Optimization of extraction of antioxidant components from Yarrow herb

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Abstract

Recently, research efforts have been directed toward medicinal plants and their extracts, as important sources of natural antioxidant. Lots of biologically active compounds are responsible for the antioxidant effects of yarrow – *Achillea millefilium* L. extracts. The aim of our study was to determine which of the process parameters of pressure enhanced solvent extraction of *Millefolii herba* is significant for its efficiency and weather there are interactions between the examined parameters. Compression time, decompression time and the number of cycles were identified as independent variables, while the content of total flavonoids, tannins and total polyphenols were selected as dependent variables. For obtaining the extract of *M. herba*, rich in antioxidative ingredients, compression time should be set on its higher level (2.0 min), decompression time on its lower level (1.30 min) and the number of cycles on its higher level (99).

Keywords: timatic micro-extractor, Yarrow, optimization, polyphenols, tannins, flavonoids.

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The functional food designates all sorts of food containing, along with their own nutritive value, constituents that may have positive effects on human health and its psychophysical condition. Modern community is changing the concept of foods. The aim of science, today, is development of such food products, which assume prevention and simultaneous reduction of risks of appearance of different diseases [1]. Oxidation reactions and the decomposition of oxidation products are major causes of deterioration of various food products. To prevent these processes, antioxidants are widely used as additives in some foods. Owing to increased safety concerns about synthetic antioxidants and their possible involvement in chronic diseases, research efforts have been directed toward natural antioxidants [2]. Medicinal plants and their extracts constitute one of the most important targets to search for new sources of natural antioxidants for consideration as components for functional ingredients and nutraceuticals, as well as feasible and natural alternatives to synthetic antioxidants in the food industry. Since plant-derived antioxidants are generally considered to be multifunctional and their activity depends on various parameters, any herb or its extract should be thoroughly tested involving several methods of assessing antioxidant activity [3].

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The genus *Achillea* L. (Yarrow) comprises over 100 perennial herb species indigenous to the Northern Hemisphere [4]. In Serbia, the *Achillea millefolium* L., the best-known and most widespread species of Yarrow, is listed among the most commonly used plant species in both folk and conventional medicine [5]. Yarrow is primarily a bitter tonic, but it can also be used to treat atonic forms of stomach disease [6]. Aerial part of *A. millefolium* contains terpenes, alkaloids and bases, tannins, coumarins, saponins, sterols, vitamins, amino and fatty acids [6,7]. Phenolic compounds, such as flavonoids and phenolcarbonic acids, constitute one of the most important groups of pharmacologically active principles in yarrow. It is suggested that anti-inflammatory [6], choleretic [8] and cytotoxic [9] activities of species of *Achillea* genus are mainly attributed to the flavonoid and phenolcarbonic acid complex. It has been shown that the anti-diabetic [10] and gastroprotective [11] properties of different extracts from *Achillea* sp. may be linked to their antioxidant potential. Therefore, it is of high importance to investigate their antioxidant effectiveness. Recent reports indicate that the *Achillea* genus displays a relevant antioxidant activity that is associated or correlated well with its flavonoid and total phenolic contents [12,13]. However, the observed similarities or close correlation between the profiles of the antioxidant capacity and of the total phenolic and flavonoid contents must be interpreted with care, since the latter parameter is usually measured using traditional spectrophotometric assays, which are based on non-specific reaction of phenolic compounds with Folin–Ciocalteu reagent and complexation of flavonoids with Al(III) ion [14].

The interest in the use of medicinal plants in food industry is continuously growing and accordingly the efforts in the improvement of the conventional solvent based extraction are made. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and improve the mass transfer [15]. For each device and/or each method of extraction, it is necessary to experimentally determine optimal conditions for making the extracts which are the richest in relevant active substances. Other researchers have presented the definition of optimal conditions for extraction of bioactive compounds (crocin, geniposide, total phenolic compounds, phenolic acids and proteins) using different extraction methods, different extractors and different optimization methodologies [15-17]. In the present study, Timatic micro-extractor was used for the extraction [18]. The aim of our study was to determine which of the process parameters of pressure enhanced solvent extraction of aerial parts of *A. millefolium* (*Millefolii herba)*, is significant for its efficiency and weather there are interactions between the examined parameters.

EXPERIMENTAL

Samples of *A. milefollum* (upper parts of the herb ~12 cm in length) were collected at full flowering stage, during the flowering period on the mountain Rtanj, Serbia (in the end of June 2009). A voucher specimen's No. 174/09 has been deposited at the Institute for Medicinal Plant Research "Dr Josif Pančić". The raw material was air dried at room temperature (20–25 °C), in a ventilated lodge, avoiding direct sunlight for two weeks. Dry material was packed into paper bags and stored in a dark room at ambient temperature. The air-dried aerial parts of *A. millefolium* were milled at room temperature and sieved using a lab sieve with 355 μm mesh.

