowned for a high total antioxidant activity with 45.82 mg GAE/g DW and an important antiradical activity with an IC<sub>50</sub> of 22.05 μg/mL. The ethanolic extract showed the best activity inhibiting the production of NO<sub>•</sub> at an IC<sub>50</sub> of 36.60 μg/mL. These results show that this plant could be a promising source of bioactive substances and can be used for the production of juices and functional food supplements.

**KEYWORDS**

*Nitraria retusa*; phenolic compounds; antioxidant; anti-inflammatory

**Introduction**

Over last decade, the practices of traditional medicine have expanded globally and are gaining popularity. The plant kingdom has become a target for the search by multinational drug companies and research institutes for new drugs and biologically active lead compounds. Among plant metabolites, phenolic compounds are the most important antioxidant extracted from medicinal plants by their ability to scavenge free radicals as well as by the inhibition of the synthesis pathways of these radicals. The extraction of phenolic compounds depends on their chemical classes, which range from simple compounds to highly polymerized ones. Plant materials can contain varying amounts of phenolic acids, flavonoids, flavonols anthocyanins, and tannins. This structural diversity is responsible for the great variability of the physicochemical properties influencing the extraction of...
polyphenols. Among other things, the solubility of phenolic compounds is affected by the nature and the polarity of the used solvent. Therefore, it is complicated to develop one extraction process suitable for extracting all phenolic compounds from the plant.\(^{8,9}\)

In this study, *Nitraria retusa* (Forsk.) Asch. was chosen because of its therapeutic potential in traditional medicine.\(^{10}\) The decoction of dried leaves was used as a tea and to make cataplasms. Ashes of this plant have the anti-inflammatory property treating the infected wounds.\(^{11}\) The fresh *Nitraria* red fruits are consumed by human beings and birds and its wood was used as fuel by the local inhabitants.\(^{12}\) In a previous chemo-systematic study of leaves and young stems of *N. retusa*,\(^{13}\) isolated and fully characterized six flavonol glycosides: isorhamnetin-3-O-4-rhamgalactosyl-robinobioside, isorhamnetin-3-O-robinobioside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-galactoside, isorhamnetin-3-O-glucoside, and, free isorhamnetin were identified. These bioactive molecules isolated from *Nitraria retusa* have been reported to promote apoptosis in human myelogenous erythroleukemia cells,\(^{14}\) and to exert anti-oxidant and anti-mutagenic activities.\(^{14}\) In this context, the objective of this study was to seek the best technique for extracting total polyphenols, flavonoids and condensed tannins from different organs of the *Nitraria retusa* aerial part, by testing and modifying the extraction methods and solvents.

**Materials and methods**

**Chemical products**

Polyphenolic standards (Luteolin 7-O-glucoside, Rutin, Kaempferol 3-O-rutinoside, and Isorhamnetin 3-O-rutinoside, Gallic acid, Quercetin, BHT, and Catechol) were purchased from Sigma Aldrich (Germany), the Folin-Ciocalteu reagent were purchased from Sigma Aldrich (USA). The other reagents and solvents are obtained from Panreac, Cheminova, Prolabo, Aldrich, Organics and Janssen.

**Plant sampling**

Aerial parts of *Nitraria* samples were collected during the flowering stage in April-May 2019 from a salt flat called “Sabkha El kelbia” (35.84295057247895, 10.271751741215757 Kairouan, Tunisia) with a semi-arid climate, kept in laboratory temperature, oven-dried at 60°C. It is then ground finely using a knife mill type (Knife Mill GM 200, Germany). The plant powder obtained was stored at room temperature for further experiments.

**Polyphenols extraction**

**Extraction by maceration**

Seventy percent aqueous solvent (ethanol, acetone, and methanol) extraction of the plant samples was conducted with 10% (w/v). The extracts were held in the dark at room temperature for 15 days, with handshaking at least once a day. The liquid fraction was then collected, filtered through a 0.45 μm filter (MILLIPORE, U.S.A.), and concentrated using vacuum concentrators (SpeedVac, Thermo Scientific. the U.S.A). The dried residue was re-dissolved in seventy percent ethanol by vortexing and stored at −20°C for further experiments.\(^{15}\)

