

Phytochemical Screening, Extraction of Essential Oils and Antioxidant Activity of Five Species of Unconventional Vegetables

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Abstract

Unconventional vegetables, in general, are plants that have been largely consumed by the population at some point and, because of changes in eating behavior, now present reduced economic and social expression and have lost ground to other vegetables. The objectives of this study were to perform phytochemical screening of the ethanol extracts of *Rumex acetosa* L., *Tropaeolum majus* L., *Solanum muricatum*, *Stachys byzantina* K. Koch and *Solanum betaceum* Cav. and evaluate their antioxidant potentials via the methods involving scavaging of the DPPH free radical and the ABTS radical, phosphomolybdenum and reducing power. In phytochemical screening, five species of unconventional vegetables tested positive for tannins; for sesquiterpene, lactones and other lactones. These tests were positive for *Tropaeolum majus* L. and *Rumex acetosa* L., *Solanum betaceum* Cav. and *Solanum muricatum* tested negative for steroids. Only *Solanum betaceum* Cav. gave positive tests for flavonoids. Among the five plant species studied, *Stachys byzantina* K. Koch presented the greatest antioxidant potential in all the methods evaluated.

Keywords

Rumex acetosa L., *Tropaeolum majus* L., *Stachys byzantina* K. Koch, Reducing Power

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1. Introduction

Unconventional vegetables are considered to be those that are currently consumed by only a few people, usually in restricted areas or communities. These vegetables have a good taste and nutritional value [1]. Because of globalization and the increasing use of processed foods, cultivation and consumption of unconventional vegetables have decreased in all the regions of Brazil, both urban and rural areas. As a result, the eating habits of Brazilians of all social classes are being modified, and the consumption of food from local and regional sources has been shrinking [2].

Tropical and subtropical countries have the greatest diversity of vascular plant species. However, the number of indigenous fruits and vegetables utilized are negligible. With respect to native vegetables, research, cultivation, use and valorization seem to be even less [3].

Among these vegetables, some stand out in Brazilian cuisine. In southeastern Brazil, more specifically in the state of Minas Gerais, there is *Rumex acetosa* L. (sorrel), which is a herbaceous perennial native to Europe and North Asia (Eurasia), including being considered a “weed” in that continent. It presents sour leaves and is used in raw salads (pure or mixed, providing freshness) or as a vegetable in soups, puree or green sauce and omelets [4].

Another widely used unconventional vegetable is *Tropaeolum majus* (nasturtium). It is an annual, herbaceous plant native to the highlands of Mexico and Peru. It is widely cultivated for ornamental purposes and for consumption in most regions of Brazil. Its flowers and young leaves can be used to prepare raw salads, green pasta, pancakes, pizzas, breads and soups, and it can be cooked with meat, among other uses. The *Stachys byzantina* K. Koch (peixinho; “little fish”) species, also known as peixinho-do-jardim, is a herbaceous perennial vegetable native to Turkey, Asia and Caucasus. It is widely cultivated in southern and southeastern Brazil for ornamental purposes in sites with strong sunlight and for consumption as a vegetable. Its leaves can be eaten after cooking and suitable culinary preparation. It has a light texture and fried-fish flavor, hence some of the popular names: peixinho, peixinho-da-horta or lambari-da-horta (“little fish”, “little-fish-of-the-garden” or “tetra-of-the-garden”) [4]. *Solanum muricatum* (melãozinho; “little melon”) is an evergreen shrub with fruits 10 to 15 cm long. It is a plant of the Solanaceae family. It has a sweet flavor and aroma similar to melon (hence the name, despite not being closely related). *Solanum betaceum* (tomate da arvore; “tree tomato”) of the Solanaceae family is an erect shrub, sublenhose, perennial, sparsely branched, 2.5 to 4.5 m high and native to Bolivia and Peru. The fruit can be consumed fresh. It has a yellow juicy pulp with a slightly sour flavor and many seeds [4].

Unconventional vegetables are not usually organized in the supply chain itself and do not arouse interest from seed, fertilizer or pesticide companies. However, the work of rescuing these plants from the point of view of increasing their use in food and research represents a cultural, social and economic gain and may stimulate the production and consumption of these foods because of their possible nutritional and pharmaceutical characteristics, in addition to their hardiness in cultivation.

