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SCREENING OF BIOFLAVONOID AND ANTIOXIDANT ACTIVITY OF *LENS CULINARIS* MEDIKUS♣

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In the phyto-chemical research of extracts, there is a rising demand for simple, quick, selective and precise quantitative analysis methods of polyphenolics. The goal of this research was to realise a quantitative meta-analysis of the experimental methods that investigate flavonoids from three types of seeds of *Lens culinaris* Medikus extracts using spectrophotometry. Polyphenol quantitative determination was done through spectrophotometric methods (based on the chelating reaction with $AlCl_3/CH_3COONa$ for flavones, and Arnow reaction for poliphenolcarboxylic acids). Determination of the antioxidant activity of the extract was realised with the 2,2–Diphenyl–1–picrylhydrazyl (DPPH) method and ORAC (antioxidative capacity of the oxygen radical) method.
The antioxidant capacity of the analysed extracts is proportional with their concentration value. In order to use as nutraceuticals, the organic solvents were replaced with alimentary ethanol of fermentative origin, and HCl was replaced with acetic acid of 0.5% concentration.

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Keywords: Lens culinaris Medikus; antioxidant activity; antiradical properties; phenolics

1. Introduction

The intensity of the modern life style, the stress, the malnutrition, the low food quality, and the interaction of the exogenous and endogenous toxic factors and agents are among the cumulative causes of premature degradation of the human organism systems: central nervous, cardiovascular, endocrine and reproductive [1]. As a result, there are frequent liver diseases, ischemic heart diseases, diabetes, and urinary system disorders, accompanied by the depreciation of the immune function of the body [2]. The modern medication pleads for the introduction in treatment and recovery protocols of preparations capable of stimulating the body's protection capacity in different pathological states [3]. In modern complex therapy and recovery, natural and chemical compounds are used [4]. These toxic factors are a direct consequence of the complex of phenomena collectively called "oxidative stress" defined as an exaggerated production of free radicals in the human body, accompanied by a decrease of antioxidant agents [5, 6]. A possible solution for this problem is to use natural antioxidants that neutralize free radicals and protect human body cells.

The antioxidants represent a group of compounds synthesized by the body, which can also be found in different natural food products [7]. They act in synchrony with the human body, consuming free radicals and maintaining its health. The role of antioxidants consists in

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annihilating free radical activity in the human body, which are produced uninterrupted and are serving as catalysts for the metabolic processes [8]. The oxidative stress represents a perturbation of the redox homeostasis or the totality of the oxidative deteriorations produced by an overproduction of reactive species (RS) or a decrease of the antioxidant (AO) system capacity at cell level or at body level.

Therefore, there is a deregulation of the pro-oxidant / antioxidant balance in favour of pro-oxidants [9, 10]. Oxide radicals have two categories of effects on the body, as follows: the beneficial effects, acting as bactericides within phagocytosis, stimulation of the activity of lymphocytes, control of vascular tonus, stimulation of the growth and proliferation of cells, stimulation of erythropoietin secretion [11] and also negative effects such as destruction/deterioration of cell structure, cell malignant alteration, cell ageing. [12]. Several DNA structures [13]; lipids, proteins, and glucides can be affected by the impact of oxidative stress [14]. Reactive oxygen species, resulted from these processes will cause the endothelial disfunction, and will induce an increase in smooth muscle cells proliferation. The phenomenon is not completely elucidated at present. This shortcoming comes from the fact that reactive species have a short life span, hence the difficulty of their dosing and of establishing the relationship between their action and the emergence of some pathological phenomena [15, 16]. It is interesting to note that flavonoids metabolise considerably, determining the alteration of their chemical structure, which means that these do not always have antioxidant effects [17, 18].

