LYTHRUM SALICARIA (PURPLE LOOSESTRIFE). MEDICINAL USE, EXTRACTION AND IDENTIFICATION OF ITS TOTAL PHENOLIC COMPOUNDS

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Abstract

Lythrum salicaria L., known as purple loosestrife (Lythraceae) has a wide range of beneficial health effects. It is well known as a medicinal plant from ancient Greek and Roman times and it has been an important drug for centuries. Its pharmacological activity is mostly due to its phenolic compounds, mainly tannins. Therefore obtaining an extract with a high percent of total phenolic compounds would be interesting for studying the biological potential of the plant. The dried herbal parts of Lythrum salicaria L. were sequentially extracted with different solvents: 96% ethanol, 70% ethanol, and distilled water, in order to select the best solvent exhibiting the maximum percent of total phenolic compounds; the measurements were performed using modified Folin-Ciocâlteu method according to Makkar et al. In addition, the total flavonoid content was also analyzed in the different extracts using Chang et al method. The quantitative analysis of the examined chemical compounds showed that 70% ethanol solution was the best solvent used in order to obtain the highest phenolic content.

Keywords: Lythrum salicaria; medicinal plant; tannins; phenolic compounds; modified Folin-Ciocâlteu method.
Introduction

The genus *Lythrum* (Lythraceae) is spread throughout the world. It is represented by almost 30 species, 10 of which are found in Europe. The generic name comes from the Greek "luthron," blood, possibly referring to the color of the flowers or to one of its herbal uses as an astringent. *L. salicaria* is originally Eurasian, but during the 19th century it was spread via the ballast of European ships not only to Europe but also into North and South America, as well as Australia. Its English name is “blooming sally”, “purple willow-herb”, “rainbow weed” and “purple loosestrife”. It is known in German as “Blutweiderich”, in French “Salicaire”, in Romanian “râchitan” and in Swedish “fackelblomster” [1-4]. *L. salicaria* is known as a medicinal plant from the ancient Greek and Roman times and it has been an important drug for centuries.

The whole flowering plant and the flowering branch tips of this plant are used not only in folk medicine but also in pharmaceuticals. It is used internally for diarrhea, chronic intestinal catarrh, in the form of a decoction or a fluid extract. Externally, it is used to treat varicose veins, venous insufficiency, bleeding of the gums, hemorrhoid and eczema [5-7].

The phytochemical examination carried on this plant reported that tannins were the main compounds in *Lythrum salicaria*. It contains a notable amount of flavons represented by flavon C-glycosides (vitexin, isovitexin, orientin and isoorientin) and anthocyanins, also. In addition, vescalagin, pedunculagin, vanoleic acid dilactone, 1,6-di-O-galloylglucose, 1-O-galloylglucose and 6-O-galloylglucose were identified. Sterols as β-sitosterol were also detected in this plant [7-10].

Antioxidant, antimicrobial, and hypoglycemic effects of *Lythrum salicaria* have been reported [11-14]. An ethanolic extract of this plant showed concentration-dependent superoxide anion radical scavenging activity and inhibitory effect on lipid peroxidation [14]. Aqueous methanolic extract showed a moderate antioxidant activity regarding the auto-oxidation of methyl-linoleate [12] and against the ABTS radical [2, 2’ aziono bis (3-ethylbenzoline)-6-sulfonic acid radical] [13]. The effect of *Lythrum salicaria* on the growth of *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* and its antifungal activity have been reported [6]. Stems and flowers of *Lythrum salicaria* show hypoglycemic activity in epinephrine-induced hyperglycemia [11].

Tannins (the main component of *Lythrum salicaria*) are extracted from the plant matrix depending on their physical properties (solubility in water, alcohol and acetone). These solvents can be used alone or as a mixture [15]. Some solvents are incompatible with some methods of
analysis, in particular acetone which inhibits all protein precipitation assays except the radial diffusion assay [16].

The addition of water to methanol (or ethanol) was found to increase the total phenolic (TP) recovery, since water allows the plant tissue to swell allowing the solvent to better penetrate the sample matrix [17].

In this study, different solvent extracts of *Lythrum salicaria* were quantitatively analyzed for their total phenolic compounds content via modified Folin-Ciocălтеu method [18] according to Makkar et al [19] measured as tannic acid equivalent, in order to select the best solvent able to extract the highest percent of total phenolic compound exhibiting the best pharmacological activity.

**Materials and methods**

**Plant material**

The plant samples were provided by Phytotherapy S.C., Romania.

**Instruments**

UV/Vis spectrophotometer Jasco V-530 (Jasco, Japan) with PC-HP 845 x UV-Visible System (Jasco, Japan) and 1 cm quartz cells were used for all absorbance measurements.

**Reagents and solutions**

Ethyl acetate, 96% ethanol, sodium carbonate, sodium tungstate, sodium molybdate, phosphoric acid, lithium sulfate, aluminum chloride hexahydrate, and potassium acetate, were provided by Fluka-chemika; we also used during the study bromine solution and concentrated hydrochloric acid.

