Total phenolic compounds, antioxidant activity and toxicity of selected medicinal and aromatic plants

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The importance of dietary antioxidant components for the prevention of some diseases and health quality improvement has attracted much research attention through the last decade. Vegetables and herbal infusions have been recognized as important antioxidant sources. Food industry shows significant interest in application of plant bioactive compounds for flavoring but also for preservation purposes, but attention should be given in case of high doses . In the current study were investigated the total phenolic content, the antioxidant activity and the toxicity of selected medicinal aromatic plants that are being consumed as decoctions or used as food additives.

Sample preparation

2 g dry mass of each plant species were:
a)steeped in 200 ml (1 cup) a) at 85 ^{0}C for 15 min,

b) at room temperature for 15 min and,

c) at room temperature with the assistance of ultrasonic (35 MHz) for 15min. The herbal infusions were then filtered though a Whatman filter No.1.

All extracts were then further extracted by petroleum ether.

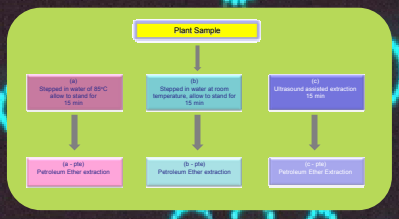

Fig 1. Schematic Plan of extraction procedures

Total phenolic content and antioxidant activity

Total phenolic content (in terms of caffeic acid) was determined using a Folin-Ciocalteu assay. Antioxidant determined applying both the ABTS assay - 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), and the DPPH assay 2, 2-diphenyl-1-picrylhydrazyl radical .

Toxicity tests

Toxicity assessment of the plant extracts has been performed using MICROTOX® toxicity analyzer. MICROTOX® uses the marine bioluminescence bacteria Vibrio fischeri as a reference test species for the measurement of bioluminescence inhibition. The protocol used was the Basic Test 81.9% (AZUR, 1997) with appropriate primary dilution of samples in order the EC_{50} (Effective Concentration where bioluminescence inhibition is 50%) not to be obtained by extrapolated data.

Table 1. Names and use of plant species examined

Code	Species	Common name	Plant part used	Use in diet
MOF	*Melissa officinalis*	Lemon balm	leaves	Decoctions
OVU	Origanum vulgare	Oregano	leaves	Herb in salads (fresh or dry), roasted, stewed
ODI	*Origanum dictamnus*	Dittany	Leaves/flowers	Decoctions
SOF	*Salvia officinalis*	Sage	Leaves	Decoctions, as a herb in cooking
HOF	*Hyssopus officinalis*	Hyssop	Leaves/flowers	Decoctions

Table 2. Toxicity of plant species expressed as EC50 (mg/ml) after 15 minutes incubation time

Sample	Treatments					
	(a)	*(a-pte)*1*	*(b)*	*(b-pte)*1*	*(c)*	*(c-pte)*1*
MOF	119.75	210.68	200.61	216.30	113.09	126.93
HOF	38.31	139.60	150.67	162.75	96.05	101.47
ODI	10.56	42.87	60.46	110.75	62.61	96.87
OVU	8.61	36.38	40.96	52.32	22.09	77.69
SOF	52.17	107.77	199.45	198.78	137.19	224.87

Table 3. Total Phenolic Content, Antioxidant Activity estimated by DPPH and ABTS method