All chemicals were of analytical grade. Folin–Ciocalteu reagent was purchased from Merck (Germany), gallic acid from Sigma–Aldrich Chemie GmbH (Germany). Acetonitrile, labelled as HPLC grade, was supplied by Sigma–Aldrich (Buchs, Switzerland). Ethanol 96.3% (*V*/*V*) was provided by Crvenka, Serbia.

For the pressure enhanced solvent extraction we used Timatic micro-extractor (Tecnolab, Spello, PG, Italy) [18]. The rapid series extractors Timatic are technologically advanced and dedicated to the extraction of active ingredients from medicinal plants. This is an alternative method for extraction in comparison to traditional methods, such as maceration or percolation. The extraction was carried out under controlled conditions (defined by temperature, pressure and time of solvent circulation). Plant material was filled into the filter bag, which was sealed and placed into the extraction chamber. Appropriate solvent is circulating through the filter bag and can be controlled by the speed of circulation and the number of cycles during the extraction process. The working cycle is fully automatic and alternates between dynamic and static phases. During the extraction, plant material is compacted under pressure, which allows better extraction efficiency. With respect to our preliminary research (data not shown), fixed amounts of the drug and 70% (*V*/*V*) ethanol, as an extraction solvent were used. The drug: extract ratio was 1:3 and the operating pressure was set between 1 and 9.9 bar. A 2^3 factorial design consisting of eight experimental points was applied to evaluate the effect of three process parameters as independent variables, namely compression time (*TP1*), decompression time (*TP0*) and the number of cycles (*CL*) on the extraction efficiency in terms of obtaining the extract with the best properties. Prepared extracts were marked as YE_1–YE_8. Characterization of extracts included determination of physicochemical characteristics: dry residue (*DR*), refractive index (*RI*), relative density (*RD*), ethanol content (*EC*) and pH value as well as determining the content of total flavonoids (*TF*), tannins (*TN*) and polyphenols (*PY*) as active compounds, important for the extract quality definition.

The total content of phenols was determined by the Folin–Ciocalteu method. A total of 100 μl of a methanolic solution of dry extract (17.5, 13.1, and 8.8 μg ml^{-1} final quantity) was mixed with 0.75 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min; 0.75 ml of sodium bicarbonate (60 g l^{-1}) solution was added to the mixture. After 90 min at 22 °C, the absorbance was measured using a Hewlett Packard 8453 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) at λ_{max} 725 nm. Results are expressed as gallic acid equivalents (GAE), and presented as mean value of three determinations.

The percentage content of tannins was calculated using the method described in the European Pharmacopoeia [19]. The content of tannins, expressed as pyrogallol percentage, is presented as the mean value of three determinations.

The percentage content of flavonoids expressed as hyperoside was calculated using the method described in the Deutsches Arzneibuch, DAB 10 [20]. Briefly, the sample was extracted with acetone/HCl under a reflux condenser; the $AlCl_3$ complex of the flavonoid fraction was extracted with ethyl acetate and measured by a UV–Vis spectrophotometer at λ_{max} 425 nm. The content of flavonoid, expressed as the hyperoside percentage, is presented as the mean value of three determinations.

Statistical analysis

The statistical analysis of experimental results was performed by the software Design Expert 7.0 (Stat-Ease, Inc., Minneapolis, MN, USA) [21].

RESULTS AND DISCUSSION

Independent variables are presented in Table 1. The conditions of making YE_1–YE_8 extracts are presented in Table 2. Dependent variables were dry residue (*DR*), refractive index (*RI*), relative density (*RD*), ethanol content (*EC*), pH value, content of total flavonoids (*TF*), tannins (*TN*) and polyphenols (*PY*).

