ORIGINAL ARTICLE

WASTES RESULTING FROM AROMATIC PLANTS DISTILLATION – BIO-SOURCES OF ANTIOXIDANTS AND PHENOLIC COMPOUNDS WITH BIOLOGICAL ACTIVE PRINCIPLES

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Abstract

We determined the total phenolic content and the antioxidant activity of hydro-alcoholic extracts obtained from waste materials resulted in the essential oil extraction process during thyme and oregano distillation. All extracts have shown high antioxidant activity that ranged between 18 to 47 mg (gallic acid equivalent) GAE/L, 1.4 to 4 mmol (Trolox equivalent antioxidant capacity) TEAC/L and a percent of inhibition: 36 - 93%. The total phenolic content determined by Folin-Ciocalteu method varied from 262 to 6926 mg GAE/L. The HPLC analysis revealed as major phenolic compounds: gallic, p-coumaric, caffeic, vanillic and syringic acids alongside rutin, quercetin, and pyrogallol. Gallic acid was detected within the range of 5.3 - 2175 mg/L and pyrocatechol within 12 - 2177 mg/L. Results of the study indicated that plant phenolic compounds (retrieved from waste materials resulted from thyme and oregano distillation) could be used as antioxidants in food products and pharmaceuticals.

Rezumat

S-a determinat conținutul total de fenoli și activitatea antioxidantă a extractelor hidroalcoolice obținute din deșeurile rezultate în procesul de extracție a uleiurilor esentiale, în timpul distilării plantelor de cimbru și oregano. Pentru toate extractele a rezultat o activitate antioxidantă ridicată, variind între 18 - 47 mg GAE/L, respectiv 1,4 - 4 mmol TEAC/L, precum și un procent de de inhibiíe de 36 - 93%. Conținutul total de fenoli determinat prin metoda Folin-Ciocalteu a variat între 262 - 6926 mg GAE/L. Analiza HPLC a evidențiat ca principali compuși fenolici următorii acizi: galic, p-cumaric, cafeic, vanilic și siringic, alături de rutină, quercetin și pirogalol. Concentrația acidului galic s-a încadrat în intervalul 5,3 - 2175 mg/L, iar a pirocatecolului între 12 - 2177 mg/L. Rezultatele studiului indică faptul că acești compuși fenolici din plante (recuperați din deșeurile rezultate prin distilarea plantelor de cimbru și oregano) pot fi utilizați ca antioxidanți în produse alimentare și produse farmaceutice.

Keywords: *Lamiaceae*, waste materials from distillation, antioxidants, phenolic compounds, waste valorisation

Introduction

Aromatic herbs and spices have made their way in our lives since ancient times, providing a diverse source of biologically active principles for food and medicinal purpose [3]. Their volatile oils and poly-phenolic content give rise to unaccounted properties such as aroma and flavour enhancers, perfumes, cosmetics and diverse anti-carcinogenic, anti-fungal, antiviral, diaphoretic, insecticidal, antimicrobial and antioxidant properties [1-5, 11, 13, 17, 20-27]. Their antioxidant properties have proved effective in retarding lipid peroxidation, making them suitable in the manufacture of edible films and other bio-active food packaging [18].

One of the most important medicinal plant families, rich in volatile oils and biologically active principles is *Lamiaceae,* which contribute with over 3000 species [17]. Within the *Lamiaceae* family, the *Thymus* genus (which comprises 215 species) is exceeded only by a few genera like *Salvia, Nepeta, Scatellaria,* etc.; meanwhile the *Satureja* and *Origanum* genus include over 30 species of herbs, shrubs and sub-shrubs widely distributed within the Mediterranean region [6, 9, 12-15, 24].

Volatile oils are organic compounds that can be obtained from all parts of the plant: flowers, roots, bark, leaves, seeds, peel, fruits, wood, and even whole plant [3, 20, 21]. Phenolic compounds are secondary metabolites that have an important role in plant

defence during stress conditions (biotic, abiotic, phenologic stages, crop management, nutrient deficiency) which can increase the production of free radicals and other reactive oxidative species in plants [1, 10, 25].

