Polyphenolic extracts from *Viola x wittrockiana* reduce fat storages of *Caenorhabditis elegans*

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Polyphenolic extracts from *Viola x wittrockiana* reduce fat storages of *Caenorhabditis elegans*.
Abstract:

Diabetes mellitus is an important health problem in our society that can cause severe complications or even death when untreated.

Caenorhabditis elegans is a model organism widely used for the evaluation of functional foods and bioactive compounds that shares 60-80% of its genome with humans.

Edible flowers own interesting properties that could make them suitable for developing new drugs. Viola x wittrockiana has proven to be one of them due to its polyphenol-based composition.

The antidiabetic potential of the extract was quantified by in vitro inhibition of lipase and α-glucosidase, and the capacity of the extract to prevent AGEs formation by a non-enzymatic reaction. To furtherly test these antidiabetic properties, an in vivo assay with C. elegans was performed.

V. x wittrockiana showed lower IC₅₀ values in the α-glucosidase assay than the reference drug acarbose and higher inhibition AGEs formation potential than the reference substance aminoguanidine, it also obtained important values inhibiting lipase. Moreover, the extract lowered fat storages of C. elegans with no significant differences (p<0.05) to positive control orlistat.

The flowers of Viola x wittrockiana can be considered as source of polyphenol bioactive compounds with interesting properties as functional foods in the prevention and improvement of chronic diseases such as diabetes.

Keywords: AGES; C. elegans; Diabetes; Polyphenols
Introduction: Diabetes Mellitus

Metabolic disease characterized by high blood sugar levels. Can cause severe complications to the organism or even death when not treated. Worldwide impact.

Treatments

Type 1 Diabetes
- Insulin subcutaneous administration

Type 2 Diabetes
- Oral antidiabetic drugs
- Health dietary habits
Introduction: Edible flowers

Edible flowers have been used through history mainly as traditional medicine, nowadays their use focuses on gastronomy.

Certain flowers such as *Viola x wittrockiana* have shown interesting properties due to its polyphenol-based composition.
Introduction: *Caenorhabditis elegans*

Model organism widely used.

Easy handling, wide mutant library.

60 – 80% homologue genes to humans.
Methodology

**IN VITRO**

Inhibition

- Digestive enzymes
  - α-glucosidase
  - Lipase

- AGEs

**IN VIVO**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+ Control</th>
<th>- Control</th>
<th>Viola x wittrockiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>5% glucose</td>
<td>5% glucose + 6 µg/ml Orlistat</td>
<td>5% Glucose + 500 µg/ml extract</td>
<td></td>
</tr>
</tbody>
</table>

48h exposure → Nile Red straining
Results and discussion: in vitro

Acarbose used as control ($IC_{50} = 425.49 \mu g/mL$).

*Viola x wittrockiana* calculated $IC_{50} = 395.77 \mu g/mL$.
Results and discussion: In vitro

Orlistat used as control ($IC_{50} = 35.10 \mu g/mL$)

*Viola x wittrockiana* $IC_{50} = 586.95 \mu g/mL$
Results and discussion: in vitro

Viola x wittrockiana

Aminoguanidiné used as control (IC$_{50}$ = 81.92 µg/mL)
Viola x wittrockiana IC$_{50}$ = 80.83 µg/mL
Results and discussion: in vivo

Fluorescence images of *C. elegans* after Nile Red staining and ultraviolet light exposure.

- **Control:** No treatment
- **Positive Control:** 5% glucose
- **Negative Control:** 6 µg/mL Orlistat
- **Viola:** 500 µg/mL extract

Fluorescence images of *C. elegans* after Nile Red staining and ultraviolet light exposure.
Results and discussion: in vivo

Reduction compared to obese worm expressed in %. Same letters show no significant differences p<0.05.
Conclusions

1. *Viola* extract showed lower IC$_{50}$ values than the reference antidiabetic $\alpha$-glucosidase inhibitor known as acarbose, and important values inhibiting enzyme lipase.

2. The polyphenolic extract showed higher activity preventing the glycation of proteins than reference substance AMG (aminoguanidine).

3. Viola extract lowered fat storages of C. elegans with no significant difference to negative control Orlistat (p<0.05)

4. Flowers from *Viola x wittrockiana* can be considered as a source of bioactive polyphenolic compounds with interesting properties in the field of nutrition.
Acknowledgments