New Extraction Technologies for *Syringa Vulgaris (Oleaceae)* Meristematic Extracts

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The use of meristematic tissues for the production of health-related extracts (meristemotherapy) is coded by the european pharmacopoeia. However, traditional macerated glycerol requires long production times, based on classical extraction by diffusion. The use of a Dynamic Extraction Accelerator (Naviglio® Extractor) allowed for extraction in significantly faster (6 h) times compared to the conventional ones for the preparation of a macerated glycerol (21 days). The quantitative results of extracting Syringa vulgaris meristematic tissues were measured in terms of the amount of total extracted polyphenols and total extracted flavonoids. The biological activity related to the inhibition of the pancreatic lipase enzyme was compared and, as expected, looking phytochemical composition, the extract IC_{50} in Naviglio® Extractor was significantly lower than that of macerated glycerol.

Keywords: meristematic, gemmotherapic, lipase pancreatic, total phenolic

Syringa spp. are scented woody angiosperms belonging to the *Oleaceae* family, represented by 27 genera and 400 species around the world, extremely used in traditional medicine and in food and cosmetology in America, Europe and Asia [1]. Among the species of particular interest in Europe, there is *S.vulgaris*, commonly known as Lilac, grown as an ornamental plant which in late spring produces large corymbs at the apex, consisting of small star-shaped flowers, lilac color, slightly fragrant. Phytochemical studies related to the phenolic profile of the bark, the leaves and flowers [2] suggest biological effect, so this plant was considered by the scientific community, very interesting in the field of herbal medicine for its pharmacological effects [3]. The flowers are extremely used in traditional medicine as antipyretic [3], while the bark, leaves and buds are considered immunomodulatory, so used to relieve pain caused by osteoarthropathy or arthritis, gout [3,4,5] and in diabetes mellitus [6]. Studies on animal models suggest an anti-inflammatory protection in case of colitis, spinal cord trauma and in cases of periodontitis [7,8] also show an interesting hypotensive activity [9]. The biological activity of *Syringa vulgaris* is due to particular phytochemical compounds [10,11]: lignans, essential oils [10,11]; phenolic compounds, glycosides phenylethanoids, phenylpropanoids and oleuropein [11-13]. Modern pharmacological studies have demonstrated the bioactivity of these metabolites as anti-tumor, anti-hypertensive, anti-oxidant and anti-inflammatory activity [14]. While, phenylpropanoids acteoside and echinacoside, have been identified in *S.vulgaris* flowers and represent important active compounds in the regulation of neuroinflammation and related signals in Parkinson's disease, and may provide a new perspective for the clinical treatment [15,16].

Our aim is to compare three different methods of extracting of *Syringa vulgaris*'s buds, which represent an interesting reserve of undifferentiated embryonic stem cells (meristems). For this purpose we have used: macerated glycerol obtained according to French pharmacopoeia guidelines, Extractor Naviglio and multi-flower honey. The comparison was obtained through the study of some phytochemical parameters as well as the related biological activity.

There are three different samples: glycerine- alcohol macerate, in ethanol/ water 50:50 and th honey extract. The sample in ethanol/ water 50:50 is obtained with an experimental extraction procedure that allows a distinction between the different active substances, through many cycles of pression and depression on the extracting solvent. Main advantages of Extractor Naviglio ® are: a fast total exctraction of the substances which can be extracted, the operating temperature is ambient temperature and also the possibility to achieve the reproducibility of the extraction. And therefore, we have standardised extracts with the same content of active substances. These properties allow the production of good-quality extracts. No procedure of solid-liquid extraction, currently in existence, can give all of these benefits.

Experimental part

Plant material and chemicals

Syringa vulgaris buds was manually harvested from Macea county, Arad departement, Romania, in march 2016 from Pavel Covaciu Bothanical Garden, from a non polluated area. A sample of the vegetal productwas mantained in the Pharmacognosy Departement, Faculty of Pharmacy, Vasile Goldis Western University of Arad.

Samples extraction

S.vulgaris' s buds extraction was made with three different method and solvents:

a) 20 g of buds in glycerin: ethanol (1:10:10) . Fresh buds collected are left to macerate 5 days in ethanol and then, another 21 days in a mixture 1:1 v/v of water and glycerin.