Due to the fact that around 80% of the human population in developing countries still rely on traditional medicine, and essential oils have many scientifically and biological uses, there is a lot of waste material resulted during distillation that has no specific purpose [7]. After volatile oils removal, wasted plant materials are still abundant in phenolic compounds, oligomers and flavonoid glycosides with diverse biologically active principles [7], therefore extraction of such compounds is important [16, 28].

The aim of this study was to determine the total phenolic content and antioxidant activity of hydro-alcoholic extracts obtained from waste materials resulted in the essential oils extraction process during thyme and oregano distillation.

Materials and Methods

Plant material

Herbal parts of *Origanum vulgare* var. *aureum, Thymus vulgaris,* var. *Doone Valley* and *Satureja hortensis* were gathered in the summer of 2017 when at least 50% of the flowers were in bloom and the volatile oil content reached its maximum. Aerial parts of *Origanum vulgare* var. *aureum* and *Thymus vulgaris,* var. *Doone Valley,* were harvested in late June from Lovrin Agricultural Research and Development Resort (20º 47' E longitude and 45º 57' N latitude), Lovrin commune; aerial parts of *Satureja hortensis* were harvested in late July from a local producer (21º 19' E longitude and 46º 9' N latitude), Arad county, Romania.

Harvested plant material was dried at 35ºC for 7 days using a drying oven (Model FD23, Binder, Germany) and voucher specimens were taken and stored for every plant taken into this study at the Institute of Technical and Natural Sciences Research-Development-Innovation of "Aurel Vlaicu" University of Arad, Romania.

Chemicals and reagents

Ethanol (96% purity) was purchased from Chemical Company S.A., Romania, and was further used as solvent for the preparation of the extracts. HPLC reference standards (rutin, quercetin, kaempferol, catechin, pyrogallol, pyrocatechol, p-coumaric acid, caffeic acid, vanillic acid, syringic acid, ascorbic acid and riboflavin) were purchased from Sigma-Aldrich (Germany) and acetonitrile (HPLC grade) was purchased from Merck (Germany). Other reagents and solvents were of analytical grade, obtained from Sigma-Aldrich (Germany) and Merck (Darmstadt, Germany).

Extraction method

Being a post-distillation extraction, the dried plant material had to undergo steam distillation using a copper Alembic distillation equipment for volatile oils removal. The deodorized waste material (W1) was dried at 70ºC for 72 h using the drying oven (Model FD23, Binder, Germany).

The extracts were prepared by using 1:10 w/v grinded W1 in ethanol solution of varied concentrations (0, 40, 60, 80 and 96%, respectively) and by using three methods of extraction: (i) Soxhlet extraction for 2 h and 30 minutes, (ii) static maceration for 7 days in a refrigerator at +4ºC, and (iii) ultrasonic extraction at 35 kHz, 100% power for 2 h and 30 min using an Elmasonic TI-H5 (Elma, Schmidbauer GmbH, Germany) followed by 7 days static maceration in a refrigerator at +4ºC. All extracts were filtered twice, first time using a MN 615 ff ¼ Ø 150 mm paper filter, followed by a secondary filtration using 25 mm Syringe filters, 0.45 µm PTFE membrane.

Chemical composition

Total phenolic content. The total amount of phenolic compounds for each extract was determined spectro-photometrically using Folin-Ciocalteu (FC) reagent [19]. Total phenolics were determined and expressed as mg gallic acid equivalents (GAE/L) using a standard curve as reference.

The extracts to be analysed were diluted with distilled water (1:25). Afterwards, in a 10 mL volumetric flask, 1 mL of sample was added, and to that 0.5 mL of Folin-Ciocalteu reagent alongside 2 mL Na_2CO_3 (20%) and 5 mL distilled water. After 90 minutes of incubation at room temperature in the dark, the absorbance was measured at λ = 765 nm using a UV-VIS double beam spectrophotometer Specord 200 (Analytik Jena AG, Germany), using a 10 mm quartz cuvette. The standard reference curve for Gallic acid had the following concentrations: 0, 20, 40, 100, 160 and 200 mg/L, respectively. The regression equation and correlation coefficient were calculated and expressed in mg GAE/L. All measurements were performed in triplicates.