Lentil (scientifically called *Lens culinaris* Medikus) is a short, hairy, paripenat-compound leaved legume whose seeds are edible [19]. Lentil does not contain cholesterol and its caloric value is about 110-160 kcal/100 g, depending on cultivar [20]. The species *Lens culinaris* Medikus is a food and medicine plant belonging to the Family Fabaceae (Leguminosae). Dry lentils contain water (10–12%), simple and complex-energetic and lumber glucides (45–47% energetic carbohydrates among which predominates starch–6% and cellulose–3%), protids (30%), lipids (1–2%), nucleic acids, mineral salts (magnesium, potassium, iron, copper, manganese), vitamins of the B complex, pro-vitamin A (carotene) [21], nucleic acids, lecithin (lentil lecithin), lectins, flavonoids. Lentil content of purine nitrate bases is relatively high (162 mg/100 g) [22]. Lentil grains contain fats in negligible amounts and have special food and diet properties, their energetic value being relatively high (353kcal/100g). Lentils contain isoflavones and iron, an essential element, better absorbed when associated with foods rich in vitamin C [13, 22]. Lentils is in the top list of vegetables having a high content of vegetal protein (24%), completely assimilable by the human organism Lentil seeds contain lumber material (vegetal fibber) that helps digestion [23].

The importance of *Lens culinaris* Medikus consists in the high content of protein, which gives it a high food value (its protein value is similar to animal proteins). Lentil protein digestibility is high and it does not produce uric acid accumulation in the body which is harmful [24]. Bioflavonoid compounds that can be found in *Lens culinaris* Medikus can bond toxic metals that are thus removed from the body; they have bacteriostatic and/or antibiotic effect acting as an anti-infectious agent; they have a synergic activity with vitamin C, helping to its fixing in the tissues [25].

Flavonoids are phenol pigments that contain in their molecule a piran or furan heterocycle condensed with a benzene ring [26]. Another benzene ring couples with the heterocycle. The rings have hydroxyl groups, and this determines the phenol character of these pigments [27]. The flavonoids are vegetal pigments which are predominant in high plants in a free state and as glycosides [28]. From a chemical point of view, flavonoid pigments are phenolic water soluble glycosides that can be found in the vacuolar juice and in the chromoplasts [29].

2. Material and Methods

Were used three types of seeds of *Lens culinaris* Medikus that will be named from now on in this paper as type 1, 2 and 3, respectively.

2.1 UV–Vis extraction and concentration assessment protocol in each extract.

A quantity of 50 ml of solvent was added over the sample (crushed seeds) and was sonicated, in the absence of light, for 24 h. The absorbance of the extracts obtained (non-

diluted/diluted 1:10, 1:100, 1:1000) was recorded for the range 200-700nm (UV–Vis) to establish total concentration of phenolics. The characteristic signal was monitored at 280 nm in phenolic acids, and at 340 nm in flavonoids. Were used previously prepared extracts from orange, green and brown seeds of *Lens culinaris* Medikus, and as well as solutions of these extracts progressively diluted in ratios of 1:10, 1:100, and 1: 1000.

2.2 Quantitative determination of total polyphenols expressed in caffeic acid.

The method is based on the formation of a blue compound between the phosphowolframic acid and the polyphenols (in an alkaline medium). Total polyphenol concentration of the samples to be analysed was calculated with the standard curve, under the same conditions as the sample solutions. For the standard scale, was used a standard solution of caffeic acid 0.025 g/L.

2.3 Quantitative determination of flavonoids expressed in rutoside and quercetol.

The method is based on the formation of a yellow-orange compound through the reaction between flavonoids and aluminium chloride. Were developed two standard scales: one with a standard solution of rutoside 0.1 g/L and one with a standard solution of quercetol 0.1 g/L.

2.4 Testing of the antioxidant activity of the extracts.

In this study, was determined the scavenger activity of the free radicals from the extract of *Lens culinaris* Medikus. To assess the antioxidant activity, was used a method based on the indirect measurement of the capacity of releasing free radicals. Antioxidant activity was measured through a spectrophotometric method using free radicals of 1,1–diphenyl–2–picrylhydrazyl (DPPH). When the DPPH radical reacts with a hydrogen donor antioxidant compound, DPPH is reduced, resulting in a decrease of absorbance at 517 nm. Amounts of 10 g of powdered vegetal material, from each of the three types of seeds, were extracted with 100 ml of solvent, at room temperature, for 1 h. The extracts obtained were filtered, and the filtrates were dry evaporated. Scavenger activity of free radicals was expressed in percents using the formula 100\x(Ai–Af)/Ai, where Ai is the absorbance before adding the tested extract and Af is the value of absorbance after 5 minutes of reaction. The neutralising effect of the DPPH free radical was measured at three different concentrations of methanol and ethanol extracts: portions of 0.05 ml of the extracts of 10 mg/ml, 5 mg/ml, and 2.5 mg/ml were mixed with 2.95 ml solution of DPPH in the spectrophotometer vat. After 5 minutes of reaction, was read the absorbance compared to that of the control vat with 3 ml methanol at 517 nm. Quercetol was used as positive control: a concentration of 2.5 mM quercetol neutralises the DPPH radical 100%.