	Treatment	Total Phenolic Content mg Caffeic acid / 200 ml	Antioxidant Activity DPPH μmole Trolox / 200 ml	Antioxidant Activity ABTS μmole Trolox / 200 ml
MOF *Melissa officinalis*	(a)	197.0	1268.3	1321.7
HOF *Hyssopus officinalis*		34.9	206.4	206.8
OVU *Origanum vulgare*		128.6	631.1	669.3
ODI *Origanum dictamnus*		63.9	299.7	321.0
SOF *Salvia officinalis*		64.5	328.2	326.1
MOF *Melissa officinalis*	(a – pte)	185.9	1240.1	1319.5
HOF *Hyssopus officinalis*		31.7	182.5	190.2
OVU *Origanum vulgare*		105.1	565.2	595.2
ODI *Origanum dictamnus*		51.3	227.7	311.9
SOF *Salvia officinalis*		62.0	306.9	323.1
MOF *Melissa officinalis*	(b)	135.8	644.5	766.8
HOF *Hyssopus officinalis*		10.7	51.5	69.2
OVU *Origanum vulgare*		63.5	290.1	309.5
ODI *Origanum dictamnus*		28.3	153.5	169.6
SOF *Salvia officinalis*		15.5	77.3	99.2
MOF *Melissa officinalis*	(b – pte)	119.1	605.5	684.3
HOF *Hyssopus officinalis*		10.4	39.2	71.0
OVU *Origanum vulgare*		53.9	262.8	267.1
ODI *Origanum dictamnus*		27.9	123.7	139.1
SOF *Salvia officinalis*		15.5	66.3	89.6
MOF *Melissa officinalis*	(c)	159.9	752.7	871.8
HOF *Hyssopus officinalis*		13.1	49.7	85.2
OVU *Origanum vulgare*		68.3	323.5	344.5
ODI *Origanum dictamnus*		34.5	177.0	179.6
SOF *Salvia officinalis*		16.9	83.7	123.1
MOF *Melissa officinalis*	(c – pte)	114.7	676.0	834.9
HOF *Hyssopus officinalis*		15.0	55.7	86.1
OVU *Origanum vulgare*		57.6	276.3	288.7
ODI *Origanum dictamnus*		32.9	155.5	160.3
SOF *Salvia officinalis*		28.0	68.6	107.9

Table 4. Concentration that cause 20% luminescence inhibition and maximum dry plant mass per ref. volume

Code	Treatment	Concentration that cause 20% luminescence inhibition mg/ml	Plant mass per ref volume that cause 20% luminescence inhibition (g)	Reference volume (ml)	Use in diet
MOF	(b)	92.33	18.47	200	
	(a)	48.00	9.60	200	Decoction
OVU	(b)	12.95	6.48	500	In salads, appetizers and meals
	(a)	3.29	**0.66**	200	Decoction
ODI	(a)		16.5	5000	During cooking process
	(b)	21.03	4.21	200	
	(a)	4.04	**0.81**	200	Decoction
SOF	(b)	83.79	16.76	200	
	(a)	18.95	3.79	200	Decoction
HOF	(a)		94.75	5000	During cooking process
	(b)	65.10	13.02	200	
	(a)	11.90	2.38	200	Decoctions

Results

Total phenolic content was estimated in both infusions and the aqueous phase of their extraction by petroleum ether, expressed in mg of caffeic acid /200 ml. Maximum values were given by *Melissa officinalis*. The ranking of antioxidant activity regardless of the method that is being determined DPPH or ABTS is the same with the ranking of the total phenolic content. Results show that there is a scaling increase in total phenolic content among the species tested. *Melissa officinalis* exhibited the higher phenolic content and *Hyssopus officinalis* the lowest regardless the extraction procedure. Ranking remains the same in the aquatic phase of the extraction by petroleum ether for all the infusions (table 2) tested.

Antioxidant activity when determined by ABTS method expressed also μmole Trolox / 200 ml (table 2). Maximum values were given by *Melissa officinalis*. The ranking of antioxidant activity regardless of the method that is being determined DPPH or ABTS is the same with the ranking of the total phenolic content.

Toxicity evaluation of the plant extracts was performed by applying the Basic Test protocol obtained after 15 min exposure expressed as EC_{50} value, at which 50% loss of luminescence is obtained. EC_{20} was also estimated, at which a 20% loss of light emission is observed, as according to the ISO guidelines (ISO 11348, 1998) a toxic sample shows an effect percentage greater than 20%. Results show that *Origanum vulgare* gave the lower EC_{50} values in all treatments. Respectively, higher values were obtained by *Melissa officinalis* in treatments (a), (a-pte), (b), (b-pte), and by *Salvia officinalis* in treatments (c) and (c-pte) (table 3).