1,1-Diphenyl-2-picrylhydrazyl radical scavenging assay. The extracts ability to scavenge the nitrogen atom from 1,1-diphenyl-2-picrylhidrazyl radical (DPPH•) was evaluated spectrophotometrically as reported in [19]. The DPPH• radical was dissolved in ethanol (0.2 mM). 0.1 mL (20 mg/mL) of sample was mixed with 3 mL DPPH• solution. The spectrophotometric readings were carried out after 60 minutes of incubation in the dark at room temperature, by using a UV-VIS double beam spectrophotometer Specord 200 from Analytik Jena (Germany) at λ = 517 nm using a 10 mm quartz cuvette. As reference, positive controls containing 2.5 - 50 mg/L Gallic acid and 0.02 - 4.00 mM Trolox, respectively, were prepared in ethanol. The data were expressed in mg GAE/L and

mmol TEAC/L, respectively. The percentage of DPPH• inhibition was calculated with Eq. (1):

$$\%Inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} x100 \quad (1)$$

where: *Abs control* - the absorbance of 0.2 mM DPPH• in ethanol; *Abs sample* - the absorbance of 0.2 mM DPPH• containing plant extract.

HPLC analysis of the extracts

HPLC analysis was performed using an ultra-high-performance liquid chromatograph (Nexera X2, Shimadzu, Tokyo, Japan) equipped with a diode array detector (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil 100-3-C18 reversed-phase column (4.0 mm i.d. x 125 mm column length, 3 µm particle size, Macherey-Nagel GmbH, Duren, Germany). The column temperature was maintained at 30ºC and the flow rate of 1 mL/min. The solvents used for the chromatographic elution consisted of ultra-pure water with 0.1% trifluoro-acetic acid (A) and acetonitrile (B). The chromatographic elution program used was as follows: 95% A and 5% B, then the linear gradient grew to 35% B and was maintained for 5 min, followed by a linear gradient of 42% B in 30 min. The injected volume of samples and standards was 10 µL and it was done automatically using an auto-sampler. The spectra were acquired in the wavelength range: 200 - 600 nm.

Results and Discussion

Chemical composition

In order to compare the chemical composition of all extracts obtained from waste materials resulted during distillation of three different species, namely *Origanum vulgare* var. *aureum, Thymus vulgaris,* var. *Doone Valley* and *Satureja hortensis*, the total phenolic content and antioxidant activity were determined in addition with HPLC analysis. In Table I are presented the procedures used to obtain the extracts and the abbreviations further used in the study.

Table I

Deodorized waste hydro-alcoholic extraction procedures used in the study and abbreviations

Abbreviations	Extraction procedure
***Origanum vulgare,* var. *aureum,* Lovrin, W1**	
OVAL W1 0 OVAL W1 40 OVAL W1 60 OVAL W1 80 OVAL W1 96	Soxhlet extraction for 150 minutes
OVAL W1 96 FUS	Static maceration for 7 days in a refrigerator at +4ºC.
OVAL W1 96 US	Ultrasonic extraction for 150 minutes followed by 7 days static maceration in a refrigerator at +4ºC
***Thymus vulgaris, var. Doone Valley*,Lovrin,W1**	
TDL W1 0 TDL W1 40 TDL W1 60 TDL W1 80 TDL W1 96	Soxhlet extraction for 2 h and 30 minutes
TDL W1 96 FUS	Static maceration for 7 days in a refrigerator at +4ºC.
TDL W1 96 US	Ultrasonic extraction for 150 minutes followed by 7 days static maceration in a refrigerator at +4ºC
***Satureja hortensis,* AMG W1**	
SH AMG W1 0 SH AMG W1 40 SH AMG W1 60 SH AMG W1 80 SH AMG W1 96	Soxhlet extraction for 150 minutes.
SH AMG W1 96 FUS	Static maceration for 7 days in a refrigerator at +4ºC
SH AMG W1 96 US	Ultrasonic extraction for 150 minutes followed by 7 days static maceration in a refrigerator at +4ºC

