Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Screening the Antioxidant and Antimicrobial Properties of the Extracts from Plantain (*Plantago Major* L.) Leaves

Ivana T. Stanisavljević[a]; Saša S. Stojičević[a]; Dragan T. Veličković[b]; Miodrag L. Lazić[a]; Vlada B. Veljković[a]
[a] Faculty of Technology, Leskovac, Serbia [b] Zdravlje-Actavis, Leskovac, Serbia

To cite this Article Stanisavljević, Ivana T. , Stojičević, Saša S. , Veličković, Dragan T. , Lazić, Miodrag L. and Veljković, Vlada B.(2008) 'Screening the Antioxidant and Antimicrobial Properties of the Extracts from Plantain (*Plantago Major* L.) Leaves', Separation Science and Technology, 43: 14, 3652 — 3662
To link to this Article: DOI: 10.1080/01496390802219091
URL: http://dx.doi.org/10.1080/01496390802219091

Separation Science and Technology, 43: 3652–3662, 2008

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390802219091

Taylor & Francis
Taylor & Francis Group

Screening the Antioxidant and Antimicrobial Properties of the Extracts from Plantain (*Plantago Major* L.) Leaves

Ivana T. Stanisavljević,[1] Saša S. Stojičević,[1] Dragan T. Veličković,[2] Miodrag L. Lazić,[1] and Vlada B. Veljković[1]
[1]Faculty of Technology, Leskovac, Serbia
[2]Zdravlje-Actavis, Leskovac, Serbia

Abstract: Antioxidant and antimicrobial activities of the extracts obtained by classical (maceration) and ultrasonic (40 kHz) extraction from dry *Plantago major* leaves were compared. The antioxidant activities of extracts obtained by ultrasonic and classical extraction were 0.87 ± 0.02 and 0.85 ± 0.02 μg/μg DPPH, respectively. Ultrasound positively affected the extractive substance yield and the kinetics of extraction, but the extract obtained by classical extraction contained higher total contents of phenolic compounds and flavonoids than that obtained by ultrasonic extraction. Extracts of *P. major* showed better antimicrobial activity against the yeasts than against the bacteria.

Keywords: Antioxidant activity, antimicrobial activity, extraction, flavonoids, *Plantago major* L., total phenols

INTRODUCTION

Common plantain (*Plantago major* L.) is a perennial plant that belongs to the Plantaginaceae family (1). The genus *Plantago* comprises of 265 species having a worldwide distribution (2), preferring light (sandy) and medium (loamy) soils, mainly on roadsides, waste places, and temperate zones (3,4). *Plantago* species are astringents, diuretics, expectorants, demulcents (2,3), emollients, antibacterials (2), and antivirals (2,5).

Received 22 October 2007; accepted 11 February 2008.
Address correspondence to Vlada B. Veljković, Faculty of Technology, Bulevar oslobodenja 124, 16000 Leskovac, Serbia. Tel.: + 381 16 247 203; Fax: + 381 16 242 859. E-mail: velijkovicvb@yahoo.com

Many people know *P. major* only as a weed, but it is an old medicinal plant known for centuries for its antioxidant and immunomodulatory activities (6). This plant is commonly used as a medicinal herb in the treatment of a number of diseases related to the skin, respiratory organs, digestive organs, reproduction, circulation, cancer prevention, infectious diseases, for pain relief, and for reducing fever (1). It has also been used as a remedy for haemorrhoids, diarrhea, and cystitis (3), while crushed leaves are used as a hemostatic (7). A transdermal patch containing an extract of *P. major* produces a diminished desire for tobacco (i.e., nicotine) (8).

P. major contains biologically active compounds, such as polysaccharides, lipids, phenolic compounds, flavonoids, iridoid glycosides, terpenoids (1,5), benzoic compound (vanillic acid) (5), tannins, saponins, and sterols (2). The main caffeic acid derivative in *P. major* is plantamajoside having anti-inflammatory, antioxidant, and antibacterial activities (1). Flavonoids, such as apigenin, baicalein, baicalin, luteolin, hispidulin, plantaginin, scutellarein, nepetin, and homoplantaginin, were isolated from *P. major* (1,7), which are known for their biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions as well as for their capability of inducing carcinoma cell death (9).