2.5 Determination of ascorbic acid

The determination of ascorbic acid was done by two methods: the electrochemical method and the 2,6–diclorphenol–indophenol method, respectively. In the case of the electrochemical method, ascorbic acid donates electrons to the working electrode during electrolysis; the voltgramme shows this anodic oxidation as a peak. Quantitative measurement was done with the standard addition for the electrochemical method. In the case of the 2,6–diclorphenol–indophenols method, we relied on the reducing activity of the ascorbic acid on the 2,6–diclorphenol–indophenol in an acid medium, when ascorbic acid turns into dehydroascorbic acid and the coloured oxidates substances turns into its colourless reduced form.

3. Results and Discussion

The seeds of *Lens culinaris* Medikus are rich in phenol substances [30]. Bioflavonoids are polyphenol chemical compounds biosynthesised by the plants, which have a chromane ring at the basis of their structure. Flavonoids, having the general chemical structure C_6–C_3–C_6, have the widest group of hydrosoluble heterocyclic phenol substance group. They are by-products of plant metabolism capable of photosynthesis, and are ranged among polyphenol structure compounds [31]. Flavonoid molecules are made up of two benzene rings (C_6) united with a heterocyclic fragment C_3 [32]. This fragment forms a third ring with three carbon atoms and one oxygen atom

[33]. Flavans (chromans) are pigments that derive from flavan (2–phenyl–benzopiran or chroman) and they have in their molecule a benzopiran ring. Flavones and isoflavones are found under the form of glycosides.

The bioflavonoids from *Lens Culinaris* are biosynthesised towards phenyl propanol, where the amino acid phenylalanine is used to produce 4-coumaroil-CoA. It condenses with manoil–CoA to produce chalcones, compounds that contain two phenyl rings. The conjugation continues until the forming of the characteristic form with three rings. Then there is a series of enzymatic alterations until the production of flavones, then of dihydroflavonols and anthocyanidines. During these processes, there is formation of intermediary compounds: flavonols, flavan–3–ols, proanthocyanidins and several polyphenolics.

This is how many products are biosynthesized, such as flavonols, flavan–3–ols, proanthocyanidins (tannins) and a host of other polyphenolics. Compounds tend to polymerise and to form catechins that make up catechin tannins [34]. From the International Union of Pure & Applied Chemistry (IUPAC) point of view, they are classified as follows:

- Flavonoids derivatives of 2 phenylchromen 4–one (structure 2 phenyl 1, 4 benzopyrone);
- Isoflavonoids derivatives of 3 phenylchromen 4–one (structure 3 phenyl 1,4 benzopyrone);
- Neoflavonoids derivatives of 4 phenylcoumarin (4 phenyl 1,2 benzopyrone).

Assessment of food products is done primarily depending on their nutritional value which is given by the total amount of substances in their composition, such as glucides, lipids, protids, mineral salts, organic acids, vitamins and enzymes. Besides organoleptic examination, we also need to determine the main physico-chemical properties of the products such as stipulated by standards under the form of quality characteristics, e.g. water content (moisture), mineral substance content (ashes), acidity, fat content, sodium chloride content, ratio of components, etc. The determinations were carried out according to in-force standards.

Ashes content is a very important quality feature for most of food products and, particularly, for vegetal products representing the percentage content of mineral salts and mineral impurities of a product. Determining ashes content is usually done by calcinating the sample under established conditions through either the slow method, considered the reference method, at 550–650°C, or the quick method at 900–920°C.

The quantitative method for fat determining is based on fat property to dissolve in volatile organic solvents. The currently used method to determine fat substances is the Soxhlet method.

The chemical composition of the three types of seeds of *Lens culinaris* whose antioxidant capacity has been analysed is shown in Table 1.