Total phenolic content

The total phenolic content examined from waste materials resulted from steam distillation varied from 262 (SH AMG W1 95% FUS) to 6926 (OVAL W1 0%) mg GAE/L (Table II). In our experiments the classic Soxhlet extraction lead to the best yields, followed by ultrasonic extraction (US) and then static maceration. A high temperature combined with stirring gives a better extraction yield compared to static extraction at lower temperatures. In case of ultrasonic extraction, although the cellular matrix was deteriorated, giving a better chance of contact with the solvent, the lack of high temperature reduces the extraction yield in some cases almost by half: 2057 and 1309 mg GAE/L for TDL W1 96 and TLD W1 96 US also 1590 and 875 mg GAE/L SH AMG W1 96 and SH AMG W1 96 US. Simple static maceration is time and space consuming resulting in a poor choice for obtaining a high total phenolic content.

In all cases, the 60% ethanol solution extraction gives the best results, being preferred also in the obtaining of valuable active compounds.

Table II

Total phenolic content (mg GAE/L) and antioxidant activity (inhibition %, mg GAE/L, mmol TEAC/L) of extracts from waste materials resulted after steam distillation

Sample	Total phenolic content	Antioxidant activity		
	mg GAE/L	Inhibition %	mg GAE/L	mmol TEAC/L
OVAL W1 0	6926 ± 66	79.781 ± 0.014	40.249 ± 0.007	3.4080 ± 0.0006
OVAL W1 40	6751 ± 61	80.323 ± 0.007	40.521 ± 0.004	3.4324 ± 0.0003
OVAL W1 60	6800 ± 24	80.286 ± 0.011	40.502 ± 0.005	3.4307 ± 0.0005
OVAL W1 80	6510 ± 34	89.220 ± 0.021	44.988 ± 0.011	3.8326 ± 0.0009
OVAL W1 96	4565 ± 6	92.874 ± 0.011	46.822 ± 0.005	3.9969 ± 0.0005
OVAL W1 96 US	3316 ± 0.8	93.557 ± 0.007	47.165 ± 0.004	4.0277 ± 0.0003
OVAL W1 96 FUS	555 ± 0.1	93.460 ± 0.004	47.116 ± 0.002	4.0233 ± 0.0002
TDL W1 0	4758 ± 1	87.151 ± 0.011	43.949 ± 0.005	3.7395 ± 0.0005
TDL W1 40	5093 ± 2	88.711 ± 0.004	44.732 ± 0.002	3.8097 ± 0.0002
TDL W1 60	5281 ± 0.5	86.652 ± 0.007	43.698 ± 0.004	3.7170 ± 0.0003
TDL W1 80	4605 ± 0.1	91.942 ± 0.007	46.354 ± 0.004	3.9550 ± 0.0003
TDL W1 96	2057 ± 0.4	92.144 ± 0.004	46.455 ± 0.002	3.9641 ± 0.0002
TDL W1 96 US	1309 ± 0.1	92.583 ± 0.014	46.676 ± 0.007	3.9839 ± 0.0006
TDL W1 96 FUS	489 ± 0.3	90.276 ± 0.011	45.517 ± 0.005	3.8801 ± 0.0005
SHAMG W1 0	2921 ± 0.3	81.947 ± 0.014	41.337 ± 0.007	3.5054 ± 0.0006
SHAMG W1 40	3287 ± 0.5	82.057 ± 0.014	41.392 ± 0.007	3.5103 ± 0.0006
SHAMG W1 60	3576 ± 1.6	83.271 ± 0.004	42.001 ± 0.002	3.5650 ± 0.0002
SHAMG W1 80	3022 ± 1	87.737 ± 0.004	44.243 ± 0.002	3.7659 ± 0.0002
SHAMG W1 96	1590 ± 0.7	86.396 ± 0.004	43.570 ± 0.002	3.7055 ± 0.0002
SHAMG W1 96 US	875 ± 0.3	86.232 ± 0.004	43.488 ± 0.002	3.6982 ± 0.0002
SHAMG W1 96 FUS	262 ± 0.1	36.319 ± 0.007	18.431 ± 0.004	1.4529 ± 0.0003