The total fatty acid content in *P. major* leaves is 286 mg per 100 g of fresh plant material; the major fatty acids were linolenic, linoleic, and palmitic acid, but smaller amounts of stearic, oleic, and myristic acid were also detected (6). *Plantago* leaves contain polysaccharides such as plantaglucide, glucomannan, PMIa (31% arabinose, 32% galactose, 6% rhamnose, and 7% galacturonic acid) and PMII (containing a smooth polygalacturonan and two different ramified regions) (1). Also, raffinose and stachyose are isolated from the leaves (0.3 and 4.5 mg/g dry weight, respectively) (1). *Plantago* contains mucilage, silicic acid, zinc, silica, and potassium (3).

The aqueous whole plant extract of *P. major* has a slight antiviral activity against herpes simplex virus (types: HSV-2) (10). This is attributed to the presence of water-soluble phenolic compounds (such as caffeic, chlorogenic, ferulic, and p-coumaric acid) exhibiting antiviral activities against HSV and adenovirus (ADV). The methanolic extract of the whole plant has no antiviral activity (11). Also, these extracts possess a broad-spectrum of antileukemia and anticarcinoma activities (5). Several studies have reported that aqueous extracts inhibited *Bacillus subtilis* (7) and *Mycobacterium tuberculosis* (12) growth, while the hexane extract inhibited the growth of *Escherichia coli* (7). The methanol and chloroform aerial part extracts weakly inhibit the growth of *Bacillus subtilis* and *Escherichia coli*, respectively (7). Also, the methanolic whole plant extract shows significant antimycobacterial activity against *Mycobacterium phlei* (12).

The purpose of this study was to compare antioxidant and antimicrobial effects of the extracts obtained from *P. major* leaves by ultrasonic and classical solvent extraction (UE and CE, respectively). An aqueous ethanol solution (70% by volume) was used as extracting solvent because of its suitable properties such as low toxicity to higher life forms and easily evaporation at a low heat. Antimicrobial effects, antiradical activity, antioxidant capacity, total phenolics, and flavonoids of extracts were determined by *in vitro* assays.

EXPERIMENTAL

Materials

Plant Materials

Dried leaves were purchased from "Adonis" (Sokobanja, Serbia). The plant material was milled by an electrical mill with a fast-rotating knife (15,000 rpm; 1 min) immediately before extraction. The content of extractive substance (ES), determined by using a Soxhlet extraction apparatus (9 hours or 12 extraction cycles; the plant material-to-solvent ratio: 1:10 g/ml) and an aqueous ethanol solution (70% by volume), was 29.0 g per 100 g of dry plant material. Moisture content, determined by drying at 105°C to constant weight, was 11.8%.

Chemicals and Reagents

Ethanol was obtained from Zorka-Pharma (Šabac, Serbia). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazil (DPPH), gallic acid, rutin, Erythromycin [114-07-8] and Tylosin tartarat [74610-55-2] were obtained from Sigma (St. Louis, MO). Sodium carbonate, potassium acetate, and aluminium chloride were purchased from Merck-Alkaloid (Skopje, FYR Macedonia).

Seven microorganisms were selected to test the antimicrobial activity: *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 9027*, *Bacillus subtilis ATCC 6633*, *Staphylococcus aureus ATCC 6538*, *Candida albicans ATCC 10231*, *Saccharomyces cerevisiae ATCC 9763* and *Aspergillus niger ATCC 16404* (Oxoid, England). Sabouraud dextrose agar – SDA (Merck) was used for the yeasts and the molds and Trypton soya agar – TSA (Merck) for culturing of the bacteria. Plate count agar (Merck) was used for determination of the total number of microorganisms (CFU/ml).

Extraction of Plants Materials

Ultrasonic Extraction

Samples of ground leaves (5 g) were extracted with 70% ethanol (50 ml). Sonication was performed in 2.5, 5, 10, 20, 40, and 60 minutes using an ultrasonic cleaning bath (Sonic, Niš, Serbia; internal dimensions: $30 \times 15 \times 20$ cm; total nominal power: 3×50 W; and frequency: 40 kHz). The temperature was maintained at $25 \pm 0.1^{\circ}$C. At the end of the extraction process, the liquid extract was separated from the solid residue by vacuum filtration. The solid residue was washed twice with fresh solvent (20 ml). The filtrates were collected and the solvent was evaporated in a rotary vacuum evaporator at 40°C.

Classical Solvent Extraction

Samples of ground leaves (5 g) were extracted with 70% ethanol (50 ml) at 25°C in 2.5, 5, 10, 20, 40, and 60 minutes. Dry extracts were obtained using the same procedure as described in the previous section.