**Extraction by decoction**

The extraction of the polyphenols by decoction was carried out according to the protocol described by Chavan, Shahidi\(^{16}\): 2 g of the plant samples were added to 20 ml of extraction solvent (70% v/v ethanol, acetone, and methanol in water). Each mixture was brought to a boil in a water bath for 30 minutes, filtered through a 0.45 μm filter, and concentrated using vacuum concentrators. The dried residue was re-dissolved in seventy percent ethanol by vortexing and stored at −20°C for further experiments. Extraction yield was calculated by the formula given by Falleh, Ksouri\(^{17}\):
\[ R(\%) = 100 \times \left( \frac{\text{the extract dry mass}}{\text{the plant dry mass}} \right) \]

**Colorimetric quantification of phenolic compounds**

**Total polyphenol content**

Colorimetric quantification of total polyphenols was determined, as described by Dewanto, Wu.\(^{[18]}\) An aliquot of 125 µL of diluted extract was added to 500 µL of distilled water and 125 µL of the Folin-Ciocalteu reagent. Before adding 1250 µL Na2CO3 (7%), the mixture was shaken, adjusted with distilled water to a final volume of 3 mL, and mixed thoroughly. After incubation for 90 min at 25°C in the dark, the absorbance versus prepared blank was read at 760 nm. A standard curve of gallic acid was used. The total phenolic content of organs was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW) through the calibration curve with gallic acid, ranging from 40 to 400 µg/mL. All samples were analyzed in triplicate.

**Estimation of total flavonoid content**

Total flavonoid content was measured by a colorimetric assay developed by Dewanto, Wu.\(^{[18]}\) An aliquot of suitable diluted samples or a standard quercetin solution was added to NaNO\(_2\) and mixed for 6 min before adding 150 µL of a freshly prepared AlCl\(_3\) (10 g/100 mL). After 5 min, 500 µL of 1 mol/L NaOH solution was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance was measured at 510 nm. Total flavonoid content was expressed as mg quercetin per gram of dry weight (mg QE/g DW) through the calibration curve of quercetin, ranging from 40 to 400 µg/mL. All samples were analyzed in triplicate.

**Condensed tannin content**

The analysis of condensed tannins was carried out according to the method of Sun, Ricardo-da-Silva.\(^{[19]}\) To 50 µL of the appropriately diluted sample, 3 mL of 4% methanol vanillin solution and 1.5 mL of concentrated hydrochloric acid were added. The mixture was allowed to stand for 15 min, and the absorption was measured at 500 nm. The total condensed tannins amount was expressed as mg Cat/g DW. The calibration curve range of catechin was established between 40 and 400 µg/mL. All samples were analyzed in triplicate.

**Assessment of antioxidant activities**

**Total antioxidant capacity**

The assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH.\(^{[20]}\) An aliquot (0.1 mL) of plant extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95°C for 90 min. After, the mixture cooled to room temperature. The absorbance of each sample was measured at 695 nm against a blank. The total antioxidant activity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW). The calibration curve range was 50–500 µg/mL. All samples were analyzed in triplicate.

**Scavenging ability on DPPH radical**

The scavenging ability of different *Nitraria* organ extracts was measured according to Hatano, Kagawa.\(^{[21]}\) One milliliter of the extract at known concentrations was added to 0.25 mL of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm and corresponded to the ability of extracts to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine.
The antiradical activity was expressed as IC$_{50}$ (μg/mL), the extract dose required to cause a 50% inhibition. A lower IC$_{50}$ value corresponds to a higher antioxidant activity of plant extract. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\text{-scavenging effect}(\%) = \frac{(A0 - A1)/A0} \times 100$$

where, A0 and A1 are the absorbance values of the control and of the sample at 30 min, respectively. All samples were analyzed in triplicate. Butylated hydroxytoluene (BHT) has been used as a standard synthetic antioxidant.

**Anti-inflammatory activity**

**Cytotoxicity study**

The cytotoxicity of the extract was performed on a murine macrophage cell line RAW 264.7 (American Type Culture Collection). This assay was based on the reduction of resazurin (blue color) to resorufin (pink color) by the enzymes of RAW 264.7 macrophages, hence a direct correlation between the reduction of resazurin and cell viability. The RAW 264.7 macrophages previously adhered in 24-well plates were treated for 24 hours with different increasing concentrations of each extract. After recovering the supernatant, 1 ml of a 2% resazurin solution in PBS (Dulbecco’s Phosphate Buffered Saline, Dominique Dutscher) was added in each well. After a 60 min incubation, fluorescence was measured and cell viability was calculated against a control of untreated cells according to the following equation.