Considering the hardiness to cultivation and desirable nutritional characteristics, the determination of the chemical composition of these species by means of a preliminary survey that can predict the groups of secondary metabolites in the extract is important. Some research has shown that certain compounds present in plant extracts can have antioxidant action, and they are indicated to reduce or prevent the deleterious effects of cellular oxidative stress [5] [6]. There is a growing interest in finding plant species that can act in the fight against free radicals.

The objectives of this study were to evaluate the antioxidant potential using the methods of sequestration of the DPPH free radical or the ABTS radical, phosphomolybdate and reducing power and to perform phytochemical screening of *Rumex acetosa* L. (sorrel), *Tropaeolum majus* L. (capuchin), *Solanum muricatum* (little melon), *Stachys byzantina* K. Koch (little fish) and *Solanum betaceum* Cav. (tree tomato).

2. Material and Methods

2.1. Collection and Extraction of Plant Material

The leaves and fruits of unconventional vegetables (considered to be those that are currently consumed by only a few people, usually in restricted areas or communities) were obtained from the collection of the Department of Agriculture of the Federal University of Lavras. Samples of *Rumex acetosa* L. (sorrel), *Tropaeolum majus* L. (capuchin), *Solanum muricatum* (little melon), *Stachys byzantina* K. Koch (little fish) and *Solanum betaceum* Cav. (tree tomato) were sent to the Organic Chemistry Laboratory–Essential oils of the Department of Chemi-

stry of the same University. The collected material (leaves or fruits) was dried in an oven (35°C) for 72 hours. To 100 mL of ethanol was added 50 g of the plant, and the mixture was heated under reflux for 8 hours. The mixture was filtered through a Buchner funnel, and the ethanol was evaporated on a Büchi B114 rotary evaporator to furnish the plant extract.

2.2. Phytochemical Screening

The presence of the major classes of metabolites was determined according to the method proposed by Matos (1988) [7]. To test for tannins, a few milligrams of extract was dissolved in 3 mL of distilled water and a drop of 1% ferric chloride was added. A change in color or formation of a precipitate indicated a positive reaction. To test for the presence of flavonoids, a few milligrams of extract was dissolved in 10 mL of methanol. If necessary, the mixture was filtered, and five drops of concentrated HCl and a one-cm piece of magnesium tape were added. A pink tint in the solution indicated a positive reaction. For these and all other tests, 1 mg of each extract of leaves was used. Tests were performed for organic acids, reducing sugars, polysaccharides, proteins and amino acids, tannins, catechins, flavonoids, cardiac glycosides, sesquiterpene, lactones and other lactones, azulenes, carotenoids, steroids, depsides, coumarin derivatives, saponins, alkaloids, purines and anthraquinones.

2.3. Essential Oils

To obtain the essential oils, 200 g of edible fruits or leaves was subjected to hydrodistillation for 2 hours, using a modified Clevenger apparatus to furnish the hydrolac [8]. The essential oil was separated from the distillate by centrifugation on a benchtop centrifuge with a horizontal crosspiece (Fanem Model 206 Baby@I BL) at 965.36 g for 5 minutes or by partition with dichloromethane. No essential oil was obtained from any of the plants studied.

2.4. Antioxidant Activity

2.4.1. ABTS Method

The antioxidant activity by the ABTS•+ method [ammonium salt of 2,2'-azinobis (3-ethylbenzenethiazoline-6-sulfonic acid)] was performed according to the method described by Guerreiro *et al.* (2013) [9]. The ABTS radical was formed by the reaction of 7 mM ABTS•+ solution with 2.4 mM potassium persulphate, incubated at 25°C in the dark for 12 - 16 hours. Once formed, the PA radical was diluted with ethanol to obtain the absorbance (from 0.7 to 0.72) at 734 nm. In a dark environment, 50 µL of each of the samples (concentrations 31.25, 62.5, 125, 250 and 500 µg·mL⁻¹) was transferred to test tubes containing 1950 µL of the ABTS•+ radical. The percentage inhibition of the ABTS•+ radical by the samples was calculated according to the following equation:

$$\% \text{ Effect on radical capture} = \left[\frac{(A_{\text{CN}} - A_{\text{sam}})}{A_{\text{CN}}} \right] \times 100$$

where:

A_{CN} = Absorbance of the negative control;

A_{sam} = Absorbance of the sample;

The values were compared with those of the BHT (butyl hydroxy toluene) standard at concentrations of 31.25; 62.5; 125; 250 and 500 µg·mL⁻¹.

