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Abstract

This chapter describes recent research studies about kohlrabi, a versatile vegetable with important health benefits (e.g. reduces risk of breast and prostate cancer, improves body metabolism, helps in weight loss diets, etc.). The investigations are focused on pale-green kohlrabi giving an accurate and precise description, from a qualitative point of view, of the bioactive compounds found in different parts of the pale-green kohlrabi: core, peel, leaves and equal combinations between these parts. All the active principles from pale-green kohlrabi are extracted following a well-established method, in an aqueous medium at a constant temperature of 4°C for 24 h. The qualitative screening of phytochemicals gives details regarding the presence or absence of chemical compounds using different colour reactions.

Keywords: Brassica oleracea, kohlrabi, aqueous extracts, bioactive compounds, qualitative screening

1. Introduction

Brassica vegetables, also known as ‘cruciferous vegetables’, consist of a large group of herbaceous plants that include some of the world’s most cultivated vegetables, namely cabbage, broccoli and cauliflower. Besides their main use as food ingredients, Brassica vegetables are full of antioxidants that help lower the potential risk of different types of cancers and coronary heart issues and are an important source of vitamin C, folic acid and numerous minerals such as iron, potassium and selenium [1].

Brassicas are also renowned for containing disease-fighting compounds, phytochemicals that occur naturally in plants and exhibit a variety of health benefits for the human body. One
of those biologically active compounds is glucosinolates, sulphur-containing phytochemicals with strong anti-cancer properties [2–4]. *Brassica* vegetables contain significant amounts of carotenoids such as zeaxanthin and lutein, two important components of the macula lutea region of the retina, and, therefore, play an important role in the prevention of age-related macular degeneration [5].

Kohlrabi (*Brassica oleracea* of the Gongylodes group) is one of the top vitamin C plants (one cup of kohlrabi contains more than 100% of the daily dose recommended for human consumption). It has European origins, being often called ‘German turnip’, with a sweet and delicate taste, rather a combination between radish and cabbage.

Kohlrabi is a bulbous vegetable available all year round and can be eaten either raw or cooked; both root and leaves are recommended in human consumption as they contain significant amounts of nutrients and are poor in calories [6, 7].

Several varieties of kohlrabi are commonly grown and commercially available, including White Vienna, Purple Vienna, Grand Duke, Gigante, Purple Danube and White Danube.

The main benefits in human health of kohlrabi are presented in Figure 1.

In the present chapter, different parts (e.g. core, peel and leaves) of pale-green kohlrabi are used to prepare five distinct aqueous extracts that are analysed by means of qualitative phytochemical content [8–10].

![Figure 1. Health benefits of pale green kohlrabi.](image)
2. Preparation of aqueous extracts from pale-green kohlrabi

Five distinct aqueous extracts are prepared from different parts of pale-green kohlrabi, as follows:

- three simple aqueous extracts from only one part, for example, core, peel and leaves; and
- two combined aqueous extracts from core and peel in equal parts, respectively core, peel and leaves in equal amounts.

The main steps involved in the preparation of aqueous extracts from pale-green kohlrabi are (Figure 2) acquiring pale-green kohlrabi from the local market, thoroughly washing it with tap water once and distilled water thrice, separating the component parts (core, peel, leaves), shade-drying it at room temperature, grinding the components into fine parts, extracting a determined quantity of the dried powder in an aqueous medium for 24 h and filtering the resulting extract until no debris are present in the aqueous extract.

All five distinct pale-green kohlrabi aqueous extracts are prepared according to the same method that was generally described above; the only difference is the amount of dried plant that resulted after the extraction and the volume of the resulting aqueous extract. In Table 1 the amount of dried plant material before and after the extraction is presented, and Table 2 contains the exact volume of different resulting aqueous extracts compared to the initial volume of distilled water.