$$\%\text{cell viability} = \frac{[\text{Fluorescence(sample)} \times 100]}{\text{Fluorescence(control)}}$$

**Anti-inflammatory activity on LPS-activated RAW 264.7 macrophages**

To evaluate the anti-inflammatory activity of the extracts, the test was carried out with murine macrophages RAW 264.7 (American Type Culture Collection). This cell line was recovered from tumor ascites induced in a male mouse following intraperitoneal injection of murine Abelson leukemia virus (A-MuLV). The cells were cultured in flasks with RPMI 1640 medium (Dominique Dutscher; w/LGlutamine), to which was added 10% fetal bovine serum (Dominique Dutscher; Origin South America) and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL) at 37°C in a humid atmosphere containing 5% CO$_2$. Macrophages, adhered to the 24-well plates, were treated with four increasing concentrations of each extract. After 1 hour of pre-treatment, the cells were stimulated with LPS (1 μg/mL) and left incubated for 24 hours at 37°C. The supernatant was then harvested and the amount of NO$^+$ produced by the cells was estimated by a colorimetric assay using the GRIESS reagent. In summary, 100 μL of the cell supernatant was incubated with 100 μL of Griess’s reagent (0.8% sulfanilamide, 0.75% N-naphthylethylene diamine in 0.5 N HCl) at room temperature for 15 minutes. The absorbance at 540 nm was measured using the Varioskan Flash plate reader (Thermo Scientific), and the presence of nitrite was quantified from a standard curve of NaNO$_2$. The percentage inhibition was calculated relative to control treated with LPS only without extract. All the measurements were carried out in triplicate.

**Identification and quantification of phenolic compounds by high-performance liquid chromatography (HPLC)**

The phenolic compounds were separated, identified, and quantified by a high-performance reverse phase liquid chromatography system (Agilent Technologies 1260, Germany), fitted with a UV diode array detector (DAD) and equipped with a chromatographic column, filled with an octadecyl-grafted silica gel, of the ZorbaxEclipse XDB-C18 type (4.6 x 100 mm, 3.5 μm). The system was controlled by “Agilent ChemStation” software. For the hydro-ethanolic extract obtained from the leaves of Nitraria
retusa, we adopted the following chromatographic conditions: In fact, the detector (DAD) was adjusted to a scan of 200 to 400 nm. The temperature of the column was maintained at 25°C. The volume injected was 3 µL and the mobile phase used contains two solvents A and B: solvent A (Methanol) and solvent B (milliQ water containing 0.1% formic acid). The flow rate of this phase was set at 0.4 ml/min. The separation mode adopted was the elution gradient, and the program was as follows: 0–5 min, 10–20% of A; 5–10 min, 20–30% A; 10–15 min, 30–50% A; 15–20 min, 50–70% A; 20–25 min, 70–90% A; 25–30 min, 90–50% A; 30–35 min, 50–10% of A. The identification of the phenolic compounds was carried out by comparing the retention times of the peaks obtained with those of the phenolic compound’s standards injected under the same chromatographic conditions.

**Results**

**Extraction yields**

It emerges from the data summarized in Figure 1 that ethanol solvent gives the best extraction yield from macerated leaves and fruits (18.22 and 32.42% of dry weight, respectively), while water solvent gives the lowest yield. Thus, by examining the variation in yield according to the organ studied, we notice that leaves and fruits have a higher yield than flowers and stems, because these organs are richer in metabolites. The observations are the same for extraction by decoction method. Extraction yields differ significantly depending on the extraction solvent (p < 0.05). In fact, the extraction by decoction increased remarkably the amount of extract.

**Total polyphenol content**

The total polyphenol contents obtained by the two extraction methods, presented in Table 1, revealed a significant difference (p > .05). However, decoction seems to be the best method of extracting total polyphenols. For *Nitraria* leaves, extraction with 70% ethanol gives the best total polyphenol content (46.97 and 42.7 mg GAE/g DW by decoction and maceration, respectively). The hydro-methanolic solution was ranked second with contents of 31.13 mg GAE/g DW by maceration and 34.24 mg GAE/g DW by decoction. The effect of acetone was noticeable for the decoction method; exposure of the extracts to relatively high temperatures can influence the quality of the extract. In fact, acetone was a selective solvent for some chemical classes of metabolites, which may be temperature sensitive. The *Nitraria* flowers showed a high content with the extraction by 70% acetone (34.69 mg GAE/g DW) by

