FLAVONOIDS AND POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF FICUS CARICA L. EXTRACTS FROM ROMANIA

ABSTRACT: The objective of this study is to determine flavonoids and polyphenols content and antioxidant activity of extracts of figs growing in Romania. The content of flavonoids and polyphenolic compounds was determined according to the Romanian Pharmacopoeia, the 10th edition, using the standard rutin for flavonoids, catechol for polyphenols and HPLC for flavonoids quantification. Determination of antioxidant activity was done by DPPH scavenging method and at cellular level by attenuation of oxidative damage in human erythrocytes. The experimental results reveal that Ficus carica extracts may be a potential source of natural antioxidants.

KEYWORDS: Ficus carica, flavonoids, rutin, antioxidant activity, DPPH (Diphenylypicrylhydrazyl)

INTRODUCTION

It is known that many natural compounds have antioxidant activity. Flavonoids belong to a special class of natural compounds being the main active substance in many medicinal herbs. Flavonoids are used in the treatment of many diseases to inhibit specific enzymes, hormones and to stimulate and reduce free radical activity.

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The antioxidant activity of flavonoids is due to a variable number of phenolic groups contained in their chemical structure and their property to form chelates with iron and other transitional metals.

The antioxidant activity is important for the human body because it protects the cells against free radicals, formed as a result in many processes that use oxygen as energy source, and plays an essential role in protection against oxidative degradation [Havsten 2002].

Flavonoids behave as antioxidants in a variety of ways, including direct trapping of the oxygen species, chelation of transition metals involved in the process radicals formation and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals [Heim et al., 2002]. Also, they are able to modify the synthesis of eicosanoids, to prevent platelets aggregation and to protect lipoproteins against oxidation.

Although some studies indicate that flavonoids have peroxidation action, but only at high doses, they also have anti-inflammatory, antiviral, anti-allergic and protection role in various pathologies [Andersen and Markham 2006].

*Ficus carica* L. (Moraceae) is a shrub native of South West Asia cultivated since antiquity; originally from Persia and Syria, spread later in Europe and America.

A research report on this species indicates the presence of flavonoids, coumarins, sterols, triterpenoids, and anthocyanins in different parts of the plant. The leaves contain bergapten, quercetin, luteolin, and 4′,5′-dihydrosporalen, the fruits contain cyanide-3-O-glucoside, cyanidin-3-O-rhamnoglucoside, rutin, gallic acid, catechin, epicatechin, saturated fat, cholesterol, sodium, protein, vitamin A, vitamin C, calcium and iron, and the root contains psoralen and bergapten [Anshul et al., 2012].

The leaves and fruits of *Ficus carica* are traditionally used as laxative, stimulant against throat diseases, cough suppressant, emollient, emmenagogue and solvent [Bellakhadar et al., 1991; Guarrera 2003]. Fig has been traditionally used for its medicinal benefits in metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory therapy. The root is used in leucoderma and herpes. The fruits are antipyretic, tonic, purgative, useful in inflammation, faintness, paralysis, hepatic and spleen diseases, angina, and also as hair growth stimulators. The milky juice of fig is expectorant and diuretic, but represents a high risk in contact with eyes.

The decoction of fig leaves can be used in hemorrhoids therapy, while fruits infusion can safely be used as a laxative for children. Fresh leaves were dabbed in wars [Baytop 1984].

The effect of fig leaves decoction on diabetes management has been studied, by maintaining a normal hypoglycemia in the short term [Serraclara et al., 1998]. An aqueous extract of *Ficus carica* leaves may induce a significant hypoglycemic effect in rats, but the mechanism involved in such an effect was not elucidated [Perez et al., 1996]. *Ficus carica* methanolic extract has potent anti-inflammatory activities at the level of cell migration and angiogenesis and may be correlated with its antioxidant potential [Eteraf-Oskouei et al., 2015].
Rutin is the major flavonoid glycoside found in fig. It is the rhamno-glucoside of the flavonoid quercetin and vitamin P. Quercetin is the major flavonoid present in fig leaves, along with luteolin, kaemferol and rutin [Vaya 2006]. Many studies focus on the antioxidant activity of *Ficus carica*. Solomon *et al.* reported that fresh fruits had an antioxidant activity, and Konyalioğlu *et al.* showed that the antioxidant capacity of leaves extract had an antioxidant activity assessed only by the phosphomolibdenum spectrophotometric method.

This study focuses on the antioxidant activity by different methods and on determination of flavonoids and polyphenols content in fig (*Ficus carica*) leaves and fruits growing in Romania.

**MATERIALS AND METHODS**

*Plant material*

The plant material (leaves/fruits of *Ficus carica*) was collected from Arad (Romania) and dried at room temperature in a dark place.

*Solvents and reagents*

Rutin and catechol were purchased from Sigma–Aldrich. The other chemicals of analytical grade were purchased from Chimopar, Bucharest.