DPPH Radical Scavenging Activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (13). Equal volumes (2.5 ml) of different concentrations of the extracts in 70% ethanol were added to an ethanolic solution of DPPH (0.3 mM, 1 ml). After 30 minutes at room temperature, the absorbance was measured spectrophotometrically at 517 nm (VARIAN Cary-100). Ethanol (1.0 ml) plus the extract solution (2.5 ml) was used as a blank, while ethanol (2.5 ml) and the DPPH solution (1.0 ml) was used as a control. DPPH scavenging capacity in percent (%) was calculated using the following equation:

$$\text{DPPH scavenging capacity}(\%) = 100 - \frac{(\text{Ab}_{\text{of sample}} - \text{Ab}_{\text{of blank}}) \times 100}{\text{Ab}_{\text{of control}}} \quad (1)$$

Total Phenols Determination

The total phenolic content of the extracts was determined using Folin-Ciocalteu reagent (14) with gallic acid as a standard. Although the

spectrophotometric method overestimates the polyphenolic content as compared to the chromatographic method, it is a useful analytical tool for the routine analysis of polyphenols and for the determination of differences among total phenols extraction efficiencies of different techniques. Each of the plant extracts (0,2 ml, 1 mg ml^{-1}) or gallic acid was mixed with Folin Ciocalteu reagent (1 ml) and aqueous sodium carbonate (0.8 ml, 7.5%). The mixture was shaken thoroughly and allowed to stand at room temperature for 30 minutes. Then the absorbance of the reaction mixture was measured at 765 nm. The concentration of total phenolic compounds in the extracts, determined as mg gallic acid equivalents (GAE)/g dry extract, was calculated by using the following standard curve ($R^2 = 0.999$):

$$\text{Absorbance at } 765\,\text{nm} = 12.722\ c_{\text{gallic acid}}(\text{mg ml}^{-1}) + 0.0034 \quad (2)$$

which was valid up to 200 mg of gallic acid per liter.

Total Flavonoids Determination

The total flavonoid content was determined by the aluminum chloride colorimetric method (15). Each extract (0.5 ml, 4 mg ml^{-1}) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the reaction mixture absorbance was measured at 415 nm. Rutin was chosen as a standard to prepare a standard curve ($R^2 = 0.992$):

$$\text{Absorbance at } 415\,\text{nm} = 7.2328\ c_{\text{rutin}}\ (\text{mg ml}^{-1}) - 0.2286 \quad (3)$$

which was valid up to 100 mg of rutin per liter. The data were then converted into mg rutin equivalents (RE)/g dry extract.

Antimicrobial Activity

An agar well-diffusion method was employed for the determination of antimicrobial activities of extracts (16).

A 24 hours old culture grown on obliquely agar (0.1 ml) with the sterile 0.9% NaCl (10 ml) was mixed with 10 ml of the nutritive medium (ca. 10^6 CFU/ml). A petridish (86 mm internal diameter) was filled with the suspension. The wells (10 mm in diameter) were cut from the agar and 30 μL of extract solution (concentration 20 mg/ml in methanol) was delivered into them. Erythromycin (997 μg/mg) and Tylosin tartarat (950 μg/mg) were used as a positive control (at the concentration in

methanol of 0.05 mg/ml). All dilutions were filtrated using 0.45 μm membrane filters (Sartorius, Germany). After incubation at 37°C for 24 hours, agar plates were examined for any zones of inhibition. Diameters of zones of inhibition (mm) were measured by a Fisher Lilly Antibiotic Zone Reader (USA).

Statistical Analysis

All data were represented as mean for three independent measurements. Comparison of means was analyzed by Student's t test and differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Antioxidant Activity

The ethanolic *P. major* leaves extracts obtained by the two extraction techniques were subjected to screening for their possible antioxidant activities. Three complementary test systems, namely DPPH free radical-scavenging, total phenolic compounds and total flavonoid content, were used for this purpose.

The radical-scavenging effects of extracts obtained by CE and UE are tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical. The percentage of DPPH reduction was plotted against the plant extract

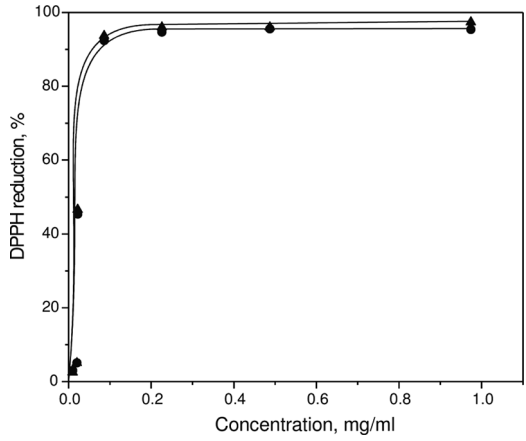

Figure 1. Antioxidant activity of the *P. major* extracts obtained by CE (triangles) and UE (circles).