Antioxidant activity

The antioxidant effects of the extracts from waste materials resulted after steam distillation were evaluated using the free radical scavenging capacity (inhibition %) of DPPH•. The ability of these extracts to act as a proton or electron donor to stable DPPH• was measured and results are presented in Table II. The inhibition varied between 93.557 to 36.319%, as well as 47.165 to 18.431 mg GAE/L and 4.0277 to 1.4529 mmol TEAC/L for OVAL W1 96 US and SH AMG W1 96 FUS, respectively. This implies that little or no difference was observed between all three extraction methods regarding the antioxidant activity of thyme and oregano varieties used in this study, namely *Origanum vulgare* var. *aureum,* and *Thymus vulgaris,* var. *Doone Valley*. Meanwhile, in the case of *Satureja hortensis,* the antioxidant activity of the extracts obtained by using Soxhlet extraction and ultrasonic extraction was more than double then that determined for extracts attained by static maceration. All extracts from waste materials resulted after steam distillation expressed strong scavenging capacity, results comparable with previous published data [7, 28].

HPLC analysis

The extracts obtained from waste materials resulted after steam distillation are rich in polyphenols as it is shown in the quantitative data from the chromatographic analysis presented in Tables III and IV.

Table III

HPLC analysis results (phenolics) of extracts from waste materials resulted after steam distillation

Ethanol conc. [%]	Phenolics [mg/L]										
	Polyphenols				Phenols		Phenolic acids				
	Flavonoids						Hydroxy cinnamic acids		Hydroxybenzoic acid		
	Flavonols										
	R	Q	K	C	PG	PC	p-CA	CA	VA	GA	SA
OVAL W1											
0	350.59	0.17	1.67	443.42	3.04	1419.04	196.30	510.10	107.03	2174.99	144.16
40	289.44	0.29	1.60	628.19	2.28	1413.97	207.38	359.98	546.80	1680.46	207.80
60	276.18	0.40	1.72	606.05	2.07	1271.87	212.31	380.60	463.01	1576.90	226.65
80	117.65	1.62	3.51	283.36	2.28	1663.06	235.96	405.81	220.32	1404.83	220.96
96	36.37	0.40	3.50	29.11	1.69	2177.05	144.09	101.39	145.49	603.86	92.65
96 US	4.25	0.01	0.01	81.87	1.39	1604.68	128.55	79.51	39.25	401.77	62.98
96 FUS	3.02	0.01	0.01	56.66	0.12	162.20	43.12	35.27	13.95	57.62	17.16

Ethanol conc. [%]	Phenolics [mg/L]										
	Polyphenols				Phenols		Phenolic acids				
	Flavonoids						Hydroxy cinnamic acids		Hydroxybenzoic acid		
	Flavonols										
	R	Q	K	C	PG	PC	p-CA	CA	VA	GA	SA
TDL W1											
0	99.88	0.19	1.84	513.18	1.26	1598.59	199.52	327.04	71.65	454.32	97.51
40	102.79	6.15	3.73	929.89	0.34	802.00	701.75	1047.08	165.76	5.30	282.75
60	130.79	8.12	3.14	905.07	0.77	1963.04	338.67	473.57	89.06	262.09	134.24
80	106.48	8.72	3.58	757.84	0.56	1633.78	285.28	429.63	74.63	211.99	117.35
96	41.88	4.12	4.62	138.01	0.88	278.98	170.81	130.13	27.56	121.06	44.08
96 US	16.98	0.58	4.12	189.13	0.57	276.75	141.55	49.43	30.72	347.28	125.81
96 FUS	4.74	0.20	1.62	4.48	9.64	54.53	46.27	12.22	13.69	185.44	28.40
SH AMG W1											
0	53.06	0.16	0.01	82.12	2.12	1257.98	104.80	148.85	40.50	677.22	69.80
40	55.61	0.51	1.44	104.14	1.59	1490.91	121.31	173.72	39.10	428.33	68.06
60	51.48	0.60	0.01	4.13	2.13	1523.83	102.99	165.58	48.16	459.90	79.25
80	30.04	0.55	0.01	176.18	1.86	1183.53	102.77	139.81	34.45	396.81	65.40
96	6.77	0.51	0.01	1189.61	0.40	92.69	295.68	60.12	23.93	97.62	34.96
96 US	1.31	0.36	0.01	161.73	0.88	36.66	100.13	34.64	18.12	59.03	24.58
96 FUS	1.26	1.00	0.01	67.34	0.83	12.24	48.05	5.87	5.36	38.97	10.54