For standard chemicals: tannic acid and quercetin (Sigma/Germany) were used.

**Preparation of plant extracts**

Two grams of the powder plant samples were refluxed separately with 50 mL of the three different solvents: 96% ethanol (E-1), 70% ethanol (E-2), and distilled water (E-3), for 30 minutes at 70°C, followed by successive re-extraction with the same extracting solvent until the disappearance of the brown extract color.

The resultant filtrates were made up to 50 mL with the same extracting solvent (solution A = S.A).

**Estimation of the total phenolic content**

Determination of total phenolic content (TPC), measured as tannic acid equivalent, was performed using the modified Folin-Ciocălтеu method according to Makkar et al [19].
To perform this analysis three reagents were used: Folin-Ciocâlțeu, sodium carbonate (20% solution), and standard tannic acid solution.

Folin-Ciocâlțeu reagent was obtained according to the literature procedure [20].

**Preparation of standard calibration curve**

The tannic acid standard solution (0.1 mg/mL) was prepared by dissolving 10 mg of tannic acid in 10 mL of distilled water, and then the volume was completed to 100 mL with the same dissolving solvent (we used freshly prepared solutions). The standard calibration curve was then developed as mentioned in the proceeding table.

All the flasks were mixed well and left in the dark at room temperature for 40 minutes, then the absorbance was read at $\lambda = 725$ nm [19].

<table>
<thead>
<tr>
<th>Flask</th>
<th>Tannic acid sol. (0.1mg/mL) Volume taken in mL</th>
<th>Distilled water Volume taken in mL</th>
<th>Folin Reagent Volume taken in mL</th>
<th>Sod. Carbonate solution Volume taken in mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.00</td>
<td>0.50</td>
<td>0.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Test Stand. 1</td>
<td>0.02</td>
<td>0.48</td>
<td>0.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Test Stand. 2</td>
<td>0.04</td>
<td>0.46</td>
<td>0.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Test Stand. 3</td>
<td>0.06</td>
<td>0.44</td>
<td>0.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Test Stand. 4</td>
<td>0.08</td>
<td>0.42</td>
<td>0.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Test Stand. 5</td>
<td>0.10</td>
<td>0.40</td>
<td>0.25</td>
<td>1.25</td>
</tr>
</tbody>
</table>

The volumes of the standard tannic acid solution together with the volumes of the reactive substances used in the preparation of the standard calibration curve are mentioned in table (I) whereas the resulted series of tannic acid concentrations with their corresponding absorbance are showed in table II. The obtained tannic acid standard curve is shown in figure 1.

**Analysis of the total phenolic compounds in plant extracts**

Sample extracts were prepared by diluting 25 mL of sol. A of each of E-1, E-2, and E-3 separately to a final volume of 50 mL with 96 % ethanol (sol. SC: stock solution of 1g dried plant /50 mL). To proceed with the Folin-Ciocâlțeu method, 0.1 mL of sol. SC (sample extract) was diluted with 0.4 mL of distilled water, followed by the addition of 0.25 mL of Folin-Ciocâlțeu reagent and 1.25 mL sodium carbonate solution. The blank was prepared using the same chemical reagents excluding the extract.

The flasks were mixed well and left in the dark, at room temperature for 40 minutes, then the absorbance was read at $\lambda = 725$ nm.
Quantification of tannins in salicaria extracts

The determination of tannins in the tested extracts was performed using the modified Makkar et al method [19].

The modification here was the use of hide powder instead of PVPP (Polyvinyl poly-pyrrolidone). To carry out the test, 100 mg of hide powder was weighed, then 1.0 mL of distilled water followed by 1.0 mL of the tannin-containing extract were added. This mixture was shaken for 60 minutes, afterwards centrifuged for 10 min and finally the supernatant was collected. The supernatant has only simple phenolic compounds other than tannins (the tannins would have been precipitated along with the hide powder).

The phenolic content of the supernatant was then measured following the same procedure described above, taking at least double the volume (preferably three times i.e. 0.3 mL) that was used for total phenol estimation, because the extract had already been diluted, in addition to the lose of tannin-phenols through binding with the hide powder.

The content of non-tannin phenols was expressed on a dry plant basis (y%), and the percentage of tannins was calculated as tannic acid equivalent, estimated as grams per 100 grams dried plant as follows:

\[
\text{Tannin percentage} = (x \%) - (y \%)
\]

where \( x \% \) is the percentage of the total phenolic compounds (g/100g dried plant), \( y \% \) is the content of the non-tannin phenols (g per 100 grams dried plant)

Estimation of the total flavonols content

The total flavonoid content in Salicaria extracts was measured using Chang et al method [21, 22] with simple modifications applied to the sample preparation. The same standard curve equation was applied in order to calculate the flavonol percent which was expressed as quercetin equivalent.