2.4.2. Sequestration of the DPPH Free Radical

Evaluation of antioxidant activity by means of the capture of the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was performed according to a modification of the method of Lutz-Lopes *et al.* (2008) [10]. A methanol solution of DPPH was prepared at a concentration of 40 µg·mL⁻¹. To each test tube was added 2.7 ml of the DPPH solution, followed by 0.3 mL of each extract dissolved in ethanol at concentrations of 31.25; 62.5; 125; 250 and 500 µg·mL⁻¹. The BHT standard was used as a positive control. The negative control was a solution containing all the reagents except the extracts. After standing for 60 minutes in the dark, absorbance readings were taken at 515 nm (Shimadzu UV-160 1PC). The antioxidant activity (AA%) was calculated from the following equation:

$$\text{AA}\% = \left[\frac{(A_{\text{CN}} - A_{\text{sam}})}{A_{\text{CN}}} \right] \times 100$$

where:

A_{CN} = Absorbance of the negative control;

A_{sam} = Absorbance of the sample.

2.4.3. Phosphomolybdate

To a test tube containing 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added an aliquot of 0.1 ml of 500 $\mu\text{g}\cdot\text{mL}^{-1}$ solution of extract in ethanol. The blank contained 1 mL of the reagent solution and 0.1 mL of ethanol. The tubes were capped and incubated in a water bath at 95°C for 60 minutes. After cooling, the test tubes were read at 695 nm. The values for the antioxidant activity of the extracts were compared with a curve obtained with various concentrations of BHT (31.25, 62.5, 125, 250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$), and the results were expressed in mg equivalents of BHT per mg of extract [11].

2.4.4. Reducing Power

To a test tube were added a 0.1 mL aliquot of a solution of plant extract with 1 mL of phosphate buffer solution (pH = 7.4) and 1.0 mL of 1% potassium ferricyanide. After incubation at 50°C for 30 minutes, 0.5 mL of 10% trichloroacetic acid, 1.5 mL of distilled water and 0.3 mL of ferric chloride were added, and the absorbance was measured at 700 nm. The antioxidant activity of the extracts were compared with a standard curve for various concentrations of BHT (31.25, 62.5, 125, 250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$). Results were expressed in equivalent mg BHT per mg of extract [12].

2.5. Statistical Analysis

Analysis of variance was performed for the ABTS and DPPH tests, followed by the F test, to verify the effect of concentration on the antioxidant activity. For both tests, a completely randomized design (CRD) was used in a 6 × 5 factorial: six samples and five concentrations of extracts (31.25, 62.5, 125, 250 and 500 $\mu\text{g}\cdot\text{mL}^{-1}$) were analyzed with three replications. Analyses were performed using the Sisvar program [13].

3. Results and Discussion

3.1. Phytochemical Screening

The results of the tests for the classes of compounds in both extracts are presented in **Table 1**.

All five species of vegetables tested positive for tannins. The tannin identification reaction is based on the formation of a green or blue coloration or precipitate. According to Cunha and Batista (2010) [14], the tannins are generally found in different concentrations in vascularized plants. They may be present in many plant organs such as leaf, fruit and bark; they are soluble in water, alcohols and acetone, and exert a protective function in plants.