Table 1. Descriptive statistics of seed yield and chemical compositions (% dry mass)

No.	Variable	Mean	Standard Deviation	Variance	Minimum	Maximum
Type 1						
1.	Dry matter	82.24	0.25	0.103	73.2	88.6
2.	Seed yield	174.2	0.17	4.607	172.1	175.5
3.	Moisture	5.76	0.12	0.024	5.4	6.3
4.	Ash	2.78	0.16	0.016	2.6	2.9
5.	Total nitrogen	3.25	0.10	0.020	2.9	3.4
6.	Crude protein	4.26	0.15	0.023	4.1	4.5
7.	Water soluble protein	2.21	0.16	0.025	2.1	2.3
8.	Total carbohydrate	47.61	0.32	0.057	44.6	52.8
9.	Crude fat	2.90	0.05	0.002	2.5	3.1
10.	Crude fibre	14.53	0.11	0.031	14.3	15.1
Type 2						
1.	Dry matter	79.34	0.35	0.103	73.3	84.1
2.	Seed yield	154.3	0.15	4.605	153.1	155.5
3.	Moisture	5.56	0.13	0.034	5.2	6.3
4.	Ash	3.58	0.16	0.016	3.1	3.9
5.	Total nitrogen	3.35	0.10	0.030	3.1	3.4

No.	Variable	Mean	Standard Deviation	Variance	Minimum	Maximum
6.	Crude protein	4.36	0.15	0.033	4.1	4.5
7.	Water soluble protein	3.31	0.16	0.035	3.1	3.3
8.	Total carbohydrate	45.61	0.33	0.055	42.6	47.8
9.	Crude fat	3.90	0.05	0.003	3.8	3.99
10.	Crude fibre	14.53	0.11	0.031	14.3	14.78
Type 3						
1.	Dry matter	79.34	0.37	0.103	73.3	82.9
2.	Seed yield	174.3	0.15	4.607	172.1	175.7
3.	Moisture	7.76	0.13	0.034	7.4	7.9
4.	Ash	3.78	0.16	0.016	3.5	3.9
5.	Total nitrogen	3.37	0.10	0.030	3.2	3.4
6.	Crude protein	4.36	0.13	0.031	4.1	4.7
7.	Water soluble protein	3.31	0.16	0.037	3.1	3.5
8.	Total carbohydrate	49.61	0.35	0.071	44.6	53.8
9.	Crude fat	3.90	0.07	0.003	3.7	4.1
10.	Crude fibre	14.73	0.12	0.032	14.3	15.1
Data are displayed with mean values ± SD (6n)						

Most frequently, flavonoids are found in plants together with ascorbic acid. They are also known as vitamins P of factor P of permeability, since they are determining factors for vascular permeability.

In *Lens culinaris* Medikus, some of these compounds, e.g. flavones, are heterosids (bound to glucides parts), called flavonosides. Bioflavonoids in *Lens culinaris* Medikus enhance the action of vitamin C by two pathways:

– forming a complex redox system together with vitamin C, cooperating with it in different redox processes;

– allowing a better digestive absorption of the ascorbic acid.

The solvents used retrieve almost the same amount of phenol compounds from the extracts of the three types of *Lens culinaris* Medikus seeds (according to the values in Table 2).

Table 2. Variation of total polyphenol content and of flavonoid values from Lens culinaris Medikus depending on the solvent used for extraction

No.	Samples	Total polyphenols expressed in caffeic acid (mg%)	Flavonoids expressed in rutoside and quercetol (mg%)
Type 1			
1.	Extract absolute methanol	358.26	1790.64/1057.41
2.	Extract 20% absolute ethanol	322.16	1852.80/1238.29
3.	Extract 40% absolute ethanol	388.26	1892.21/1381.61
4.	Extract 60% absolute ethanol	384.81	2482.24/1862.21
5.	Extract absolute ethanol 96%	331.68	2342.20/1740.72
6.	Extract 10% glacial acetic acid	345.35	2283.08/1614.51
7.	Extract aqueous	338.34	2049.18/1527.13
8.	Vitamin E	0.008 mg/ml	
9.	Vitamin C	0.052 mg/ml	
Type 2			
1.	Extract absolute methanol	422.26	2870.14/2058.42
2.	Extract 20% absolute ethanol	422.36	2852.10/2248.27
3.	Extract 40% absolute ethanol	482.56	2872.12/2482.62
4.	Extract 60% absolute ethanol	462.82	2482.14/2862.22