R = rutin; Q = quercetin; K = kaempferol; C = catechin; PG = pyrogallol; PC = pyrocatechol; p-CA = p-coumaric acid; CA = caffeic acid; VA = vanillic acid; GA = gallic acid; SA = syringic acid; AA = ascorbic acid; B2 = riboflavin

The components: rutin, quercetin, kaempferol, catechin, pyrocatechol, pyrogallol, p-coumaric acid, gallic acid, caffeic acid, vanillic acid, syringic acid, ascorbic acid and riboflavin were identified and quantified.

Table IV

HPLC analysis results (vitamins) of extracts from waste materials resulted after steam distillation

Ethanol concentration [%]	Vitamins [mg/L]	
	AA	B2
OVAL W1		
0	29.57	4198.80
40	33.93	10382.34
60	39.94	10531.71
80	60.81	8441.83
96	62.85	6900.27
96 US	60.35	5417.82
96 FUS	17.67	531.78
TDL W1		
0	29.19	2146.91
40	61.02	175.26
60	57.33	477.95
80	38.99	372.05
96	62.71	503.44
96 US	52.39	295.75
96 FUS	57.41	55.83
SH AMG W1		
0	30.24	1059.80
40	45.30	1059.80
60	74.12	987.91
80	64.27	605.83
96	40.68	140.51
96 US	40.37	67.32
96 FUS	17.66	14.77

The major phenolic components in all three investigated extracts from waste materials resulted after thyme and oregano distillation were as follow: pyrocatechol, gallic acid, p-coumaric acid, caffeic acid, vanillic acid, rutin and syringic acid. From all the phenolic compounds, gallic acid was detected within the range of 5.3 (TDL W1 40) - 2175 (OVAL W1 0) mg/L and pyrocatechol within 12 (SH AMG W1 96 FUS) - 2177 (OVAL W1 96) mg/L. Gallic acid (3,4,5-trihydroxybenzoic acid) is an outspread phyto-chemical with numerous and notable biological activities: anticancer, antitumor and antiviral activity, but also used in the pharmaceutical industry as an astringent and styptic agent. It is also used to treat allergic symptoms, control inflammation, cell differentiation and proliferation, being a potent anti-inflammatory and antihistamine agent [8]. Caffeic acid blocks the biosynthesis of leukotrienes involved in immuno-regulation diseases, asthma and allergic reactions [8]. Vanillic acid has anthelmintic and over-comes hepatic fibrosis in chronic liver injuries [8].

Vitamins like ascorbic acid and riboflavin were also present in high amounts in all extracts with the highest amount of riboflavin of 10531.71 mg/L determined in OVAL W1 60.

The ethanolic concentrations used for the extraction influenced the amount of different phenolic compounds. For example, in the case of catechin (a flavonoid) a maximum of extraction is obtained at 40% ethanol solution, yielding 104 - 930 mg/L (Figure 1A), while for gallic acid (hydroxybenzoic acid), the most efficient extraction would have been water with 454 - 2175 mg/L (Figure 1B). Furthermore, all extracts have shown that the concentration of compounds of interest rises when the ethanol

solution concentration decreases and such affinity for water and low ethanol concentrations has been shown in [7, 19], 45% and 60% being the most efficient ones.