The test for steroids was positive for *Rumex acetosa* L., *Tropaeolum majus* L. and *Stachys byzantina* K. Koch. The residue from the *Solanum muricatum* extract was not soluble in chloroform, so it was not possible to perform the test with this extract. The test with the *S. betaceum* extract was negative. The extracts from *Rumex acetosa* L. and *Tropaeolum majus* L. tested positive for sesquiterpene lactones and other lactones,

Tests for flavonoids were negative with the extracts from *Rumex acetosa* L., *Tropaeolum majus* L., *Solanum muricatum* and *Stachys byzantina* K. Koch. A positive result was observed for the extract from *Solanum betaceum* Cav. According to Souza *et al.* (2010) [15], flavonoids have an antioxidant action by reason of their

Table 1. Metabolites whose results were positive in phytochemical screening for ethanol extracts of *Rumex acetosa* L., *Tropaeolum majus* L., *Solanum muricatum*, *Stachys byzantina* K. Koch and *Solanum betaceum* Cav.

| Compound | <i>Rumex acetosa</i> L. | <i>Stachys byzantina</i> K. Koch | <i>Tropaeolum majus</i> L. | <i>Solanum betaceum</i> Cav. | <i>Solanum muricatum</i> |
|--------------------------------------------|-------------------------|----------------------------------|----------------------------|------------------------------|--------------------------|
| Tannins | + | + | + | + | + |
| Steroids | + | + | + | - | - |
| Sesquiterpene, lactones and other lactones | + | - | + | - | - |
| Flavonoids | - | - | - | + | - |

chemical structures and reducing properties. They are phenolic compounds that have a perfect structure for scavenging free radicals and can be more effective as antioxidants than some vitamins.

The antioxidant activity of flavonoids is related to their oxidation power [16]. Suhartono *et al.* (2012) [17] studied the antioxidant activity and the presence of flavonoids in some medicinal plants (Kelakai, Kasturi, pasak bumi and ferns) and found that all fractions of the plants tested had a high flavonoid content, unlike four of the five species analyzed in this study. Porto *et al.* (2014) [18] found a high concentration of flavonoids 728.00 mg. 100 g⁻¹ when they analyzed the chemical composition of Jenipapo (*Genipa americana* L.), unlike Barreto, Benassi and Mercadante (2009) [19] who obtained a flavonoid concentration of 73.3 mg. 100 g⁻¹ for buriti (*Mauritia flexuosa*), 103.8 g/mg. 100 g⁻¹ for bacuri (*Platonia insignis* M.), 319.4 mg. 100 g⁻¹ for murici (*Byrsonima crassifolia* L.) and 741.2 mg. 100 g⁻¹ for pequi (*Caryocar brasilia* C.). According to Porto *et al.* (2014) [18], only the *Caryocar brasilia* C. contained a concentration of flavonoides near that obtained in the previous study, whereas the values for the other plants were well below those recorded in that survey. The remaining phytochemical screening tests with the extracts of these five vegetables were negative.

3.2. Essential Oils

No essential oils were obtained from the five species of non-conventional vegetable surveyed here.

3.3. Antioxidant Activities

The results of evaluation of the antioxidant activity (AA) through the method involving inhibition of the ABTS radical are shown in **Figure 1**. The percent of AA increased with increasing concentration for all the samples. Thus, the highest percentage of antioxidant activity was obtained at a concentration of 500 µg·mL⁻¹.

Among the five species studied, *S. brizantina* presented the greatest activity, and this fact is due to the high concentration of phenolics (tannins) present in the plant. *Solanum muricatum* had the lowest antioxidant potential, and its activities at concentrations of 62.5, 125 and 250 µg·mL⁻¹ were statistically equal. Although tannins were found in that extract, these compounds might be present in lower concentrations or complexed that the hydroxyl hydrogen is unable to react with the ABTS radical (**Figure 2**).

Results for the antioxidant activity determined by scavaging of the DPPH free radical are shown in **Figure 3**. The percentages of the antioxidant activity of all the species were similar to those observed with the previous method. There was an increase in the antioxidant activity with increasing concentration of the extract, there by demonstrating a dose-dependent effect (**Figure 4**).