No.	Samples	Total polyphenols expressed in caffeic acid (mg%)	Flavonoids expressed in rutoside and quercetol (mg%)
5.	Extract absolute ethanol 96%	428.28	2442.10/2840.82
6.	Extract 10% glacial acetic acid	422.42	2284.18/2624.52
7.	Extract aqueous	424.28	2047.18/2528.24
8.	Vitamin E	0.021 mg/ml	
9.	Vitamin C	0.055 mg/ml	
Type 3			
1.	Extract absolute methanol	438.37	3570.74/3055.13
2.	Extract 20% absolute ethanol	439.18	3553.50/3345.17
3.	Extract 40% absolute ethanol	453.57	3573.33/3453.13
4.	Extract 60% absolute ethanol	473.51	3453.34/3573.13
5.	Extract absolute ethanol 96%	435.85	3443.30/3540.13
6.	Extract 10% glacial acetic acid	433.47	3354.05/3734.13
7.	Extract aqueous	434.35	3047.35/3535.14
8.	Vitamin E	0.023 mg/ml	
9.	Vitamin C	0.057 mg/ml	
Data are displayed with mean values ± SD (6n)			

Extracts from *Lens culinaris* Medikus in ethanol 40% and 60%, respectively, are remarkable due to their significantly higher content of phenolics, for each of the three types of seeds. The calibration curve has a good linearity with a regression coefficient R^2=0.9982.

The extract from type 1 of seeds of *Lens culinaris* Medikus, in ethanol 40% has a concentration value of 388.26 mg%, while extract in alcohol 60% has a concentration value of 384.81 mg%. For type 2 of seeds, the extract in ethanol 40 % has a concentration value of 482.56 mg %, and the extract in 60% ethanol has a concentration value of 462.82 mg %. Regarding the type 3 of *Lens culinaris* seeds, the extract in the extract in ethanol 40 % has a concentration value of 453.57 mg %, and the extract in 60% ethanol has a concentration value of 473.51 mg %.

As shown in Table 2, there are high amounts of flavonoids in all analysed extracts from *Lens culinaris* Medikus, for all three types of seeds ; For the type 1, it is to be noted the flavonoid content of the extract in ethanol 60% (expressed in rutoside 2482.24 mg% and in quercetol 1862.21 mg%, respectively). For the type 2, the highest value of flavonoid content expressed in rutoside resulted for the extract in ethanol 40%, while the flavonoid content expressed in quercetol the highest vauel was recorded for the extract in ethanol 60%. In the case of the third type of seeds, it stand out the flavonoid content expressed in rutoside for the extract in 40% absolute ethanol, 3573.33 mg%, and the flavonoid content expressed in quercetol, for the extract in glacial acetic acid 10%, 3734.13 mg%.

Spectrophotometric analysis of extracts from *Lens culinaris* Medikus pointed out a significant content of bioflavonoids (1790.64/1057.41 mg%–2482.24/1862.21 mg% for type 1, 2047.18/2058.42 mg% - 2872.12/2862.22 mg % for type 2, and 3047.35/3055.13 mg% -3573.33/ 3734 mg% for type 3 of seeds). The calibration curve for the dosing of flavonoids expressed in rutoside has a good linearity with a regression coefficient R^2=0.9879. Calibration curve for the dosing of flavonoids expressed in quercetol has a good linearity with a regression coefficient R^2=0.9871.

The amount of flavonoid compounds can be correlated with the protective role of flavonoids and the adaptability of the plant to the environment. Results of the scavenger activity of the free radicals are shown in Table 3.

Table 3. Neutralising effect of the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical in the tested extracts