Depending on the use of each plant tested in nutrition, was calculated the maximum dry plant mass, in 200ml for decoctions, in 500ml in case of use in salads, and in 6 lt when is being used in cooking processes (table 4). Furthermore, was calculated the synergism ratio (SR) as follows:

$SR_{total\ phenolics}$ = Total phenolics – pte / Total phenolic mixture

$SR_{antioxidant\ activity}$ = Antioxidant activity – pte / Antioxidant activity mixture

$SR_{toxicity}$ = EC_{50}-pte / EC_{50} mixture

Table 5. $SR_{toxicity}$, $SR_{total\ phenolic}$, SR_{DPPH}, SR_{ABTS} indexes for sample tested

Sample Code	Treatment	SR total phenolic	Effect *1	SR DPPH	Effect *1	SR ABTS	Effect *1	SR toxicity*2	Effect
MOF	(b)	0.9	Synergism	0.9	Synergism	0.9	Synergism	1.1	Synergism
	(c)	0.7	Synergism	0.9	Synergism	1.0	Additive	1.1	Synergism
	(a)	0.9	Synergism	1.0	Additive	1.0	Additive	1.8	Synergism
HOF	(b)	1.0	Additive	0.8	Synergism	1.0	Additive	1.1	Synergism
	(c)	1.1	Antagonism	1.1	Antagonism	1.0	Additive	1.1	Synergism
	(a)	0.9	Synergism	0.9	Synergism	0.9	Synergism	**3.6**	Synergism
ODI	(b)	1.0	Additive	0.8	Synergism	0.8	Synergism	1.8	Synergism
	(c)	1.0	Additive	0.9	Synergism	0.9	Synergism	1.5	Synergism
	(a)	0.8	Synergism	0.8	Synergism	1.0	Additive	**4.1**	Synergism
OVU	(b)	0.8	Synergism	0.9	Synergism	0.9	Synergism	1.3	Synergism
	(c)	0.8	Synergism	0.9	Synergism	0.8	Synergism	3.5	Synergism
	(a)	0.8	Synergism	0.9	Synergism	0.9	Synergism	**4.2**	Synergism
SOF	(b)	1.0	Additive	0.9	Synergism	0.9	Synergism	1.0	Additive
	(c)	1.7	Antagonism	0.8	Synergism	0.9	Synergism	1.6	Synergism
	(a)	1.0	Additive	0.9	Synergism	1.0	Additive	2.1	Synergism

**1 : In case of Total Phenolic Content and Antioxidant Activity, SR<1 indicates synergism, SR> indicates antagonism and SR = 1 indicates additive action*
**2: In case of toxicity, $SR_{toxicity}$ <1 indicates antagonism, $SR_{toxicity}$ > 1 indicates synergism and SR = 1 indicates additive action*

Conclusions. Results of this study indicate that between plant species examined, *Melissa officinalis* presented the higher, and *Hyssopus officinalis* the lower phenolic content and actioxidant activity. Extraction procedure seems to influence significantly the extraction of phenolic compounds and antioxidant activitiy respectively, as great differences were observed between extractions in high and room temperature. A small increase was observed in ultrasound assisted extraction. Similar results were obtained when infusions extracted by petroleum ether. Linear positive correlation has been found between total phenolic content and antioxidant activity in the infusions studied.

Toxicity of infusions seems to be also influenced by the temperature and extraction procedure, but no correlation was detected between toxicity and the total phenolic content or antioxidant activity. Interactions of soluble substances and the essential oil of plants were remarkable in case of toxicity in contrast to the case of total phenolic content and antioxidant activity where interaction was almost neutral.

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