Table 1. Process parameters/independent variables

Factor	Level	
	–1	+1
X1 (*TP1*) compression time, min	1.00	2.00
X2 (*TP0*) decompression time, min	1.30	2.00
X3 (*CL*) number of cycles	20	99

Table 2. The conditions of making YE_1–YE_8 extracts

Sample of YE	*TP1* / min	*TP0* / min	*CL*
YE_1	1.00	1.30	20
YE_2	2.00	1.30	20
YE_3	1.00	2.00	20
YE_4	2.00	2.00	20
YE_5	1.00	1.30	99
YE_6	2.00	1.30	99
YE_7	1.00	2.00	99
YE_8	2.00	2.00	99

Various extraction conditions, resulted in some differences in the physicochemical characteristic of the manufactured extracts and different contents of total phenols, flavonoids and tannins (Table 3). Value of dry residue of manufactured extracts varied within the limits from 2.17 to 3.86. Dry residue content depended on the number of cycles. Increasing the number of cycles led to an increase in the value of dry residue (the maximum value of *DR* was registered in YE_5). The same extract had the highest value of relative density. There was no significant difference in the refractive index and pH values between manufactured extracts. Ethanol content in extracts YE_1–YE_8 varied within the limits from 63.28 (YE_1) to 69.88 (YE_3) and did not depend on the number of cycles.

Statistical analysis of the experimental data indicated that all three examined process parameters generally have the greatest impact on *DR*, while individually, *TP1* influences mostly *TF* and *RI*, *TP0*, *TF* and *RD*, and *CL*, *DR*, pH, *TN* and *PY*. When considering active principle content, it may be noted that *TP1* and *TP0* have the biggest influence on *TF* (except that *TP1* has a positive, and *TP0* negative effect on *TF*), while *CL* on *TN* (positive effect) and *PY* (negative effect). Comparing active principle contents among themselves, the greatest impact of all three process parameters is on *PY*. Nevertheless, interactions observed between *TP1* and *TP0* indicate that with *TP0* set on its lower value (1.30 min) the increase of *TP1* increases *TN* and *PY*. Taking all of this in consideration, it can be concluded that for obtaining the extract of *A. millefolium* rich in active ingredients, *TP1* should be set on its higher level (2.0 min), *TP0* on its lower level (1.30 min) and *CL* on its higher level (99).

Since the selected responses were not affected in the same manner an additional optimization procedure was needed. In order to optimize eight responses with different targets, the multicriteria methodology was employed by means of Derringer's desirability function [22–24].

The Derringer's desirability function, *D*, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions (d_i). The expression that defines the Derringer's desirability function is:

$$D = \sqrt[n]{\prod_{i=1}^{n} d_i^{p^i}} \quad (1)$$

where n is the number of responses, p^i is the weight of the responses and d_i is the individual desirability function of each response. The scale of the individual desirability function ranges between 0 for a completely undesired response, to 1 for a fully desired response.

Table 3. Physicochemical characteristics and contents of total phenols, flavonoids and tannins of YE_1–YE_8

Sample of YE	*DR* / %	*RI*	pH	*RD* / g cm^{-3}	*EC* / vol.%	*TF* / %	*TN* / %	*PY* / μg GA mg^{-1}
YE_1	2.31	1.3647	5.59	0.9098	63.28	0.0216	0.077	0.23
YE_2	2.50	1.3676	5.69	0,8999	67.64	0.0206	0.094	0.27
YE_3	2.52	1.3674	5.64	0.8993	69.88	0.0230	0.115	0.27
YE_4	2.17	1.3662	5.64	0.9016	65.44	0.0200	0.076	0.24
YE_5	3.86	1.3667	5.44	0.9292	66.84	0.0310	0.108	0.20
YE_6	3.61	1.3667	5.49	0.9060	66.20	0.0290	0.094	0.26
YE_7	3.27	1.3667	5.48	0.9065	65,44	0.0220	0.102	0.18
YE_8	3.15	1.3679	5.62	0.9031	67.64	0.0326	0.108	0.18

Weight of the response is the relative importance of each individual functions d_i and may range from 0.1 to 10. Weights lower than 1 give less emphasis to the goal, whereas weights greater than 1 give more emphasis to the goal (in both cases, d_i varies in a non-linear way while approaching to the desired value). In the present report, weights equal to 1 for all the eight responses was chosen.

The value of *D* close to 1 means that the combination of different criteria is globally optimal. If any of the responses or factors falls outside their desirability range, the overall function becomes zero.

The criteria for the optimization of each individual response are shown in Table 4. Desirability function calculations were performed using Design-Expert® 7.0. Obtained results are graphically presented (Figure 1). For better visualization of the results, the global desirability function, *D*, was presented in a form of a three-dimensional plot and presented in Figure 2. The coordinates related to the functions maximum are selected as the best operating conditions. The coordinates producing the maximum desirability value (*D* = 0.846) were: compression time (*TP1*), 2.00 min; decompression time (*TP0*), 1.30 min; number of cycles (*CL*), 99.0.