No.	Samples	Scavenger effect of the free radicals (%)		
		10 mg/ml	5 mg/ml	2.5 mg/ml
Type 1				
1.	Extract absolute methanol	77.29 ± 1.57	44.43 ± 1.17	24.15 ± 0.64
2.	Extract 20% absolute ethanol	73.70 ± 0.60	34.25 ± 0.63	24.97 ± 0.45
3.	Extract 40% absolute ethanol	75.96 ± 2.03	49.30 ± 0.96	27.51 ± 0.57
4.	Extract 60% absolute ethanol	77.42 ± 1.16	47.39 ± 0.52	26.21 ± 0.56
5.	Extract absolute ethanol 96%	77.36 ± 1.60	46.64 ± 0.47	27.62 ± 0.79
6.	Extract 10% glacial acetic acid	76.79 ± 0.79	60.27 ± 0.76	37.73 ± 0.55
7.	Extract aqueous	71.13 ± 0.42	40.98 ± 0.35	21.23 ± 0.23
Type 2				
1.	Extract absolute methanol	87.29 ± 0.48	55.83 ± 1.38	25.15 ± 0.65
2.	Extract 20% absolute ethanol	83.80 ± 0.60	35.25 ± 0.63	25.98 ± 0.45
3.	Extract 40% absolute ethanol	85.96 ± 0.03	59.30 ± 0.96	28.51 ± 0.58
4.	Extract 60% absolute ethanol	81.52 ± 0.06	58.39 ± 0.52	26.21 ± 0.56
5.	Extract absolute ethanol 96%	83.36 ± 0.70	56.65 ± 0.58	28.62 ± 0.89
6.	Extract 10% glacial acetic acid	87.89 ± 0.30	60.28 ± 0.46	38.83 ± 0.45
7.	Extract aqueous	82.13 ± 0.51	50.98 ± 0.35	21.23 ± 0.23
Type 3				
1.	Extract absolute methanol	89.27 ± 0.71	56.72 ± 0.16	26.31 ± 0.17
2.	Extract 20% absolute ethanol	84.80 ± 0.30	36.31 ± 0.12	26.75 ± 0.15
3.	Extract 40% absolute ethanol	86.76 ± 0.07	58.44 ± 0.15	29.17 ± 0.18
4.	Extract 60% absolute ethanol	84.52 ± 0.71	59.43 ± 0.13	27.13 ± 0.13
5.	Extract absolute ethanol 96%	85.36 ± 0.30	57.71 ± 0.17	28.72 ± 0.12
6.	Extract 10% glacial acetic acid	88.87 ± 0.17	61.33 ± 0.15	38.91 ± 0.15
7.	Extract aqueous	84.33 ± 0.72	52.71 ± 0.14	24.73 ± 0.13

The extracts show a high neutralising activity of the DPPH radical. The extract in acetic acid has an intensive scavenger activity, even at low concentrations. Vitamin C plays an important role in food metabolism, since the human organism cannot synthesise or store it [34].

It was calculated the inhibiting concentration 50 (IC_{50}–extract concentration that neutralises 50% of the DPPH radical) and the results are presented in Table 3. For type 1 of seeds of *Lens Culinaris*, it is to be noted the ethanol extract 60% (77.42±1.16) whose antioxidant capacity is maximal and the aqueous extract (21.23±0.23) with the lowest antioxidant activity (remarkable, nevertheless). Ethanol extracts have the most intense scavenger effect, in direct correlation with both total phenol content of determined samples and the total determined flavonoid content.

For the second type of seeds, the maximum antioxidant activity is reached for the extract 10% glacial acetic acid, 87.89 ± 0.30 %, and the minimum corresponds to the aqueous extract 21.23±0.23.

Regarding the third type of seeds, the highest antioxidant capacity resulted for the absolute methanol extract 89.27 ± 0.71, while the minimum, is again, corresponding to the aqueos extract with the value of 24.73 ± 0.13

All extracts considered, for each of the three types of seeds, have a remarkable antioxidant activity.

To determine total flavonoid content of the extracts from the studied *Lens culinaris* Medikus, was used the standard curve for quercetol, obtained from solutions having diverse concentrations.

In phenolic acids, was monitored the characteristic signal at 280 nm (Figure 1), and in flavonoids, was monitored the characteristic signal at 340 nm (Figure 2).