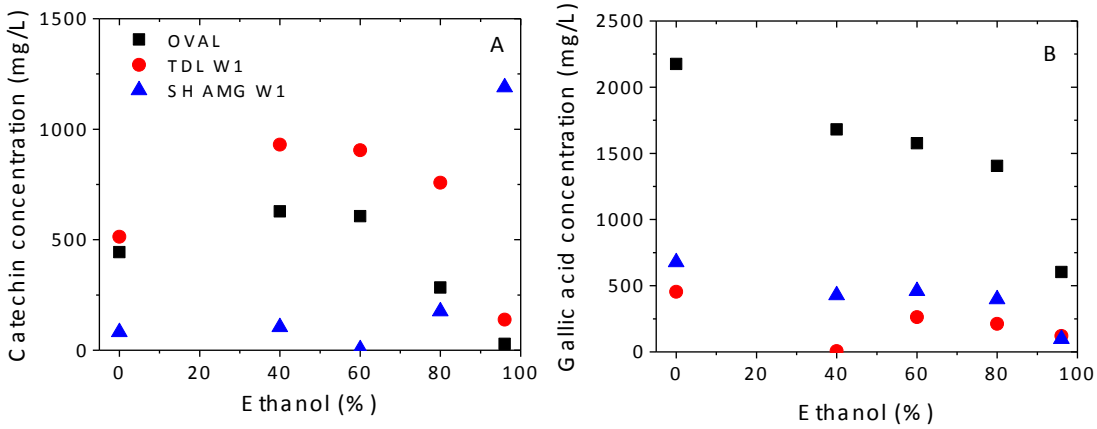

Figure 1.
The correlation between catechin (A), or gallic acid (B) concentration with percentage of ethanol solution used for extraction.

Conclusions

It is recommended to choose the best extraction method implying the solvent concentration, temperature, duration, etc., depending on the compounds that have to be extracted.

The hydro-ethanolic extracts obtained from waste materials resulted after thyme and oregano distillation present high phenolic content which is a valuable source of biologically active compounds. Furthermore, new green practices employ using the whole raw material with as little waste as possible, demanding for a more efficient extraction of compounds of interest or a further usage of the generated waste materials. Plant phenolics recovered from these waste products (thyme and oregano in the present study) including that obtained after essential oil production from aromatic plants, with zero, or low-cost raw material prices, could be used as antioxidants in food products, pharmaceuticals, for a more sustainable approach on the whole plant.

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References

1. Banerjee S, Rajamani P, Cellular, molecular, and biological perspective of polyphenols in chemoprevention and therapeutic adjunct in cancer, in Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes, Springer Berlin Heidelberg, 2013; 2175-2254.
2. Bosabalidis AM, Structural features of *Origanum sp.*, in: *Oregano:* The genera *Origanum* and *Lippia*, Taylor & Francis, London, 2003; 11-64.
3. Bungău SG, Popa VC, Between religion and science: some aspects: concerning illness and healing in antiquity. *Transylv Rev.*, 2015; XXIV(3): 3-19.
4. Gîrd CE, Duțu LE, Costea T, Nencu I, Popescu ML, Balaci TD, Olaru OT, Research regarding obtaining herbal extracts with antitumour activity. Note II. Phytochemical analysis, antioxidant activity and cytotoxic effects of *Chelidonium majus* L., *Medicago sativa* L. and *Berberis vulgaris* L. dry extracts. *Farmacia*, 2017; 65(5): 703-708.
5. Copolovici D, Bungău S, Boscencu R, Țiț DM, Copolovici L, The fatty acids composition and antioxidant activity of walnut cold press oil. *Rev Chim-Bucharest*, 2017; 68(3): 507-509.
6. Garcia-Rellan D, Blazquez MA, Boira H, Differential essential oil composition and morphology between perennial *Satureja* species growing in spain. *Rec Nat Prod.*, 2015; 9(4): 623-627.
7. Gavaric N, Kladar N, Misan A, Nikolic A, Samojlik I, Mimica-Dukic N, Bozin B, Postdistillation waste material of thyme (*Thymus vulgaris* L., *Lamiaceae*) as a potential source of biologically active compounds. *Ind Crops Products*, 2015; 74(15): 457-464.
8. Goleniowski M, Bonfill M, Cusido R, Palazon J, Phenolic acids, in: Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes, Springer Berlin Heidelberg, 2013; 1951-1973.
9. Kremer D, Kosir IJ, Koncic MZ, Cerenak A, Potocnik T, Srecec S, Randic M, Kosalec I, Antimicrobial and antioxidant properties of *Satureja montana* L. and *S. subspicata* Vis. *Curr Drug Targets*, 2015; 16(14): 1623-1633.
10. Lattanzio V, Phenolic compounds: Introduction, in: Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. Springer Berlin Heidelberg, 2013; 1543-1580.
11. Lawless J, Roche S, The encyclopedia of essential oils: the complete guide to the use of aromatic oils in aromatherapy, herbalism, health & well-being. Conari Press, San Francisco, 2013.