![Figure 1](image-url)  
**Figure 1.** Variation in yields as a function of the *nitraria* organs and as a function of the extraction solvent. Values represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for *nitraria* organs and the extraction solvent respectively at P < 0.05.
the maceration method, this content decreased by adopting the decoction method with acetone (14.18 mg GAE/g DW). On the other hand, decoction with 70% methanol gives a content of 36.42 mg GAE/g DW, which was the highest content for this organ. The lowest levels were recorded following the extraction by water for the two studied methods. Nitraria stems show a high polyphenol content using 70% acetone as an extraction solvent with 16.07 and 17.68 mg GAE/g DW for maceration and decoction, respectively. These values are less important than those of Nitraria leaves and flowers. The lowest levels were recorded with aqueous extraction (3.91 and 4.69 mg GAE/g DW for maceration and decoction, respectively). The fruit of Nitraria was characterized by orange-brown color, after the drying of this organ, the color tended to be brown. Extraction with 70% ethanol gives the best polyphenol yields (32.42 and 35.66 mg GAE/g DW for maceration and decoction, respectively). Methanol and acetone give similar levels for both extraction methods; the lower levels were associated with water extraction.

**Total flavonoid content**

The total flavonoid content in the different extracts of N. retusa areal parts was determined by the aluminum chloride method. These compounds have a wide range of biological activities, especially potent antioxidant activity.\(^{[25,26]}\) and their effects on human nutrition and health are considerable.\(^{[27]}\) The total flavonoid contents obtained by the two extraction methods are presented in Table 2. In general, extraction by decoction appears to be more effective. The values vary significantly depending on the extraction parameters. Indeed, the difference between the flavonoid contents according to organs was highly significant (p < .01). On the other hand, the difference between flavonoid contents according to the extraction solvent was not always significant (p < .01). Statistically, the extraction with ethanol and methanol demonstrated similar flavonoid amounts for all the Nitraria organs studied except for the stems. The highest content was recorded with the 70% ethanol decoction (24.86 mg EC/g DW). The aqueous extracts give the lowest contents (0.98 mg EC/g DW for the stems).

**Total condensed tannin content**

The condensed tannin content analysis was reported in Table 3. It revealed that the decoction was more effective for the extraction of the tannins. The increase in temperature promoted the diffusion and solubility of the extracted substances. Additionally, it destroyed certain sensitive substances .\(^{[8]}\) The increase in the condensed tannins contents with the decoction method can be explained by the thermal degradation of the polyphenol oxidases, which decreased the polyphenols content. Thus, the breaking of bonds between polyphenols and other substances (proteins, polysaccharides, etc.) leading to accessibility to these active ingredients may explain their abundance. Statistically, the difference between condensed tannins contents according to the plant organs was highly significant (p < .01).

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**Table 1. Total polyphenol content variation from different organs of Nitraria retusa aerial part.**

| Method     | Leaves          | Flowers         | Stems           | Fruits          |
|------------|-----------------|-----------------|-----------------|-----------------|
| Maceration |                 |                 |                 |                 |
| 70% ethanol| 42.7\(^{a}\) ± 1.12 | 18.6\(^{c}\) ± 0.93 | 11.2\(^{d}\) ± 0.56 | 32.4\(^{a}\) ± 1.62 |
| 70% methanol| 31.1\(^{c}\) ± 1.56 | 13.5\(^{c}\) ± 0.68 | 10.1\(^{d}\) ± 0.51 | 22.5\(^{b}\) ± 1.13 |
| 70% acetone| 34.39\(^{b}\) ± 1.72 | 34.6\(^{a}\) ± 1.73 | 16.07\(^{c}\) ± 0.8 | 22.45\(^{b}\) ± 1.12 |
| Water      | 30.26\(^{c}\) ± 1.51 | 10.26\(^{c}\) ± 0.51 | 4.00\(^{c}\) ± 0.2 | 14.86\(^{c}\) ± 0.74 |
| Decoction  |                 |                 |                 |                 |
| 70% ethanol| 47.00\(^{a}\) ± 2.35 | 19.6\(^{b}\) ± 0.98 | 12.34\(^{d}\) ± 0.62 | 35.66\(^{b}\) ± 1.78 |
| 70% methanol| 34.24\(^{c}\) ± 1.71 | 36.42\(^{c}\) ± 0.71 | 11.12\(^{b}\) ± 0.56 | 24.76\(^{b}\) ± 1.24 |
| 70% acetone| 41.27\(^{b}\) ± 2.06 | 14.18\(^{c}\) ± 1.82 | 17.68\(^{a}\) ± 0.8 | 24.7\(^{b}\) ± 1.23 |
| Water      | 36.31\(^{c}\) ± 1.82 | 12.31\(^{c}\) ± 0.62 | 4.69\(^{c}\) ± 0.23 | 17.83\(^{c}\) ± 0.89 |