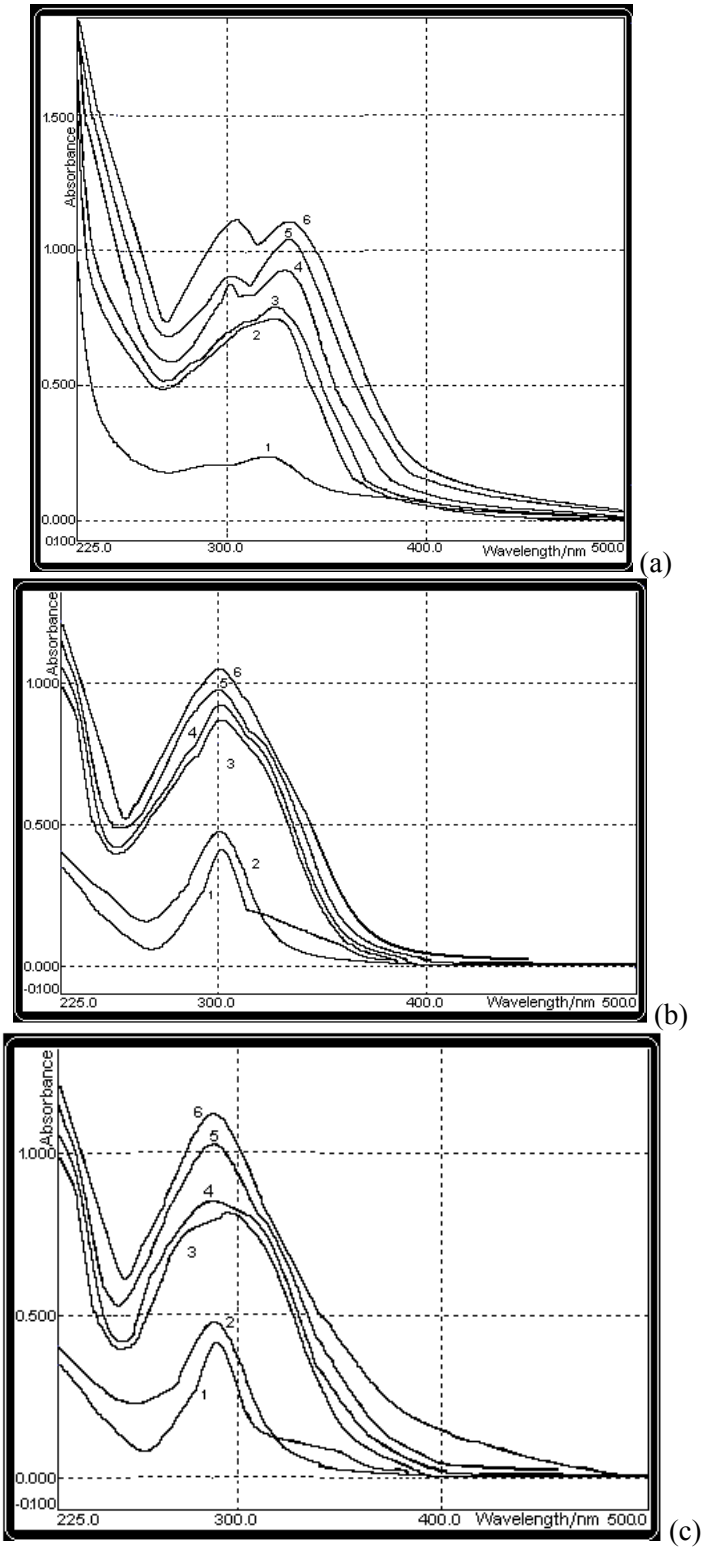
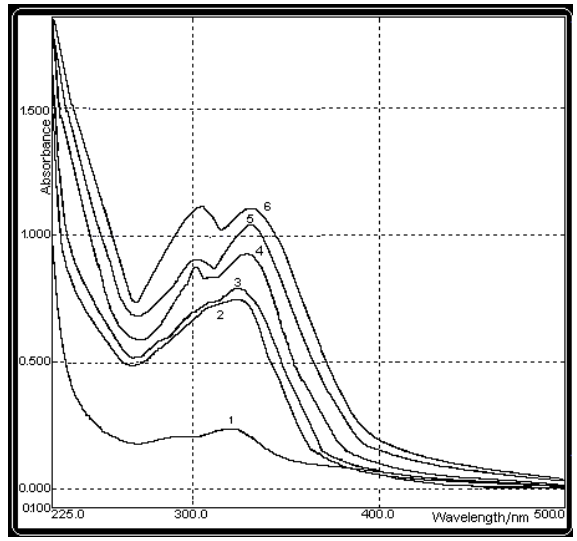
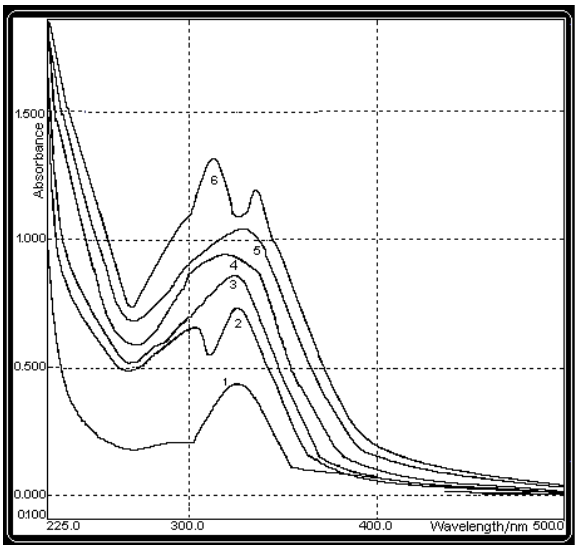