*Preparation of extracts*

The dried and finely ground samples of leaves and fruits (20 g each) were extracted with 250 mL ethanol 70% for 24h at room temperature. The extract was filtered and evaporated under vacuum to about 20 ml.

The extract was stored at 4 °C to prevent any further degradation [Cacig 2007; Trifunschi and Ardelean 2013].

* Determination of polyphenol content*

Determination of polyphenol content was made according to Romanian Pharmacopoeia, the 10th edition. Catechol was used as standard. 5 mL of plant extract was mixed with 5 mL of phosphotungstic acid, and 5 mL of this mixture was diluted to 10 mL with sodium carbonate (200 g/L). The absorbance was measured at 430 nm (Metertech SP-8001 UV/Visible spectrophotometer) one minute later. The polyphenol concentration was expressed as the equivalent concentration of catechol. All determinations were performed in triplicate. The calibration curve was prepared with catechol solution ranging from 0.005–0.5 mg/mL.
Determination of flavonoids content

Flavonoids content was determined according to Romanian Pharmacopoeia, the 10th edition. Rutin was used as standard. 5 mL of plant extract was mixed with 5 mL of sodium acetate (100 g/L), 3 ml AlCl₃ (25 g/L) and diluted to 25 mL with methanol. The absorbance was measured at 430 nm (Metertech SP-8001 UV/Visible spectrophotometer). The flavonoid concentration was expressed as the equivalent concentration of rutin. All determinations were performed in triplicate. The calibration curve was prepared with rutin solution ranging from 0.5–5 mg/mL.

HPLC analysis of flavonoids

The HPLC analysis was performed using the HPLC YL 900 series instrument coupled with a binary pump, a diode-array detector (DAD), an autosampler, and a column compartment. Flavonoids from extracts were separated on a Polaris C18 column (5μm, 4 x 125 mm) with a sample injection volume of 20 μl. A gradient elution was used with mobile phase consisting of (A) distilled water : phosphoric acid = 98 : 2 (pH=2.5) and (B) acetonitrile: 50% A and 50% B at the beginning; 80% B (after 12 min), and 50% B (15–20 min). The flow rate was 1.5 ml/min. The capillary temperature was set at 30 °C and λ = 380 nm.

Determination of free radical scavenging activity by DPPH method

DPPH assay is a method for measuring the antioxidant capacity of vegetable products involving the use of free radical 2,2-diphenyl-1-picrylhydrazyl. It is used to evaluate the ability of compounds to act as free radical scavengers or hydrogen donors and evaluate the extracts antioxidant activity. The reaction involves color change from violet to yellow which can be monitored to some degree with a spectrophotometer at 517 nm.

It was added in a micro plate 0.25 mL extract and 4 mL DPPH solution (20 mg/dL) and monitored the variation of absorbance at 517 nm for 30 minute at room temperature [Prakash 2001]. Synthetic antioxidants of acid ascorbic and flavonoids standards (quercetin and rutin) were used. The scavenging effect of DPPH was calculated using the equation:

\[
\text{DPPH scavenging effect (\%)} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

All determinations were performed in triplicate.

Antioxidant activity at the cellular level

Attenuation of oxidative stress at the cellular level (leukocytes from healthy blood) was tested for flavonoids and for extracts of Ficus carica.
Determination of the antioxidant effect in pretreatment of human leukocytes of flavonoids extracts at different concentrations was based on the “comet tail” that followed the principle of electrophoresis in agarose gel [Lean et al., 1999; Norozi et al., 1998].

Approximately 100 mL fresh blood collected from a healthy human donor was treated in a polypropylene test tube with 1 ml of RPMI and 10% FCS. The mixture has been left at low temperature for 30 minutes, and then immersed in 1 ml of Ficoll using a micropipette. The obtained sample was centrifuged at 3600 rpm for 15 minutes at 4 °C. The fraction of white blood cells in the form of a pale pink and gray ring was removed after centrifugation by a Pasteur pipette, and immersed again in 1 ml of PBS homogenized using Vortex (solution A).

In six Ependorf labeled tubes have been added 85 ml of solution A (containing between 20,000 to 30,000 leukocytes) to which have been added the following:

- **E1** (control) − 100 mL of PBS,
- **E2, E3** − 100 mL concentrations of standard solutions of flavonoids (2.7% quercetin and rutin 0.15%) in PBS,
- **E4, E5** − 100 mL alcoholic leaves extract and fruits extract of *Ficus carica* L respectively,
- **E6** − 100 mL of 0.1% H$_2$O$_2$.

Samples E1–E5 were incubated at 37 °C for 30 min. The sample E6 was kept in the dark at 4 °C during the incubation of the others. After the incubation period, samples E2–E5 were treated with 0.1% H$_2$O$_2$ in PBS and stored in the dark for 5 min at low temperature.

The experimental measurements have been made using microscope by direct examination on the lamellas with a wavelength radiation emitted at 520 nm and 620 nm respectively.