After 21 days the buds are decanted and filtered, the residue is squeezed and left to rest for 48 hours, then filtered again before being diluted (1:10) with a new mixture of

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water-alcohol and glycerin.

b) 40 g of buds in multi-flower honey (1:20), 21 days

c) 10 g of buds in ethanol and water 50:50 with Extractor Naviglio®: It has required a complete 3,20-h cycle, then leaving the extract in maceration overnight in 2L of ethanol-water solution. The solvent has been removed through the use of a rotary evaporator, which in condition of low pression, promotes the lowering of the boiling temperature of the solvent, so its evaporation. To preserve the chemical composition of the extract we have used a rotary evaporator with a water-bath at 35°C.

Each cycle works in two phases: dynamic and static phase. In the dynamic phase, pistons pressings on the plant material 30 times, while the static phase last 10 min for a total of 13 min per cycle that repeats 20 times.

Then, the yield has been determined as a percentage of extraction, through the following formula:

Yield (%)= [(extract weight (g) / fresh weight (g))]*100

Phytochemical tests

Determination total poliphenol content

0,75 mL of Folin-Ciocalteau have been added to our extract (concentration of 1 mg/mL. The solution has been left to stand at 22°C and later mixed with a sodium hydrogen carbonate solution. The samples have been left to stand for 90 min at 22°C and then with a spectrophotometer at 765 nm it has been measured the absorbance, using a blank solution as comparison.

Straight line calibration: $y = 0.0091x - 0.0155$, $R^2=0.999$

Determination total flavonoid content

The total flavonoid content of crude extract was determined on the same extracts used for total phenols determination by the AlCl3 colorimetric method [17]. In brief, 1 mL of EtOH was added to 2 mg of crude extract. After 5 min of incubation, 1 mL di 2% $AlCl_3$ aqueos solution was added and the mixture was allowed to stand for 15 min. The calibration curve was determined with eight standard concentrations, ranging from 25 to 900μg/mL. The absorbance was measured at 430 nm. Total flavonoid content was expressed as mg quercetin equivalent (QE) per g of fresh material (FW).

Biological tests

DPPH Assay

Antioxidant properties The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay was adapted from Marrelli and others [18]. In an ethanol solution of 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical (final concentration = 1.0×10^{-4} M), extracts at different concentrations were added. The reaction mixtures were shaken vigorously and then kept in the dark for 30 min. The absorbance of the resulting solutions was measured at 517 nm, against a blank with DPPH. Decreasing absorbance values of the DPPH solutions indicated an increase of DPPH radical scavenging activity. The DPPH solution without sample solution was used as control. Ascorbic acid was used as positive control. This activity was given as % DPPH radical scavenging, calculated by the following equation: % DPPH radical scavenging = (absorbance control - absorbance sample)/ absorbance control × 100.

Pancreatic lipase activity

A water solution (3 mg/mL) was prepared from type II crude porcine pancreatic. Then a 7.5 mmol/L solution of 4-nitrophenyl octanoate (NPO) in dimethyl sulfoxide was prepared. The composition of the reaction mixture was the following: 100 μL of 7.5 mmol/L NPO, 4 mL of Tris-HCl buffer (*p*H = 8.5), 100 μL of extract (5; 3.5; 2; 1; 0.8; 0.6; 0.3; 0.1; 0.05; 0.025; 0.0125; 0.00625 mL) and 100 μL of enzyme solution. The mixture was incubated at 37°C. In the control, the extract was replaced with the same volume of dimethyl sulfoxide (DMSO). The absorbance was measured at 412 nm. A blank sample without the enzyme was prepared for each extract. Orlistat was used for comparison.

α – amylase activity

The inhibition of the enzyme α-amylase was evaluated using a methodology described by Casacchia et al (19).

100 μL of a solution with different concentrations (5; 3.5; 2; 1; 0.8; 0.6; 0.3; 0.1; 0.05; 0.025; 0.0125; 0.00625 mg/mL) was added to 500 μL of 0.5 mg/mL enzyme solution in cold distilled water and to 500 L of 1% (w/v) starch solution in 0.01 M phosphate buffer at *p*H 7.0. The reaction mixture was incubated at 37°C for 5 min; the reaction was stopped after the addition of 1 mL of the reagent dye DNS (3,5-dinitrosalicylic acid and 1% potassium sodium tartrate in 2% NaOH 0.4 M). The reaction mixture was incubated at 100°C for 5 min and the absorbance measured at 540 nm.

Statistical analysis

Data were analyzed using SPSS r.11.0.0 statistical software (SPSS, Inc., Chicago, IL, USA). All measurements were carried out in replicates (n=5). Significant differences were calculated at $P\leq 0.05$ level among means by one-way ANOVA, using Tukey's test. The values of IC50 (half maximal inhibitory concentration) for each measured parameter was calculated by means of scatter charts (where the X-axis indicates the concentration and the Y axis is the % activity or % inhibition). Trend lines were plotted and IC50 calculated by a linear trendline ($Y = a X + b$) by the formula $IC50 = (0.5 – b) / a$.