Table 1. Total antioxidant capacity, phenolic compounds and flavonoids of *P. major* extracts obtained by UE and CE*

Extraction tehnique	EC_{50}, μg/μg DPPH	Total phenolic content, mg GAE/g dry extract	Total flavonoids, mg RE/g dry extract
UE[a]	0.87 ± 0.02	58.7 ± 0.1	35.2 ± 0.9
CE[a]	0.85 ± 0.02	60.9 ± 0.1	40.0 ± 0.9
CE[b]	0.32	31 ± 4	25.15 ± 0.2

*Data were expressed as the mean of three replicates ± standard deviation.

[a]Ethanol, 70%; 1:10 g/ml; 25°C; and 40 minutes.

[b]Methanol; 1:10 g/ml; temperature: not available; and 48 hours; taken from Pourmorad, F.; Hosseinimehr, S.J.; Shahabimajd, N. (2006) *Afr. J. Biotechnol.*, *5*(11): 1142.

concentration in Fig. 1. For comparison of the antioxidant activities of the extracts, the concentration of ES producing 50% reduction of the radical absorbance (EC_{50}) was used as an index. The EC_{50} values were calculated by the sigmoid non-linear regression method. The EC_{50} value of each extract was converted to μg/μg DPPH (Table 1). The EC_{50} value was slightly higher for the extract obtained by UE than for that obtained by CE, but the difference observed was not statistically significant with 95% confidence interval.

The data given in Table 1 shows also that the extract obtained by CE had a 3.6% higher amount of phenolics compound and 12% higher content of flavonoids than that obtained by UE. The differences observed were statistically significant with 95% confidence interval. This was explained by oxidation and degradation of the some bioactive compounds in the interaction with highly reactive hydroxyl radicals formed during sonication of the aqueous solution (18).

Compared to the previous study (17), both extracts obtained in the present work had a higher antioxidant activity and higher contents of total phenolic and flavonoids compounds. This was attributed to different climate and soil conditions affecting growth of the plants used in the two studies.

Antimicrobial Activity

The antimicrobial effect of *P. major* extracts obtained by the two different extraction techniques were tested against two Gram-positive bacterial species (*Bacillus subtilis, Staphylococcus aureus*), two Gram-negative bacterial species (*Escherichia coli, Pseudomonas aeruginosa*), two yeast species (*Saccharomyces cerevisiae, Candida albicans*), and one mould (*Aspergillus niger*). Methanol had no inhibitory effect on any of the test

Table 2. Antimicrobial activity of *P. major* extracts and antibiotic sensitivity of microorganisms (zone size, mm)

	Extracts, 20 mg/ml		Antibiotics, 0.05 mg/ml	
Test microorganism	UE[a]	CE[a]	Erytromycin	Tylosin tartarat
Escherichia coli ATCC 25922	11.7 ± 0.3	10.9 ± 0.1	21.2 ± 0.1	18.4 ± 0.0
Pseudomonas aeruginosa ATCC 9027	11.3 ± 0.4	11.2 ± 0.2	25.2 ± 0.9	17.6 ± 0.1
Bacillus subtilis ATCC 6633	10.7 ± 0.1	11.0 ± 0.2	19.1 ± 0.1	17.3 ± 0.7
Staphylococcus aureus ATCC 6538	10.7 ± 0.4	11.3 ± 0.4	23.6 ± 0.0	18.5 ± 0.6
Candida albicans ATCC 10231	16.0 ± 0.2	17.6 ± 0.1	23.0 ± 0.0	16.2 ± 0.3
Saccharomyces cerevisiae ATCC 9763	19.2 ± 0.1	23.5 ± 0.1	–[b]	–
Aspergillus niger ATCC 16404	–	10.1 ± 0.1	20.5 ± 0.7	18.1 ± 0.1

[a]Ethanol, 70%; 1:10 g/ml; 25°C; and 40 minutes.
[b]No antimicrobial activity.

microorganisms in the control treatment. The results of these tests, as well as the effects of two control antibiotics, are presented in Table 2.