The extractive value (yield percentage) of the kohlrabi (peel, core, leaves, equal amounts of peel and core, equal amounts of peel, core and leaves) samples was calculated before and after

Figure 2. General method for preparation of aqueous extract from pale-green kohlrabi.
<table>
<thead>
<tr>
<th>Crt. No.</th>
<th>Aqueous extract</th>
<th>Weight before extraction (g)</th>
<th>Weight after extraction (g)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pale-green kohlrabi core</td>
<td>25</td>
<td>19.06</td>
<td>76.24</td>
</tr>
<tr>
<td>2</td>
<td>Pale-green kohlrabi peel</td>
<td>25</td>
<td>21.26</td>
<td>85.04</td>
</tr>
<tr>
<td>3</td>
<td>Pale-green kohlrabi leaves</td>
<td>25</td>
<td>20.64</td>
<td>82.56</td>
</tr>
<tr>
<td>4</td>
<td>Pale-green kohlrabi core and peel (equal amounts)</td>
<td>25 (12.5 g core + 12.5 g peel)</td>
<td>17.63</td>
<td>70.52</td>
</tr>
<tr>
<td>5</td>
<td>Pale-green kohlrabi core, peel and leaves (equal amounts)</td>
<td>30 (10 g core + 10 g peel + 10 g leaves)</td>
<td>22.85</td>
<td>76.17</td>
</tr>
</tbody>
</table>

Table 1. Quantities of solid vegetal material before and after the extraction.

<table>
<thead>
<tr>
<th>Crt. No.</th>
<th>Aqueous extract</th>
<th>Distilled water (mL)</th>
<th>Volume of aqueous extract (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pale-green kohlrabi core</td>
<td>250</td>
<td>202</td>
</tr>
<tr>
<td>2</td>
<td>Pale-green kohlrabi peel</td>
<td>250</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>Pale-green kohlrabi leaves</td>
<td>250</td>
<td>192</td>
</tr>
<tr>
<td>4</td>
<td>Pale-green kohlrabi core and peel</td>
<td>250</td>
<td>190</td>
</tr>
<tr>
<td>5</td>
<td>Pale-green kohlrabi core, peel and leaves</td>
<td>300</td>
<td>208</td>
</tr>
</tbody>
</table>

Table 2. Volume of resulted aqueous extracts from pale green kohlrabi.

the preparation of the aqueous extracts using the formula and the results are also presented in Table 1 [11]:

\[
\text{Extract yield \%} = \left(\frac{W_1}{W_2}\right) \times 100.
\]

where \(W_1\) = net powder weight (grams) after extraction and \(W_2\) = total powder weight (grams) used for the preparation of aqueous extracts.

3. Qualitative screening of phytochemicals from pale-green kohlrabi aqueous extracts

Various standard qualitative phytochemical analyses are known that allow the determination of chemical groups or compounds in aqueous extracts from different plants. The majority of these qualitative tests is based on the change of colour or precipitation as a clear response to the presence of that specific chemical compound [12, 13]. It is important to mention that these colour reactions allow only to highlight the presence or absence of various chemical groups and not the amount in which they are present in different aqueous extracts.

Standard phytochemical methods are used to analyse from a qualitative point of view all the five aqueous extracts prepared as mentioned in the previous section [14, 15].
3.1. Qualitative screening of carbohydrates

Carbohydrates, the sugars and fibres that can be found in every fruit or vegetable, represent one of the basic food groups of great importance for human health. Carbohydrates are among the top three macronutrients, along with protein and fats.

A large number of analytical techniques have been used to determine the concentration and different types of carbohydrates found in foods.

There are four different standard phytochemical methods used for the qualitative screening of carbohydrates found in aqueous extracts [16] (Table 3):

a. A 1 ml Molisch reagent (a solution of α-naphthol in ethylic alcohol) is added to 2 ml aqueous extract to which a few drops of concentrated sulphuric acid are slowly dripped until a purple-reddish colour appears;

b. To 1 ml of aqueous extract, 5 ml of Benedict’s reagent (a complex solution of sodium carbonate, sodium citrate and copper sulphate pentahydrate) was added and boiled for 5 min. The bluish-green colour indicates the presence of carbohydrates;

c. To 1 ml of aqueous extract, a few drops of Fehling A reagent (aqueous solution of copper sulphate) are added, which gives green colouration;

d. To 1 ml of aqueous extract, a few drops of Fehling B reagent (a solution of potassium sodium tartrate in sodium hydroxide) are added, and a brown colour appears.