Values expressed in mg GAE g/DW and represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for nitraria organs and the extraction solvent respectively at P < 0.05.
Table 2. Total flavonoid content variation from different organs of Nitraria retusa aerial part.

|        | Leaves       | Flowers     | Stems       | Fruits      |
|--------|--------------|-------------|-------------|-------------|
| **Maceration** |              |             |             |             |
| 70% ethanol | 22.60<sup>±</sup> ± 0.9 | 15.23<sup>±</sup> ± 0.76 | 2.56<sup>±</sup> ± 0.13 | 16.86<sup>±</sup> ± 0.84 |
| 70% methanol | 22.31<sup>±</sup> ± 1.12 | 15.14<sup>±</sup> ± 0.76 | 3.26<sup>±</sup> ± 0.16 | 17.00<sup>±</sup> ± 0.9 |
| 70% acetone | 19.00<sup>±</sup> ± 1.0 | 6.32<sup>±</sup> ± 0.32 | 2.00<sup>±</sup> ± 0.1 | 12.87<sup>±</sup> ± 0.64 |
| Water | 6.59<sup>cC</sup> ± 0.33 | 3.68<sup>±</sup> ± 0.18 | 0.98<sup>cC</sup> ± 0.05 | 7.32<sup>cD</sup> ± 0.37 |
| **Decoction** |              |             |             |             |
| 70% ethanol | 24.86<sup>±</sup> ± 1.24 | 16.00<sup>±</sup> ± 0.8 | 2.82<sup>±</sup> ± 0.14 | 18.55<sup>±</sup> ± 0.93 |
| 70% methanol | 24.54<sup>±</sup> ± 1.23 | 16.00<sup>±</sup> ± 0.8 | 3.59<sup>±</sup> ± 0.18 | 18.60<sup>±</sup> ± 0.93 |
| 70% acetone | 22.75<sup>±</sup> ± 1.14 | 6.64<sup>±</sup> ± 0.33 | 2.18<sup>cC</sup> ± 0.11 | 14.16<sup>aC</sup> ± 0.71 |
| Water | 8.00<sup>cD</sup> ± 0.4 | 4.42<sup>cC</sup> ± 0.22 | 1.18<sup>cD</sup> ± 0.06 | 8.78<sup>cD</sup> ± 0.44 |

Values expressed in mg QE/g DW and represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for nitraria organs and the extraction solvent respectively at P < 0.05.

Table 3. Total condensed tannin content from different organs of Nitraria retusa aerial part.

|        | Leaves       | Flowers     | Stems       | Fruits      |
|--------|--------------|-------------|-------------|-------------|
| **Maceration** |              |             |             |             |
| 70% ethanol | 21.8<sup>±</sup> ± 1.21 | 0.02<sup>±</sup> ± 00 | 1.32<sup>±</sup> ± 0.07 | 1.08<sup>±</sup> ± 0.06 |
| 70% methanol | 18.26<sup>±</sup> ± 1.01 | 0.31<sup>±</sup> ± 0.02 | 1.53<sup>±</sup> ± 0.08 | 1.00<sup>cD</sup> ± 0.05 |
| 70% acetone | 21.13<sup>±</sup> ± 1.17 | 0.43<sup>cA</sup> ± 0.02 | 1.37<sup>±</sup> ± 0.08 | 1.31<sup>±</sup> ± 0.07 |
| Water | 19.24<sup>aB</sup> ± 1.07 | 0.12<sup>cC</sup> ± 0.01 | 0.9<sup>cC</sup> ± 0.05 | 1.04<sup>bB</sup> ± 0.06 |
| **Decoction** |              |             |             |             |
| 70% ethanol | 26.06<sup>aA</sup> ± 1.34 | 0.02<sup>±</sup> ± 00 | 1.45<sup>cC</sup> ± 0.08 | 1.19<sup>±</sup> ± 0.07 |
| 70% methanol | 25.36<sup>aA</sup> ± 1.41 | 0.45<sup>aB</sup> ± 0.03 | 1.84<sup>±</sup> ± 0.1 | 1.43<sup>cA</sup> ± 0.08 |
| 70% acetone | 20.09<sup>cC</sup> ± 1.11 | 0.33<sup>cB</sup> ± 0.02 | 1.68<sup>bB</sup> ± 0.09 | 1.08<sup>cB</sup> ± 0.06 |
| Water | 23.08<sup>aB</sup> ± 1.28 | 0.14<sup>cC</sup> ± 0.01 | 1.08<sup>cC</sup> ± 0.06 | 1.25<sup>bB</sup> ± 0.07 |

Values expressed in mg CE/g DW and represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for nitraria organs and the extraction solvent respectively at P < 0.05.