Fig. 1. UV–Vis spectrum of the extracts from Lens culinaris Medikus (specific print within the range 255–526 nm with details concerning maximum absorbance peak specific to phenol acids) (a) type 1, (b) type 2, (c) type 3



(a)



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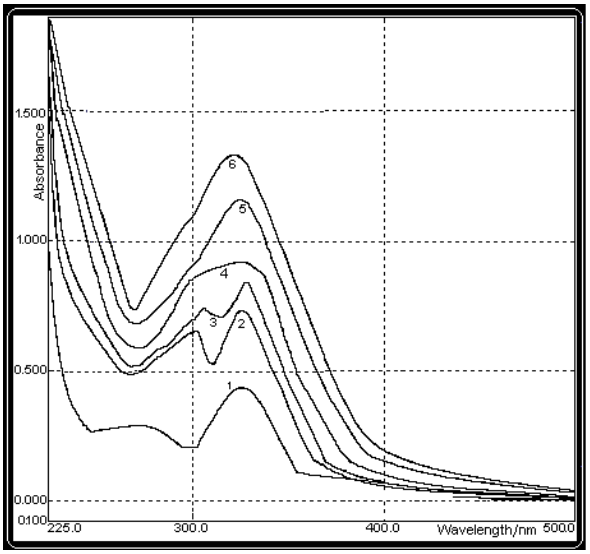

(c)

Fig. 2. UV–Vis spectrum of the extracts from Lens culinaris Medikus (specific print within the range 255–526 nm with details concerning maximum absorbance peak specific to flavonoids) (a) type 1, (b) type 2, (c) type 3

Absorption spectra in the UV-VIS domain are involving electrons transitions between the ground state and an excited electronic state of molecules, transitions are allowed between the same types of molecular orbit, which occur with electron spin conservation.

In addition to spin conservation, there is some selection rules, the transitions that are allowed by these rules leading to absorption characterized by molar extinction coefficients *e,* defined by the Lambert-Beer law, with values ranging from 10^4–10^5, the transitions with molar extinction coefficients between 1-10^3 being "forbidden".

The substituents that make possible the UV–VIS absorption are chromofore groups that are, in general, groups of electrons π that can interact with the electron system π in the rest of the molecule, thus expanding the dislocation of $–NO_2$, –NO, –CHO, –COOH. The substituents that, together with chromophores, produce both an alteration of the position and the intensity of the absorption are auxochrome groups. They are saturated groups of atoms of the –OH, $–NH_2$, –Cl type. The UV–VIS electronic spectra, though less characteristic and supplying general information concerning the molecular structure of the compounds concerning the presence or absence in the molecule of chromofore groups, play, nevertheless, an important role in the study and clarification of the structure of organic compounds.

In this study, we have evaluated the antioxidant activity of the extracts from three types of seeds of *Lens culinaris* Medikus by assessing the neutralising effect of the DPPH radical. Antioxidant activity of the analysed extracts was directly proportional with their concentration value. Extracts from *Lens culinaris* Medikus can be considered promising sources of natural antioxidants in the prevention of diseases mediated by free radicals.

4. Conclusions

Extracts from *Lens culinaris* Medikus were evaluated by assessing the neutralizing effect of the DPPH radical. Tested extracts showed a remarkable antioxidant activity, the extract from *Lens culinaris* Medikus, type 3, in methanol had the most intense scavenger effect, (Table 3). The antioxidant activity of the analysed extracts was directly proportional with their concentration value.

In conclusion, extracts from *Lens culinaris* Medikus can be considered promising sources of natural antioxidants in the prevention of diseases mediated by free radicals.

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