It is clear from the colour reaction described above that, with the only exception of pale-green kohlrabi peel, carbohydrates can be found in all the other four aqueous extracts.

3.2. Qualitative screening of tannins and phlobatannins

Tannins are a group of phenol compounds usually found in plants, part of a group of chemicals called ‘polyphenols’, and almost all of them are soluble in water. Phlobatannins are largely considered a novel class of ring-isomerized condensed tannins [17].

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates—Molisch</td>
<td>Purple solution</td>
<td>Yellow-mustard solution</td>
<td>Purple solution</td>
<td>Purple solution</td>
<td>Purple solution</td>
</tr>
<tr>
<td>Carbohydrates—Benedict</td>
<td>Blue-green solution</td>
<td>Turquoise solution</td>
<td>Blue-green solution</td>
<td>Blue-green solution</td>
<td>Blue-green solution</td>
</tr>
<tr>
<td>Carbohydrates—Fehling A</td>
<td>Green solution</td>
<td>Turquoise opalescent solution</td>
<td>Green solution</td>
<td>Green solution</td>
<td>Green solution</td>
</tr>
</tbody>
</table>

Table 3. Qualitative screening of carbohydrates.
According to the literature [18], the test for tannins consists of the following steps: to 1 ml of aqueous extract 2 ml of 5% ferric chloride is added and a dark blue or greenish black colour appears.

Phlobatannins are tested as follows: To 1 ml of aqueous extract few drops of diluted HCl (1%) are added and a red precipitate appears (Table 4).

Tannins are absent from all the five pale-green kohlrabi aqueous extracts while small traces of phlobatannins can be found in three aqueous extracts: pale-green kohlrabi core, pale green kohlrabi leaves and in the aqueous extract prepared from equal amounts of core and peel.

### 3.3. Qualitative screening of saponins

The general method is 2 ml of aqueous extract and 2 ml of distilled water are shaken in a graduated cylinder for 15 min. A 1 cm foam layer indicates the presence of saponins (see Table 5).

### 3.4. Qualitative screening of flavonoids and phenolic flavonoids

Flavonoids are a class of polyphenolic compounds with important functions in plants: attract pollinating insects, fight against different microbial infections and control cell growth [19].

Flavonoids are tested as follows: 2 ml of aqueous extract and 1 ml of 2 N sodium hydroxide are mixed. A yellow colour indicates the presence of flavonoids. The test for phenolic flavonoids involves the reaction between 1 ml of aqueous extract and 2 ml of 10% lead acetate solution reacting to give a brown precipitate (see Table 6).

Flavonoids are present in two aqueous extracts (pale-green kohlrabi peel and pale-green kohlrabi leaves), while phenolic flavonoids occur in pale-green kohlrabi core and in the two complex aqueous extracts that contain it.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>Brown-yellow opalescent solution</td>
<td>Yellow-brown solution</td>
<td>Brown-yellow solution</td>
<td>Brown solution</td>
<td>Brown solution</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>Red-brown opalescent solution</td>
<td>White opalescent solution</td>
<td>Red-brown opalescent solution</td>
<td>Red-brown solution</td>
<td>Yellow solution</td>
</tr>
</tbody>
</table>

Table 4. Qualitative screening of tannins and phlobatannins.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>2 cm foam layer</td>
<td>3 cm foam layer</td>
<td>2.5 cm foam layer</td>
<td>3.5 cm foam layer</td>
<td>3 cm foam layer</td>
</tr>
</tbody>
</table>

Table 5. Qualitative screening of saponins.
3.5. Qualitative screening of alkaloids

Alkaloids are naturally occurring compounds that contain basic nitrogen atoms. They have a large variety of pharmacological applications: antimalarial, antiasthmatic, anticancer, analgesic, and so on [20].