Independently of the extraction technique employed, the water-ethanolic extracts of *P. major* leaves showed better antimicrobial activity against yeasts than against both Gram-positive and Gram-negative bacteria. No statistically significant difference (95% confidence interval) in antimicrobial activities of the two extracts obtained by the different recovery techniques was shown in the cases of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Saccharomyces cerevisiae* and *Candida albicans*. There was a difference between the antimicrobial activity of the ethanolic extract obtained from aerial parts of *P. major* which were collected from Jordan and the extracts obtained in the present study. The former extract showed no activity against *C. albicans* (19), probably due to different growth and extraction (96% ethanol; 5 days maceration at room temperature) conditions. The extract obtained by UE was more effective against *Escherichia coli*, while the extract obtained by CE exhibited a slightly higher antimicrobial activity against *Bacillus subtilis* and both yeasts (with 95% confidence interval). The most susceptible microorganism was *Saccharomyces cerevisiae* (19.2 ± 0.5 and 23.5 ± 0.1 mm diameter for the extracts obtained by CE and UE, respectively). The UE extract was not active and the CE extract exhibited a minimum activity against the mould tested. Our test confirmed previously reported inhibition of *Bacillus subtilis* growth by an aqueous *P. major* leaves extract (7).

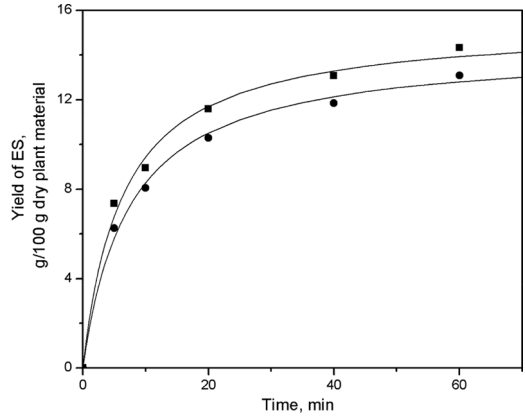

Figure 2. Time course variation of the extractive substances yield from the *P. major* leaves (CE: circles and UE: squares).

Kinetics of Solvent Extractions

Variations of the ES yield with the progress of CE and UE from *P. major* are shown in Fig. 2. The yield of ES increased with extraction time in a characteristic way. In accordance with the two-step mechanism (20), the increase of the ES yield was rapid in the initial stage of the process (up to about 10 minutes), due to dissolution of the ES near the particle surface (so called washing). In the latter stage, it slowed down because of slow ES diffusion through the solid particles towards the liquid extract (so called slow extraction). Based on this simplified mechanism, the kinetics of UE and CE was modeled by the unsteady-state diffusion through plant material; the kinetic equation of this two-parametric model and its linearized forms are as follows (20):

$$q/q_0 = (1 - b)\exp(-kt) \quad (1)$$

$$\ln(q/q_0) = \ln(1 - b) - kt \quad (2)$$

where b is the washing coefficient (1); k is the slow extraction coefficient (min^{-1}); q is the ES content in the plant material during the extraction (g/100 g); q_0 is the ES content initially present in the plant material ($= 29.0$ g/100 g); and t is time (minutes).

Parameters of the kinetic model were calculated from the experimental data by the linear regression method using equation 2 (values of the linear correlation coefficient were 0.94 and 0.99 for CE and UE, respectively). Both parameters were higher for the UE ($b = 0.35$ and $k = 4.4 \cdot 10^{-3}$ min^{-1}) than for the CE ($b = 0.34$ and $k = 2.7 \cdot 10^{-1}$ min^{-1}).

According to these results, sonication greatly affected diffusion of ES through the plant material but little washing. The ultrasonic enhancement of extraction was attributed to the cell destruction, better solvent penetration, and mass transfer intensification (21). For herbal materials, both washing and slow extraction are affected by sonication (18). The UE gave a higher ES yield than CE as a benefit of ultrasound action; the yields in 60 minutes were 14.3 and 13.1 g/100 g of dry plant material, that is about 50 and 45% of the initial content, respectively.

CONCLUSION

The present study suggests that 70% water-ethanolic extracts of *P. major* leaves are a potential source of active natural and non-toxic substances such as natural antioxidants and antimicrobials. Ultrasound affected positively both the kinetics of extraction and the yield of ES from ground leaves of *P. major*. The contents of total phenolic compounds and flavonoids were reduced under sonication, probably because of degradation of some bioactive compounds by interaction with highly reactive hydroxyl radicals formed by ultrasound action, but there are no statistically significant differences between the antioxidant activities of the two extracts. The extracts of *P. major* leaves, independently of the recovery technique employed, were effective in inhibition on the growth of the all tested strains, except on the fungal growth.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Environmental Protection, Republic of Serbia projects OI 142073B.