The great distinction between the organs appears at the level of the richness of the leaves and the deficiency of the other organs. Ethanol 70% leaves extracts retained the highest condensed tannins contents (26.06 mg Cat/g DW by decoction), followed by methanol 70% and water with 25.36 and 23.08 mg Cat/g DW, respectively. Nitraria flowers showed very low condensed tannins contents 0.22 mg Cat/g DW on average. Likewise, stems and fruits do not reveal an important content of tannins; the highest value was recorded with the decoction of stems in 70% Methanol (1.84 mg Cat/g DW).

**Antioxidant activities**

**Total antioxidant capacity**

The variation of the total antioxidant capacity was illustrated in Figure 2. It showed that the maceration method was more suitable to ensure an extract with stable antioxidant activity. In fact, for most of the tests carried out, a decrease in the antioxidant capacity was noted by increasing the extraction temperature. On the other hand, by studying the variation according to the type of extraction solvent used, 70% ethanol was the best maceration solvent for the extraction of leaves, flowers and fruits with capacities of 45.82, 22.13, and 18.99 mg GAE/g DW, respectively. In contrast, 70% methanol and 70% acetone were the most effective in treating Nitraria stems. Maceration and decoction with pure water show low capacities of the order of 15.22, and 4.42 mg GAE/g DW for the maceration of the leaves and the decoction of the stems, respectively. Statistically, the difference between the antioxidant capacities according to the type of the plant was highly significant (p < .01). The variation according to the organ of Nitraria proved that the leaves represent the best extract
with capacities of 45.82 mg GAE/g DW. Flowers come second with capacities close to that of fruit extracts. The stems do not present a potential source of antioxidants compared to the other parts of this plant.

**DPPH radical scavenging ability**

The variation of the anti-free radical capacity is illustrated in Figure 3. The results showed that the extraction method influences the DPPH radical scavenging ability. Therefore, maceration was more recommended for effective extracts. Indeed, for all the tests carried out in this study an increase in IC₅₀ was recorded when using high temperatures for extraction. In addition, by studying the variation depending on the type of extraction solvent used, 70% ethanol was the best maceration solvent for the extraction of leaves, flowers and fruits with IC₅₀ of 22.05, 54.36, and 96.7 μg/mL, respectively. On the other hand, 70% methanol and 70% acetone give statistically similar results by the different extraction methods. Maceration and decoction with pure water showed relatively very high IC₅₀, which varied from 431.07 μg/mL for the stems decocted in water to 202.16 μg/mL for the leaves macerated in water. Statistically the difference between the anti-free radical capacities according to the part of the plant was highly significant (p < 0.05). The variation according to the organ of *Nitraria* proved that the leaves

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**Figure 2.** Evaluation of total antioxidant capacity of *N. retusa* maceration and decoction extracts. Values represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for extraction solvent and plant organs respectively at P < 0.05.

**Figure 3.** Evaluation of DPPH radical scavenging ability of *N. retusa* maceration and decoction extracts. Values represented the mean of three replicates (n = 3); letters (a–d, A–D) indicated significant differences for extraction solvent and plant organs respectively at P < 0.05.
represent the best extract with IC<sub>50</sub> of 22.05 μg/mL. This value was comparable to that found by a standard synthetic antioxidant (BHT) with IC<sub>50</sub> of 21 μg/mL, which shows that this plant has a significant anti-radical power.

**Identification and quantification of phenolic compounds by reverse phase-high performance liquid chromatography**

Chromatographic analysis of the 70% ethanol extract is shown in Table 4 and Figure 4. The identification verified the presence of four compounds. The major compound was Luteolin 7-O-glucoside with a content of 10.51 mg/g residue, followed by isorhamnetin 3-O-rutinoside with 7.96 mg/g residue. Rutin and kaempferol 3-O-rutinoside 0.859 and 0.69 mg/g residue, respectively. The levels found are greater than the detection limits (LOD) and quantification (LOQ) with a height correlation coefficient.