Sample preparation

The total flavonol content in Salicaria extracts was measured using Chang et al method with simple modifications applied to the sample preparation as such: 25 mL of S.A for each of E-1, E-2, and E-3 were first hydrolyzed with a solution of 4 N HCl for 30 minutes, then the filtrates were partitioned with ethyl acetate (15 mL x 3) after that evaporated to dryness and re-dissolved in 96% ethanol to a final volume of 25 mL, solution D (S.D). The test was applied on 1 mL of S.D to which 3 mL of 96% ethanol (\( V/V \)), 0.2 mL 10% aluminium chloride (\( m/V \)), 0.2 mL of 1 mol/ L potassium acetate and 5.6 mL distilled water were added. A volume of 10 % (\( m/V \)) aluminum chloride was substituted by the same volume of distilled...
water in the blanks. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at $\lambda = 415$ nm.

**Statistical analysis**

The results of all experiments were expressed as the Mean ± standard deviation (SDOM) upon three independent trials.

**Results and discussion**

The total phenolic content in different *Salicaria* extracts was expressed as tannic acid equivalent using the standard curve equation:

$$Ab = A + B \times Conc. \quad (A = 0.0055; B = 0.2666); \quad r^2 = 0.9999$$

![Figure 1](image)

*Tannic acid standard curve*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Conc.% (mg/100 mL)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.400</td>
<td>0.1119</td>
</tr>
<tr>
<td>2</td>
<td>0.800</td>
<td>0.2178</td>
</tr>
<tr>
<td>3</td>
<td>1.200</td>
<td>0.3270</td>
</tr>
<tr>
<td>4</td>
<td>1.600</td>
<td>0.4321</td>
</tr>
<tr>
<td>5</td>
<td>2.000</td>
<td>0.5379</td>
</tr>
</tbody>
</table>

Then the final test sample concentration was obtained using the following formula:

$$(R \times DF \times V \times 100)/W$$
where R= result obtained from the standard curve equation; DF= dilution factor; V= volume of stock solution; 100= for 100 grams dried plant; W= weight of plant used throughout the experiment in (g).

Flavonols were estimated by means of Chang et al method using the same standard curve equation \( y = 0.0603 x + 0.0007, r^2 = 0.9997 \) and values were calculated as g % (g Quercetin per100g of dried plant material).

Modifications of the original Chang et al method were performed due to the presence of turbidity which was noted after the addition of potassium acetate to the unhydrolyzed test sample that could be attributed to a reaction between one or more extract constituents with the applied potassium acetate. Therefore, a hydrolysis process to the test sample was needed in order to exclude the interference of other plant chemicals with the applied reagents, thus the analysis was applied to the aglycone part of the extracts.

The quantities of each of the examined chemicals are represented in the table bellow.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic compounds percent expressed as tannin equivalent (g/100 g dried plant)</th>
<th>Tannins% (g/100g of dried plant)</th>
<th>Total flavonols percent expressed as quercetin equivalent (g/100 g dried plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-1</td>
<td>9.2 g % ± 0.22</td>
<td>3.062 g% ± 0.220</td>
<td>0.632 g % ± 0.012</td>
</tr>
<tr>
<td>E-2</td>
<td>17.5 % ± 0.111</td>
<td>6.153 g% ± 0.213</td>
<td>0.594 g % ± 0.142</td>
</tr>
<tr>
<td>E-3</td>
<td>14.0 g % ±0.031</td>
<td>7.579 g % ± 0.301</td>
<td>0.214 g % ± 0.223</td>
</tr>
</tbody>
</table>

The presented data indicates that the order of increasing phenolic content, expressed as tannic acid equivalent (g) per gram of dry plant was: 70% aqueous ethanol > water > ethanol. The addition of water to ethanol was found to increase the total phenolic recovery, since water allows the plant tissue to swell, allowing the solvent to better penetrate the sample matrix. It is clear from the results obtained that a mixture of ethanol: water in a ratio of 70:30 (v/v) evoked the best results.

An interesting difference in tannin content was noted from the above data showing the independency of tannins percentage as related to the total phenolic content. This may be attributed to the fact that alcohol solvents have a harmful effect on the recovery of hydrolysable tannins (represented by ellagitannins in Salicaria extracts). Viriot et al. found that ellagitannins undergo hydrolysis to give ellagic acid in aqueous ethanol solution [23], where as Puech and coworkers demonstrated that the hydrolysis of ellagitannins increase with the ethanol concentration [24].
They also found that hydrolysis was not the only degradation process associated with ellagitannins in aqueous ethanol solutions. Oxidation also occurs, giving ellagitannins derivatives containing ethoxy groups [24]. Methanol was also found to cleave ester bonds in gallotannins at room temperature through methanolysis, suggesting that methanol was also not a suitable solvent for extracting ellagitannins [25].

The aqueous extracts show a higher percent of recovery of tannins than the other examined solvents.

**Conclusions**

This study assured that *Lythrum salicaria* extracted with 70% ethanol solution showed the highest percent of total phenolic compound, whereas the maximum tannin percent was obtained in aqueous extracts of this plant.

**References**

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