The classification of cells was made by a quick visual evaluation, using a scale from 0–4 as follows:

Grade 0 − cells degraded, less than 5% of the total;
Grade 1 − cells poorly degraded, between 5–25% of the total;
Grade 2 − cells with an average of degradation, between 25–45%;
Grade 3 − cells strongly degraded, between 45–70%;
Grade 4 − cells damaged, more than 70%.

* The concentrations of standard solutions of flavonoids have been the same as the ethanolic extract of *Ficus carica* concentration.

**Statistical Analysis**

The experimental results are expressed as ± means of standard deviation (SD). Data represent the average of three replicates and Microsoft Excel program was used for the statistical analysis.
Table 1. Flavonoid/phenolic contents and antioxidant activity of leaves and fruits of *Ficus carica* extracts

<table>
<thead>
<tr>
<th><em>Ficus carica</em> Extract</th>
<th>Polyphenol content (mg catechol/g)</th>
<th>Flavonoid content (mg rutin/g)</th>
<th>DPPH scavenging effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of leaves</td>
<td>25.25 ±0.001</td>
<td>2.62 ±0.003</td>
<td>44.22</td>
</tr>
<tr>
<td>Ethanol extract of fruits</td>
<td>18.63 ±0.001</td>
<td>1.96 ±0.002</td>
<td>34.23</td>
</tr>
<tr>
<td>Quercetin</td>
<td>-</td>
<td>-</td>
<td>57.9</td>
</tr>
<tr>
<td>Rutin</td>
<td>-</td>
<td>-</td>
<td>59.6</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>-</td>
<td>-</td>
<td>53.9</td>
</tr>
</tbody>
</table>

Table 2. HPLC analysis of extracts of *Ficus carica*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ethanol extract of leaves of <em>Ficus carica</em></th>
<th>Ethanol extract of fruits of <em>Ficus carica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tr [min]</td>
<td>Concentration [%]</td>
</tr>
<tr>
<td>Quercetin</td>
<td>10.587</td>
<td>2.5</td>
</tr>
<tr>
<td>Luteolin</td>
<td>12.293</td>
<td>0.07</td>
</tr>
<tr>
<td>Kaemferol</td>
<td>13.587</td>
<td>trace</td>
</tr>
<tr>
<td>Rutin</td>
<td>14.827</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 3. The effect of Standard solutions of flavonoids on the degradation of leukocytes

<table>
<thead>
<tr>
<th>The type of treatment used</th>
<th>The percentage of cells with different degrees of damage [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gr.0</td>
</tr>
<tr>
<td>quercetin (2.7 %) +100μL H₂O₂ 0.1%</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>rutin (0.15 %) +100μL H₂O₂ 0.1%</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Luteolin (0.07%) +100μL H₂O₂ 0.1%</td>
<td>16.2 ± 0.1</td>
</tr>
<tr>
<td>kaempferol (0.02%) +100μL H₂O₂ 0.1%</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>The control sample</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>cells + solution of H₂O₂ 0.1% in PBS (100 μL)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 4. Effect of extracts of leaves and of fruit extracts on degradation of leukocytes

<table>
<thead>
<tr>
<th>The type of treatment used</th>
<th>The percentage of cells with different degrees of damage [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gr.0</td>
</tr>
<tr>
<td>Extract of fruits +100 μL H₂O₂ 0.1%</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>Extract of leaves +100 μL H₂O₂ 0.1%</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td>The control sample</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>Cells + solution H₂O₂ 0.1% in PBS (100 μL)</td>
<td>18.0 ± 0.2</td>
</tr>
</tbody>
</table>
REFERENCES

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Vaya J, Mahmood S (2006): Flavonoid content in leaf extracts of the fig (Ficus carica L.), carob (Ceratonia siliqua L.) and pistachio (Pistacia lentiscus L.), Biofactors, 28: 169–175.
САДРЖАЈ ФЛАВОНОИДА И ПОЛИФЕНОЛА И ОДРЕЂИВАЊЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ ЕКСТРАКАТА FICUS CARICA L. ИЗ РУМУНИЈЕ

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РЕЗИМЕ: Циљ овог истраживања је утврђивање садржаја флавоноида и полифенола као и антиоксидативне активности екстраката смокве која расте у поднебљу Румуније. Садржај флавоноида и полифенолних јединњења одређен је на основу Румунске фармакопеје, 10. издање. Коришћен је стандардни рутин за флавоноиде, катехол за полифеноле и HPLC метода за квантификовање флавоноида. Одређивање антиоксидативне активности урађено је помоћу органског јединњења DPPH (2,2-дифенил-1-пикрилхидразил), а на ћелијском нивоу путем слабљења оксидативног оштећења у људским еритроцитима. Експериментални резултати показују да екстракти Ficus carica могу бити потенцијални извор природних антиоксиданата.

КЉУЧНЕ РЕЧИ: Ficus carica, флавоноиди, рутин, антиоксидативна активност, DPPH (Diphenylpicrylhydrazyl)