There are two different standard phytochemical methods:

a. To 1 ml of aqueous extract, 1 ml of Wagner’s reagent (iodine in potassium iodide solution) is added leading to the formation of a reddish brown precipitate.

b. To 1 ml of aqueous extract, 2 ml of concentrated hydrochloric acid and a few drops of Mayer reagent are added, resulting in a green colour or white precipitate (the results are presented in Table 7).

According to the results presented in Table 7, alkaloids are absent from all the aqueous extracts from pale-green kohlrabi, whatever method was used for the qualitative screening.

3.6. Qualitative screening of anthraquinones and anthocyanosides

The standard method used for the qualitative screening of anthraquinones involves the reaction of 1 ml of aqueous extract with a few drops of 10% ammonia solution, leading to the formation of a pink precipitate. Anthocyanosides are observed when 1 ml of aqueous extract is mixed with 5 ml of dilute hydrochloric acid and a pink colour appears (see Table 8 for the results).

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Red-brown solution</td>
<td>Pale-yellow solution</td>
<td>Pale-yellow opalescent</td>
<td>Red-brown solution</td>
<td>Brown solution</td>
</tr>
<tr>
<td>Phenolic flavonoids</td>
<td>Brown precipitate</td>
<td>White precipitate</td>
<td>Pale-yellow precipitate</td>
<td>Pale-brown solution</td>
<td>Opalescent brown-yellow solution</td>
</tr>
</tbody>
</table>

Table 6. Qualitative screening of flavonoids and phenolic flavonoids.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids—Wagner</td>
<td>Opalescent red-brown solution</td>
<td>Opalescent brown solution</td>
<td>Opalescent yellow-brown</td>
<td>Clear red-brown solution</td>
<td>Opalescent red-brown solution</td>
</tr>
<tr>
<td>Alkaloids—Mayer</td>
<td>Opalescent orange-yellow solution</td>
<td>Opalescent beige solution</td>
<td>Brown-yellow opalescent</td>
<td>Red-brown solution</td>
<td>Opalescent beige solution</td>
</tr>
</tbody>
</table>

Table 7. Qualitative screening of alkaloids.
3.7. Qualitative screening of proteins and aminoacids

There are two different standard methods used (see results in Table 9):

a. 1 ml of aqueous extract reacts with 5–6 drops of Millon’s reagent, and a white precipitate appears that changes its colour to red upon heating;

b. To 3 ml of aqueous extract, 3 ml of 4% sodium hydroxide solution and few drops of 1% copper sulphate are added to form a purple solution.

3.8. Qualitative screening of steroids and terpenoids

The general procedure to test the presence of steroids is To 1 ml of aqueous extract, add 10 ml of chloroform and slowly drip 10 ml of sulphuric acid. The upper layer turns red and the sulphuric acid layer turns yellow green. Similarly, terpenoids are analysed by reacting 1 ml of aqueous extract with 2 ml of chloroform and then slowly few drops of concentrated sulphuric acid. An interface with a reddish brown colouration appears (Table 10).

The qualitative screening of steroids revealed that these phytochemicals are absent from all the extracts while very small traces of terpenoids could be visually observed in three aqueous extracts: pale-green kohlrabi core and the other two extracts that contain this part.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthraquinones</td>
<td>Red-beige solution</td>
<td>Pale-yellow opalescent solution</td>
<td>Green-yellow precipitate</td>
<td>Opalescent red-brown solution</td>
<td>Opalescent red-beige solution</td>
</tr>
<tr>
<td>Anthocyanosides</td>
<td>Red-yellow opalescent solution</td>
<td>Yellow opalescent solution</td>
<td>Orange-red solution</td>
<td>Pale-beige solution</td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Qualitative screening of anthraquinones and anthocyanosides.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins and aminoacids—Millon</td>
<td>Red-beige solution</td>
<td>Opalescent solution, white-yellow after heating</td>
<td>Green-yellow precipitate</td>
<td>White brown solution</td>
<td>Opalescent beige solution, red-brown after heating</td>
</tr>
<tr>
<td>Proteins and aminoacids—Biuret test</td>
<td>Red-yellow opalescent solution</td>
<td>Green-yellow solution, blue precipitate</td>
<td>Dark-brown solution</td>
<td>Violet-green solution</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Qualitative screening of proteins and aminoacids.
3.9. Qualitative screening of cardiac glycosides