CONCLUSION

In the present study, Timatic micro-extractor was used for the extraction of yarrow and it may be concluded that various extraction conditions result in some differences in the physicochemical characteristic of the manufactured extracts and content of active substances. When considering active principle content with

Table 4. Criteria for multivariate optimization of the individual responses

Response	Goal	Weight	Lower limit	Upper limit	Importance
TF	Maximize	1	0.02	0.0326	5
DR	In range	1	2.17	3.86	3
RI	In range	1	1.3647	1.3679	3
pH	In range	1	5.44	5.69	3
RD	In range	1	0.899	0.9292	3
EC	In range	1	53.28	69.88	3
TN	Maximize	1	0.076	0.115	5
PY	Maximize	1	0.18	0.27	5

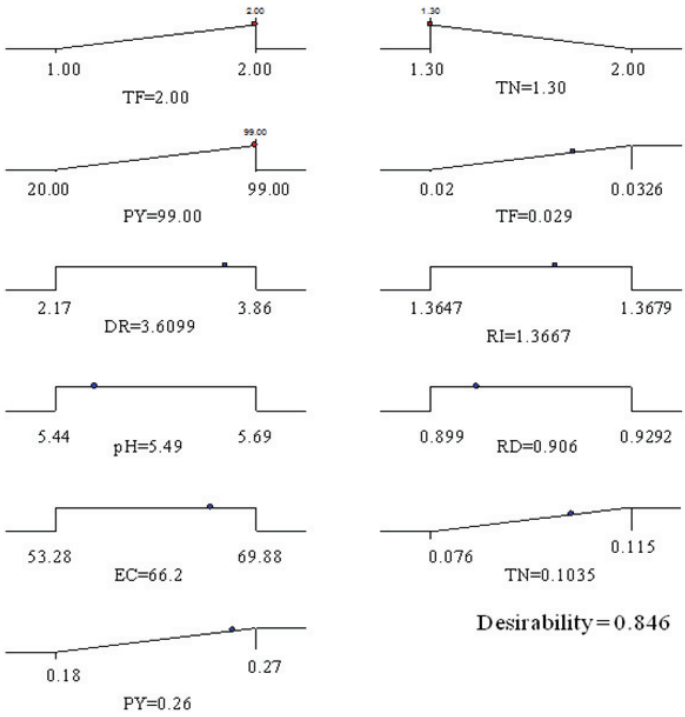

Figure 1. Graphical representation of the constraints accepted for the determination of global desirability and obtained optimal conditions.