REFERENCES

1. Samuelsen, A.B. (2000) The traditional uses, chemical constituents and biological activities of *Plantago major* L. A review. *J. Ethnopharmacol.*, *71* (1–2): 1.
2. Jurišić Grubešić, R.; Vuković, J.; Kremer, D.; Vladimir-Knežević, S. (2005) Spectrophotometric method for polyphenols analysis: Prevalidation and application on *Plantago* L. species. *J. Pharmaceut. Biomed.*, *39* (3–4): 837.
3. The Australian Naturopathic Network. (2002) May 20, 2002., May 28, 2007., <http://www.ann.com.au/herbs/Monographs/plantmaj.htm>
4. Plants For A Future. (2000), May 28, 2007., http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Plantago+major

5. Chiang, L.; Chiang, W.; Chang, M.; Lin, C. (2003) In vitro cytotoxic, antiviral and immunomodulatory effects of *Plantago major* and *Plantago asiatica*. *Am. J. Chinese Med.*, *31* (2): 225.
6. Liu, L.; Ho, P.; Zhou, Y.; Hocart, C.; Zhang, R. (2002) Fatty acid profiles of leaves of nine edible wild plants: An Australian study. *J. Food Lipids*, *9*: 65.
7. Velasco-Lezama, R.; Tapia-Aguilar, R.; Román-Ramos, R.; Vega-Avila, E.; Pérez-Gutiérrez, M.S. (2006) Effect of *Plantago major* on cell proliferation in vitro. *J. Ethnopharmacol.*, *103* (1): 36.
8. Cody, M.E. (1998) *Plantago major* transdermal patch for use in treating a tobacco or nicotine habit. U.S. Patent 5716635, February 10.
9. Pietta, P. Flavonoids as antioxidants. (2000) *J. Nat. Prod.*, *63* (7): 1035.
10. Chiang, L.C.; Chiang, W.; Chang, M.Y.; Ng, L.T.; Lin, C.C. (2002) Antiviral activity of *Plantago major* extracts and related compounds in vitro. *Antivir. Res.*, *55* (1): 53.
11. McCutcheon, A.R.; Roberts, T.E.; Gibbons, E.; Ellis, S.M.; Babiuk, L.A., Hancock, R.E.W.; Towers, G.H.N. (1995) Antiviral screening of British Columbian medicinal plants. *J. Ethnopharmacol. 49* (2): 101.
12. Gautam, R.; Saklani, A.; Jachak, S.M. (2007) Indian medicinal plants as a source of antimycobacterial agents. *J. Ethnopharmacol.*, *110* (2): 200.
13. Choi, C.W.; Kim, S.C.; Hwang, S.S.; Choi, B.K.; Ahn, H.J.; Lee, M.Y.; Park, S.H.; Kim, S.K. (2002) Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant. Sci. 163* (6), 1161.
14. Singleton, V.L.; Rossi, J.A. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, *16* (3): 144.
15. Chang, C.; Yang, M.; Wen, H. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Chern, J. Food Drug Anal.*, *10* (3), 178.
16. NCCLS (National Committee for Clinical Laboratory Standards). (1999) Performance Standards for antimicrobial susceptibility testing. 9th International Supplement. M100-S9.
17. Pourmorad, F.; Hosseinimehr, S.J.; Shahabimajd, N. (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.*, *5* (11): 1142.
18. Vinatoru, M. (1999) An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrason. Sonochem.*, *8* (3): 303.
19. Hassawi, D.; Kharm, A. (2006) Antimicrobial activity of some medicinal plants against. *Candida albicans.*, *J. Biol. Sci.*, *6* (1): 109.
20. Veljković, V.; Milenović, D. (2002) Analiza ekstrakcije rezinoida kantariona (*Hypericum perforatum* L.) II. Poređenje modela kinetike ekstrakcije. *Chem. Ind.* (*Belgrade*), *56* (2): 60.
21. Vinatoru, M.; Toma, M.; Mason, T. (1999) Ultrasonically assisted extraction of bioactive principles from plants and their constituents. *J. Adv. Sonochem.*, *5*: 209.