There are two different standard phytochemical methods:

a. 1 ml of aqueous extract, 1 ml of FeCl\textsubscript{3} reagent (1 ml of 5\% FeCl\textsubscript{3} solution mixed with 99 ml of glacial acetic acid) and few drops of concentrated H\textsubscript{2}SO\textsubscript{4} gives a greenish-blue colour that appears in time;

b. 5 ml of aqueous extract, 2 ml of glacial acetic acid, a drop of FeCl\textsubscript{3} solution and 1 ml of concentrated H\textsubscript{2}SO\textsubscript{4} forms a brown ring and often a purple ring appears below (see results in Table 11).

Regardless of the method used in the screening, cardiac glycosides are absent from all the aqueous extracts prepared from pale-green kohlrabi.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids</td>
<td>Colourless layer, brown ring, colourless layer</td>
<td>Colourless layer, beige ring, colourless layer</td>
<td>Colourless layer, light-brown ring, colourless layer, brown ring</td>
<td>Colourless layer, brown ring, colourless layer</td>
<td>Colourless layer, brown ring, colourless layer</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Colourless layer, yellow-brown ring</td>
<td>Colourless layer, white ring (precipitate)</td>
<td>Pale-yellow layer, beige ring</td>
<td>Yellow-brown layer, red-brown ring</td>
<td>Colourless layer, brown-yellow opalescent ring</td>
</tr>
</tbody>
</table>

Table 10. Qualitative screening of steroids and terpenoids.

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Pale-green kohlrabi core</th>
<th>Pale-green kohlrabi peel</th>
<th>Pale-green kohlrabi leaves</th>
<th>Pale-green kohlrabi core and peel</th>
<th>Pale-green kohlrabi core, peel and leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac glycosides—FeCl\textsubscript{3} reagent</td>
<td>Colourless layer, thin brown ring, beige clear solution</td>
<td>Colourless clear layer, yellow suspension</td>
<td>Colourless layer, pink-beige suspension</td>
<td>Colourless layer, brown ring, opalescent beige layer</td>
<td>Colourless layer, opalescent yellow-beige ring</td>
</tr>
<tr>
<td>Cardiac glycosides—Keller-Killani test</td>
<td>Colourless layer, brown ring, beige opalescent layer, red opalescent layer</td>
<td>Colourless layer, red-beige opalescent solution</td>
<td>Colourless layer, yellow-brown layer, brown-red layer</td>
<td>Colourless layer, brown ring, red-brown layer, beige precipitate layer</td>
<td>Colourless layer, brown ring, red-brown layer</td>
</tr>
</tbody>
</table>

Table 11. Qualitative screening of cardiac glycosides.

4. Conclusions

This chapter describes the qualitative phytochemical screening of five distinct aqueous extracts prepared from different parts of pale-green kohlrabi, a versatile vegetable part of Brassica
genus with numerous benefits for human health. The qualitative screening is achieved by standard methods that are able to determine whether a phytochemical is present or not in a specific aqueous extract.

The qualitative screening of carbohydrates revealed that, except for pale-green kohlrabi peel aqueous extract, in all the other extracts carbohydrates are present. It can be clearly stated that tannins are absent from all the five pale-green kohlrabi aqueous extracts. Phlobatannins can be found, in small traces, in three aqueous extracts: pale-green kohlrabi core, pale-green kohlrabi leaves and in the aqueous extract prepared from equal amounts of core and peel.

In smaller or larger quantities, saponins are present in all five aqueous extracts, according to the height of the resulting foam layer, while alkaloids, cardiac glycosides and steroids are clearly absent from all the extracts.

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Conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

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