12. Mandal S, Debmandal M, Chapter 94: Thyme *(Thymus vulgaris L.)* Oils A2 - Preedy, Victor R, Essential Oils in Food Preservation, Flavor and Safety, Academic Press, San Diego, 2016; 825-834.
13. Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira ICFR, Decoction, infusion and hydroalcoholic extract of *Origanum vulgare* L.: Different performances regarding bioactivity and phenolic compounds. *Food Chem.*, 2014; 158: 73-80.
14. Maskovic P, Velickovic V, Mitic M, Durovic S, Zekovic Z, Radojkovic M, Cvetanovic A, Svarc-Gajic J, Vujic J, Summer savory extracts prepared by novel extraction methods resulted in enhanced biological activity. *Ind Crops Products*, 2017; 109: 875-881.
15. Morales R, The history, botany and taxonomy of the genus *Thymus*, in: *Thyme,* The Genus *Thymus*, Taylor & Francis, New York, 2002; 1-43.
16. Navarrete A, Herrero M, Martin A, Cocero MJ, Ibanez E, Valorization of solid wastes from essential oil industry. *J. Food Eng*., 2011; 104(2): 196-201.
17. Nikolic M, Jovanovic KK, Markovic T, Markovic D, Gligorijevic N, Radulovic S, Sokovic M, Chemical composition, antimicrobial, and cytotoxic properties of five *Lamiaceae* essential oils. *Ind Crops Products*, 2014; 61: 225-232.
18. Ortega-Ramirez LA, Rodriguez-Garcia I, Silva-Espinoza BA, Ayala-Zavala JF, Chapter 71 - Oregano *(Origanum spp.) Oils*, in: Essential Oils in Food Preservation, Flavor and Safety, Academic Press, San Diego, 2016; 625-631.
19. Pag AI, Radu DG, Drăgănescu D, Popa MI, Sirghie C, Flaxseed cake - a sustainable source of antioxidant and antibacterial extracts. *Cell Chem Technol*, 2014; 48(3-4): 265-273.
20. Pallag A, Bungău S G, Ţiţ D M, Jurca T, Sîrbu V, Honiges A, Horhogea C, Comparative study of poly phenols, flavonoids, and chlorophylls in *Equisetum arvense* T. populations. *Rev Chim (Bucharest)*, 2016; 67(3): 530-533.
21. Rios JL, Essential oils*:* What they are and how the terms are used and defined, in: essential oils in food preservation*, Flavor and Safety. Elsevier Inc.*, 2016; 3-9.
22. Rizea GD, Popescu M, Ionescu D, Mihele D, Manea S, Comparative determinations of antioxidant free radical scavenging polyphenols in certain natural products by HPLC methods and UV-Vis spectrophotometry. *Rev Chim (Bucharest)*, 2012; 63(11): 1085-1088.
23. Sajilata MG, Singhal RS, Quality indices for spice essential oils, in: Handbook of Herbs and Spices, Vol. 1, 2nd Ed., Woodhead Publ. Ltd, Cambridge, 2012; 42-54.
24. Stahl-Biskup EV, Thyme, in: Handbook of herbs and spices, Woodhead Publishing Ltd and CRC Press LLC, Cambridge - UK, 2004.
25. Tekeli Y, Antioxidant Activities and phenolic compounds of two endemic taxa of *Labiatae sideritis*. *Rev Chim-Bucharest*, 2012; 63(5): 465.
26. Tohidi B, Rahimmalek M, Arzani A, Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran. *Food Chem*., 2017; 220: 153-161.
27. Velescu BS, Anuţa V, Nițulescu GM, Olaru OT, Orțan A, Ionescu D, Ghica MV, Drăgoi CM, Dinu Pîrvu CE, Pharmaceutical assesment of Romanian crops of *Anthriscus sylvestris* (*Apiaceae*). *Farmacia*, 2017; 65(6): 824-831.
28. Vladic J, Zekovic Z, Jokic S, Svilovic S, Kovcevic S, Vidovic S, Winter savory: Supercritical carbon dioxide extraction and mathematical modeling of extraction process. *J Supercrit Fluids*, 2016; 117: 89-97.