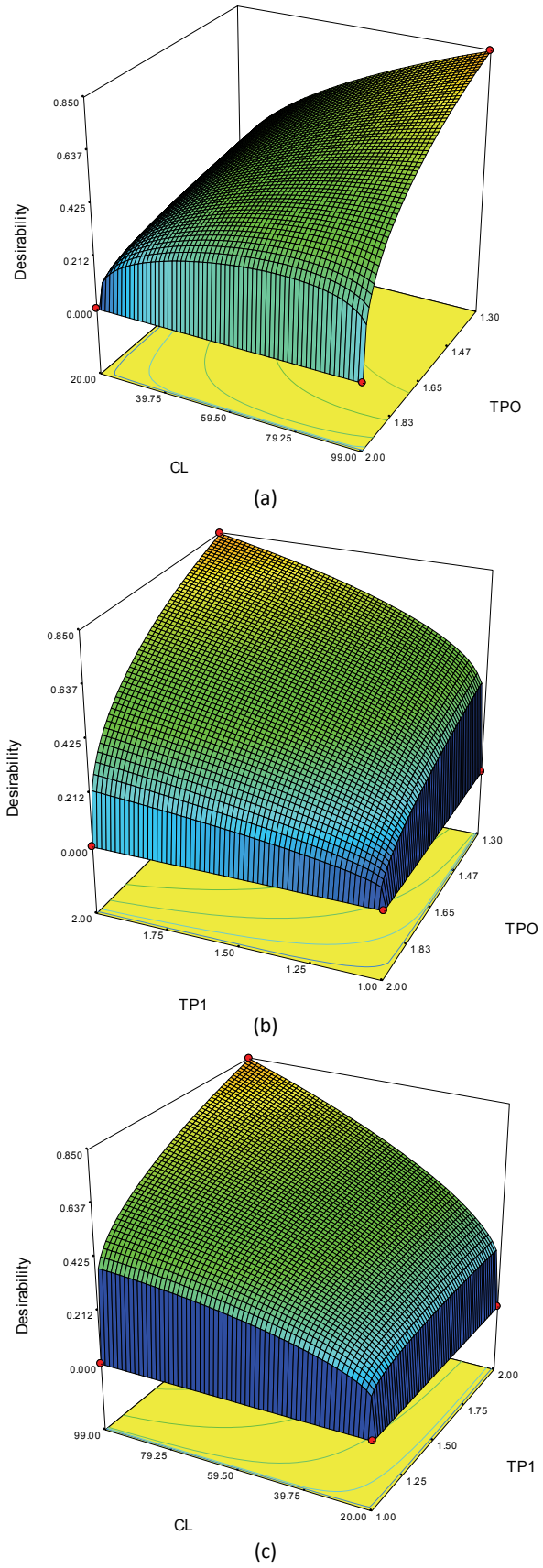

Figure 2. 3-D plots of the Derringer's desirability function in correlation with number of cycles and decompression time (a), decompression time and compression time (b) and compression time and number of cycles (c).

antioxidative effects (total phenols, flavonoids and tannins content), it may be noted that *TP1* and *TP0* have the biggest influence on *TF* (except that *TP1* has a positive, and *TP0* negative effect on *TF*), while *CL* on *TN* (positive effect) and *PY* (negative effect). Taking all of this in consideration, it can be concluded that using Timatic micro-extractor, for obtaining the extract of *A. millefolium* rich in active ingredients, *TP1* should be set on its higher level (2.0 min), *TP0* on its lower level (1.30 min) and *CL* on its higher level (99).

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IZVOD

OPTIMIZACIJA EKSTRAKCIJE ANTIOKSIDANTNIH KOMPONENATA IZ HERBE HAJDUČKE TRAVE

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(Naučni rad)

Oksidativna degradacija sastojaka hrane dovodi do promene njenog mirisa i ukusa, čime se narušava njen nutritivni i senzorni kvalitet. Iz navedenog razloga je neophodno dodavati antioksidante tokom tehnološkog procesa izrade namirnica. Lekovito bilje i njihovi izolati predmet su istraživanja, pored ostalog i kao izvor antioksidantnih materija i alternativa sintetičkim antioksidansima za primenu u industriji hrane. Istraživanja velikog broja biljaka ukazala su da skoro sve poseduju određena antioksidativna svojstava. Hajdučka trava – *Achillea millefilium* L. (Asteraceae) i njeni ekstrakti, zahvaljujući prisustvu, pre svega, polifenola, flavonoida i tanina, ispoljavaju antioksidativni efekat. Sadržaj ovih materija u izolatima zavisi od njihove koncentracije u polaznom biljnom materijalu ali i od načina/uslova ekstrakcije. Cilj našeg ispitivanja bio je da utvrdimo optimalne vrednosti procesnih parametara za izvođenje pritiskom ubrzane ekstrakcije iz herbe hajdučke trave (*Millefolii herba*), radi dobijanja ekstrakata bogatih antioksidativnim materijama, kao i da utvrdimo zavisnost koja postoji između procesnih parametara. Ekstrakcija je obavljena u Timatic mikro ekstraktoru. Takozvano vreme kompresije, vreme dekompresije i broj ciklusa kruženja ekstrakcionog sredstva predstavljaju nezavisno promenljive veličine, dok su sadržaj ukupnih flavonoida, tanina, ukupnih polifenola kao i indeks refrakcije, suvi ostatak, relativna gustina, sadržaj etanola i pH vrednost zavisno promenljive veličine. Vreme kompresije bilo je 1 odnosno 2 minuta, vrednost vremena dekompresije 1,3 i 2 min, dok su ekstrakcije obavljene uz minimalno 20 odnosno maksimalno 99 ciklusa. Kao ekstragens korišćen je 70% (*V*/*V*) etanol. Droga: ekstrakt odnos iznosio je 1:3. Radni pritisak kretao se u opsegu od 1 do 99 bar. Primenjen je 2^3 faktorski dizajn, odnosno urađeno je osam ekstrakcija herbe hajdučke trave. Statistička analiza eksperimentalnih rezultata izvršena je upotrebom softvera *Design Expert* 7.0. U cilju potpune optimizacije primenjena je multivarijantna metodologija upotrebom *Derringer* funkcije poželjnih odgovora. Proces optimizacije postupka ekstrakcije pokazao je da je za izradu ekstrakata herbe hajdučke trave bogatih sadržajem antioksidativnih materija (flavonoidi, tanini i polifenoli), potrebno podesiti vreme kompresije na najviši nivo (2,0 min), vreme dekompresije na najniži (1,30 min) i vršiti ekstrakciju uz maksimalni broj ciklusa (99).

Ključne reči: *Timatic* mikroekstraktor • Hajdučka trava • Optimizacija • Polifenoli • Tanini • Flavonoidi