Results of the tests of the extracts from the five vegetables for antioxidant activity performed by means of the

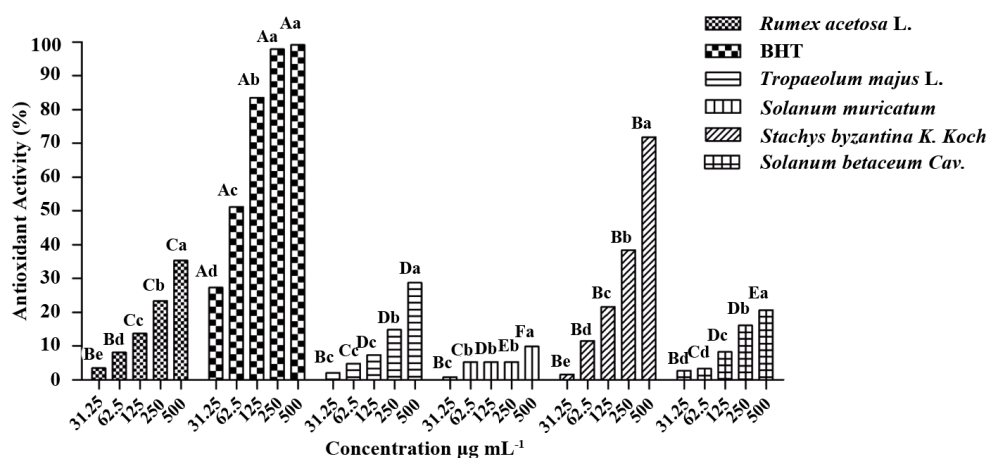


Figure 1. Percentage of the antioxidant activity of plants *Rumex acetosa* L., *Tropaeolum majus* L., *Solanum muricatum*, *Stachys byzantina* K. Koch and *Solanum betaceum* Cav. the method of inhibiting the ABTS radical. The means followed by the same letter: in lower case are the comparison of concentrations in each plant and the upper letter of each concentration compared between different plants do not differ significantly at probability of 5%, by Scott-Knott test. *BHT = Butyl hydroxy toluene.

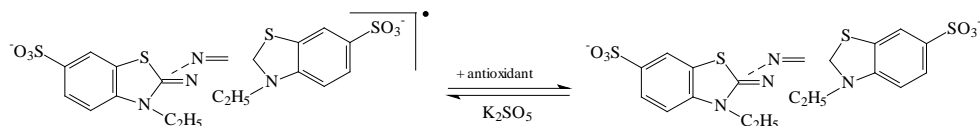


Figure 2. Antioxidant stabilizing ABTS radical and formation of potassium persulfate.

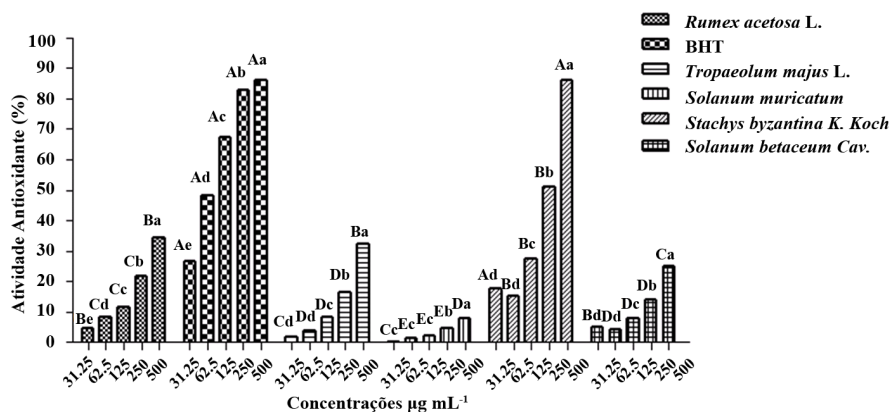


Figure 3. Percentage of the antioxidant activity of plants *Rumex acetosa* L., *Tropaeolum majus* L., *Solanum muricatum*, *Stachys byzantina* K. Koch and *Solanum betaceum* Cav. by DPPH radical inhibition method. The means followed by the same letter: in lower case are the comparison of concentrations in each plant and the upper letter of each concentration compared between different plants do not differ significantly at probability of 5%, by Scott-Knott test. *BHT = Butyl hydroxy toluene.