**Anti-inflammatory activity**

The cytotoxicity of N. retusa leaves extracts was evaluated and illustrated in Figure 6. RAW 267.4 cells were treated with different N. retusa extract concentrations (25–100 μg/mL). Nitraria extract did not reveal any significant cytotoxicity against RAW 267.4 macrophage cells until 100 μg/mL. Thus, the effect of N. retusa leaves extracts on NO<sup>•</sup> production was illustrated in Figure 5. N. retusa extract decreased NO<sup>•</sup> production in a dose-dependent manner. The results showed that the different extracts exhibited significant anti-inflammatory activity. The ethanolic extracts showed the best activity inhibiting the production of NO<sup>•</sup> at an IC<sub>50</sub> of 36.60 μg/mL. The second best IC<sub>50</sub> was recorded with water extract (42.52 μg/mL). The Nitraria leaves extracted with 70% Methanol gave an activity of an IC<sub>50</sub> at 51.47 μg/mL. The 70% acetone extract gave 109.95 μg/mL as a NO<sup>•</sup> IC<sub>50</sub> inhibition.

**Discussion**

Regarding the extraction yields, summarized in Figure 1, the ethanol 70% was the best solvent for the maceration and decoction method. These results agreed with the study of Salem, Chevalot,[12] where they found that the yield of extraction of Nitraria leaves can reach 19% of dry leaves weight by carrying out an aqueous maceration. Likewise, These results were comparable to those obtained by Lezoul, Belkadi,[28] where they proved that the extraction yield depends on solvent and the studied plant organ. These extracts were the subject of a research of antioxidant and anti-inflammatory activities, the dosage of total polyphenols, total flavonoids and condensed tannins showed that the extraction solvent is a determining factor; ethanol was the most suitable solvent for extracting these phenolic compounds. This observation was explained by the ability of ethanol to extract a wide range of compounds, this ability was explained by Galanakis, Goulas[29] through the prediction of the activity coefficients of 15 natural phenols in 7 solvents and 3 assayed extraction temperatures. This study reveals that the polyphenols’ hydroxyl groups can develop hydrogen bonds with the electronegative oxygen of ethanol. Ethanol-water combinations have been reported for their advanced recovery of

**Table 4. Quantification of flavonoids in the extract (70% ethanol) from the leaves of N. retusa.**

| Phenolic compound                  | (LOD) (μg/mL) | (LOQ) | Regression equation | (R<sup>2</sup>) | Quantification (mg/g residue) |
|-----------------------------------|--------------|-------|---------------------|-----------------|-----------------------------|
| Luteolin 7-O-glucoside            | 0.622        | 2.073 | Y=7.334X+15.4       | 0.999           | 10.51±0.68                  |
| Rutin                             | 0.693        | 2.313 | Y=4.252X+19.8       | 0.998           | 0.859±0.97                  |
| Kaempferol 3-O-rutinoside         | 0.795        | 2.651 | Y=7.968X+50.3       | 0.998           | 0.69±0.35                   |
| Isoflavone 3-O-rutinoside         | 0.658        | 2.194 | Y=2.887X-3.17       | 0.998           | 7.96±0.77                   |

** correlation coefficient
phenolic compounds, while hydro-methanolic combinations have been referred to extract poly-
phenols with the highest yield and widest array compared to pure ethanol and ethyl-acetate solvents.
The use of 70% aqueous ethanol for the flavonoids extraction from olive leaves among ethanol,
water, acetone, and water-acetone have been recommended. Acetone has the ability to solubilize
pro-anthocyanidins that are not soluble in methanol. However, the problem according to
Rosales and Jokić, Veljić was that water and acetone, especially at high temperatures, could
extract also unwanted substances like proteins, lipids and non-phenolic dyes that interfere with the
determination of tannins. Contrariwise, Mariem, Sameh reported that the water extraction gave the
highest amount of phenolic compounds (647.00 mg GAE/100 g extract), the lowest ones were obtained
in the aqueous ethanol extract and the aqueous acetone extract. In accordance with our results, the
highest levels of flavonoids were found in aqueous ethanol extract (39.98 mg QE/100 g extract),
aqueous acetone extract (38.71 mg QE/100 g extract) and aqueous extract (35.96 mg QE/100 g extract).
The extraction of condensed tannins depends on their chemical nature, the solvent used and the
operating conditions.