Results and discussions

Our purpose was to compare several extraction techniques in order to improve what can be considered the rate determining step of making a meristematic extract.

For this purpose, our results have allowed us to evaluate how the realization of a hydroalcohol extract, obtained through a dynamic extraction accelerator (Extractor Naviglio) represents an improvement of the technique both in quantitative and in terms of time. In fact, a total polyphenolic content of approximately 11.41mg / g (CAE) was obtained with the technique employed, compared with 8.4mg / g (CAE) obtained through the conventional GM (table 1).

The quality of the extractive method is also related to the portion of extracted flavonoids: there is statistically significant difference between the extract extracted from the traditional method compared to the innovative method (table 1).

It should be noted that macerated glycerol was obtained by maceration of the vegetable matrix in ethanol:glycerol in a ratio of 1: 1 (for the requested period of time by the French pharmacopoeia since 1965), while the procedure established for an extraction in Naviglio requires 3.2 h for each extraction, and for a total of two.

These values are definitely consistent with the antioxidant activity found if the higher polyphenolic concentration of the extract in Naviglio corresponds to an IC 50 (μg / mL) lower than that found for the macerated glycerol extract (table 1).

These results can be related to the innovative process adopted, where the forced extraction is induced by generation of a negative pressure gradient between the

Table 1

TOTAL POLYPHENOL AND FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY EVALUATED BY DPPH METHOD OF *SYRINGA VULGARIS* EXTRACTS

EXTRACT	Total polyphenols (mg CAE / g FW)	Total flavonoids (mg QE / g FW)	DPPH (IC_{50}) [a] (µg/mL)
Naviglio ®	11.41±0.0045 [a]	0,285± 0.02[a]	63.59 ± 1.09 [a]
Glicerinic Extract	8,407 ± 0.005[b]	0,237± 0.010 [b]	72.25 ± 2.61[b]
Syringa in honey	3,132 ± 0.007 [c]	0.285± 0.008[a]	132.63 ±2.14[c]
Honey	1,923 ± 0.010[d]	0.03± 0.016 [c]	178.61±1.04 [d]

Total phenolics are expressed as chlorogenic acid equivalents (CAE) per g of fresh material (FW).Total flavonoids are expressed as quercetin equivalents (QE) per g of fresh material (FW).Means (n = 5) ± SD with different letters within the same column are significantly different at p< 0.05. Positive reference DPPH: ascorbic acid.

Table 2

LIPASE AND α-AMYLASE INHIBITORY ACTIVITY OF *SYRINGA VULGARIS* EXTRACT

Extract	lipase IC_{50} (µg/mL)	α –amylase IC_{50} (µg/mL)
Naviglio	32,32 ± 0.02 [a]	104,37 ± 0.10 [a]
Glicerinic Extract	37,14 ± 0.12 [b]	185,67 ± 0.18 [b]
Syringa Honey	344,7 ± 0.72 [c]	232,43 ± 0.02 [c]
Honey	349,1 ± 0.81 [c]	362,11 ± 0.14 [d]

Orlistat IC_{50} (positive control) = 22,11 µg/mL; Acarbose IC_{50} (positive control) = 27,31 µg/mL.
Means (n = 5) ± SD with different letters within the same column are significantly different at p < 0.05.

inside and outside of the solid matrix containing the compounds to extract.

This principle allows quicker and more efficient extraction even at ambient and sub-ambient temperature because there is no diffusion effect as in the solid-liquid extraction techniques.

Polyphenols in the matrix have a structure compatible with possible uses in enzymatic inhibition, particularly pancreatic lipase. The results demonstrate, in fact, the significant inhibitory effect of the lipase enzyme whose IC50 is consistent not only with the plenty of polyphenols and total flavonoids, but also show a significant inhibition according to the standard used (table 2).

There is no significant inhibition of the alpha amylase enzyme (table 2).

Conclusions

In conclusion, the extract from *Syringa vulgaris*'s meristems is indicated for health purposes (to control hypercholesterolemia) in traditional gemmotherapy formulations, but also in innovative phytotherapeutic formulations (supplements and enriched foods).

For this purpose, all the study was also conducted using honey as extracting matrix, which hasn't been characterised by significant concentrations in polyphenols and total flavonoids, and consequently desired/ related biological activity.

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