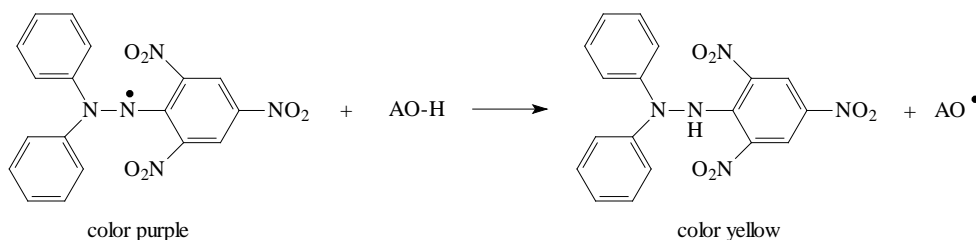


Figure 4. Antioxidant stabilizing DPPH• radical. *AO-H = representation of an antioxidant substance.

formation of complexes with phosphomolibdenum and by determination of the reducing power were expressed in mg equivalent BHT/mg of plant extract (**Table 2**). The extracts from *Tropaeolum majus* L. and *Stachys byzantina* K. Koch presented the highest antioxidant activities as measured by formation of the phosphomolibdenum complex. This method is a simple and inexpensive way to measure the total antioxidant capacity of a complex mixture of compounds containing both lipophilic and hydrophilic substances, such as extracts obtained from plants [20].

The *Stachys byzantina* K. Koch extract presented the highest antioxidant activity by the reducing power method (**Table 2**). This method is based on the reduction of ferricyanide to ferrocyanide ion in the presence of ferric ion (derived from FeCl_3) (**Figure 5**) [21].

According Meir *et al.* (1995) [22], the reducing capacity of a compound may serve as an important indicator of its antioxidant potential. Sudha *et al.* (2012) [23] found that the reducing power of the ethanol extracts of ripe and unripe exotic cucumbers in concentrations of 0.190; 0.177 to 1 $\text{mg}\cdot\text{mL}^{-1}$ and 0.921; 0.672 to 5 $\text{mg}\cdot\text{mL}^{-1}$, respectively. The values observed in this study were lower than those found by Sudha *et al.* (2012) [23].

4. Conclusion

The extracts of five species of unconventional vegetables tested positive for tannins, whereas only the extracts from *Rumex acetosa* L. and *Tropaeolum majus* L. tested positive for sesquiterpene lactones and other lactones.



Figure 5. Reduction of ferricyanide ion to ferrocyanide.

Table 2. Antioxidant activity of *Rumex acetosa* L. (sorrel), *Tropaeolum majus* L. (Capuchinha), *Solanum muricatum* (melaozinho), *Stachys byzantina* K. Koch (peixinho) and *Solanum betaceum* Cav. (tree tomato), by phosphomolybdenum complex and the reducing power methods.

| Plants | Phosphomolybdenum complex | Reducing power |
|-----------------------------------------|----------------------------------------|----------------|
| | mg BHT Equivalent/mg of sample extract | |
| <i>Rumex acetosa</i> L. (leaf) | 0.11 | 0.06 |
| <i>Tropaeolum majus</i> L. (leaf) | 0.30 | 0.07 |
| <i>Solanum muricatum</i> (fruit) | 0.07 | 0.00 |
| <i>Stachys byzantina</i> K. Koch (leaf) | 0.36 | 0.28 |
| <i>Solanum betaceum</i> Cav. (fruit) | 0.11 | 0.04 |

No steroids were found in the tests of the extracts of *Solanum betaceum* Cav. and *Solanum muricatum*, and only the extract from *Solanum betaceum* Cav. tested positive for flavonoids. The results were negative in the remaining tests. The percentage of antioxidant activity determined by sequestering of the DPPH and ABTS radicals increased with increasing concentration, thereby showing a dose-dependent effect for the five species studied. The highest antioxidant activity was observed for *Tropaeolum majus* L. and *Stachys byzantina* K. Koch using the phosphomolibdenum method. Of the five species of vegetables evaluated, *Stachys byzantina* K. Koch presented antioxidant activity by all the methods evaluated. The plants appeared to be devoid of essential oils.

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