Total antioxidant capacity by phosphomolybdenum method was based on the reduction of Mo
(VI) to Mo (V) by the Nitraria antioxidants and the subsequent formation at acidic pH of green
phosphate/Mo (V) complex, which has an absorption at 695 nm. The variation of total
antioxidant capacity according to the extraction solvents was in agreement with the results of the
assays of the phenolic compounds, 70% ethanol was found to be the most effective. The leaves of Nitraria showed a greater total antioxidant activity than the other organs. The scavenging
activity on DPPH radicals test was largely used as a basic screening method for analysis the
antiradical activity of a huge range of compounds. DPPH* is a stable free radical characterized
by a maximum absorption between 515 and 517 nm, which is diminished in the presence of a
compound capable of reducing it to its hydrazine form by a hydrogen/electron transfer reaction.
A lower IC_{50} value corresponds to a higher scavenging activity on DPPH*.

Figure 4. RAW 267.4 cells viabilities treated with 25 to 100µg of N. retusa leaves extract.
Figure 5. NO radical inhibition IC₅₀ of cell treated with 25 to 100 µg of *N. retusa* leaves extract.
by the accumulation of phenolic compounds in certain plant organs. The most active extract was chosen for the remainder of this work, hence the need to seek its composition in phenolic compounds.

Chromatographic analysis showed that the hydroethanolic extract from the leaves of *N. retusa* was rich in glycosylated flavonoids (luteolin 7-O-glucoside, rutin, kaempferol 3-O-rutinoside and isorhamnetin 3-O-rutinoside) (Table 4 and Figure 6). These molecules are secondary metabolites which have been previously identified in the *Nitraria* species, of which several phytochemical studies have shown that the leaves of *Nitraria* are rich in flavonoids, more particularly in glycosylated flavonoids which are derivatives of quercetin, apigenin, luteolin, kaempferol and isorhamnetin. More particularly, this species is known for its richness in isorhamnetin and its derivatives such as isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-galactoside, isorhamnetin-3-O-ribonobioside, isorhamnetin-3-O-xylosylrobobioside and isorhamnetin-3-O-4Rhm galactosyl-ribobioside. These molecules are well known for their biological properties. In particular, as antioxidants and natural anti-inflammatory.

Increased production of reactive oxygen species and antioxidant enzyme systems are associated with inflammatory syndromes. This relationship was widely studied by several authors, which explains the choice of the leaf extract to investigate the anti-inflammatory activity. Luteolin is
a flavonoid found in medicinal plants that has important anti-inflammatory effect in vitro and in vivo studies. Some of its derivatives, luteolin-7-O-glucoside, have also demonstrated anti-inflammatory action. The action mechanism of luteolin varies, its main target transcription factors include Src in the nuclear factor (NF)-B route, MAPK in the activator protein (AP) 1 pathway, and SOCS3 in the signal transducer and activator of transcription 3 (STAT3) pathway. Furthermore, isorhamnetin has anti-inflammatory properties that can inhibit inflammatory reactions in a number of diseases, including osteoarthritis and periodontitis. Isorhamnetin’s anti-inflammatory activity is also involved in anti-acute lung injury,[49,50] anti-tuberculosis,[51] and kidney protection.[52] The mechanism is linked to the regulation of inflammatory mediators, cytokines, and ROS. Chi, Zhong[49] revealed that isorhamnetin inhibited the phosphorylation of ERK, JNK, IBa, and NF-B (p65) activated by LPS in vivo by affecting the MAPK and NF-B signaling pathways and alleviated neutrophil infiltration and edema in an ALI model.

**Conclusion**

The extraction of polyphenolic compounds is a crucial step for valorizing these active ingredients. It depends on the method and the appropriate solvent, which preserves their biological properties. This study emerged that the maceration of *Nitraria* leaves with 70% ethanol and 70% methanol was the best experimental condition for polyphenols and flavonoid extracting. In contrast, the *Nitraria* leaves aqueous decoction was preferable for extracting condensed tannins. The extraction of *Nitraria* leaves with a hydro-alcoholic solution led to an extract with remarkable antioxidant and anti-inflammatory activity. These activities are associated with a composition rich in glycosylated flavonoids. *Nitraria* extracts have shown that this plant is a source of active molecules, which can be valued in several industrial fields, particularly pharmaceuticals and cosmetic applications.

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No potential conflict of interest was reported by the author(s).

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