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Antimutagenicity and phytoconstituents of Egyptian *Plantago albicans* L.Sahar Salah El Din El Souda^{1*}, Reda Sayed Mohammed^{2*}, Mona Mohamed Marzouk³, Maha Aly Fahmy⁴, Zeinab Mohamed Hassan⁴, Ayman Ali Farghaly⁴¹Department of Chemistry of Natural Compounds, National Research Centre, El-Tahrir St., Dokki, 12622, Cairo, Egypt²Department of Pharmacognosy, National Research Centre, El-Tahrir St., Dokki, 12622, Cairo, Egypt³Department of Phytochemistry and Plant Systematics, National Research Centre, El-Tahrir St., Dokki, 12622, Cairo, Egypt⁴Department of Genetics and Cytology, National Research Centre, El-Tahrir St., Dokki, 12622, Cairo, Egypt

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ABSTRACT

Objective: To evaluate the safety and the possible antimutagenic effect of the defatted aqueous ethanol extract and polysaccharide extract of *Plantago albicans* L. areal parts on the genotoxicity of the anticancer drug cyclophosphamide and to investigate the chemical constituents of these extracts.

Methods: Two doses of each extract (10 and 15 mg/kg body weight) were administered orally to albino mice 7 d prior to treatment of cyclophosphamide (20 mg/kg body weight, *i.p.*). Chromosomal aberration analysis and micronucleus test were performed. Phytochemical investigation of defatted aqueous ethanol extract was carried out through chromatographic tools, chemical and physical analysis to know the active constituents of these extracts.

Results: Groups of mice administered with defatted aqueous ethanol extract and polysaccharide extract prior to cyclophosphamide treatment showed statistically significant reduction in the percentage of chromosomal aberrations and micronuclei in bone marrow cells in a dose-dependent manner. Defatted aqueous ethanol extract gave more effective protection in comparison with polysaccharide extract. Phytochemical investigation of defatted aqueous ethanol extract allowed isolation of gallic acid, methylgallate, tri, tetra, penta-galloyl- β -glucopyranoside, kaempferol-3-O- β -sophro pyranoside, quercetin-3-O- β -glucopyranoside-7-O- α -rhamnopyranoside, kaempferol and quercetin, for the first time from this species.

Conclusions: The results of the present work demonstrated that *Plantago albicans* phytoconstituents play a protective role against genotoxicity of the drug cyclophosphamide.

1. Introduction

Antimutagenic properties elicited by plant species have full range of prospective applications in human healthcare. Herbal remedies and phytotherapeutic drugs containing active principles are currently developed to protect against free radicals that attack DNA and the widespread outcomes such as aging and cancer[1]. The genus *Plantago* belonging to family Plantaginaceae comprises about 265 species of small and inconspicuous plants commonly called plantains[2]. Extensive traditional use and modern medicinal applications of several *Plantago* species are

a consequence of their remarkable variety of curative properties: astringent, styptic, antimicrobial, expectorant, diuretic and demulcent[3,4]. *Plantago* species were also reported for different biological activities: hepatoprotective, anti-inflammatory[5,6], antiviral, immunomodulatory[7], antiadipogenic, antidiabetic[8], antirheumatic[9], antioxidant and anticancer[10–13]. Furthermore, some *Plantago* species are included in human diet as fresh salads, soups, side dish, and they can also be used as herbal tea[14]. Plantains contain several classes of biologically active compounds such as polysaccharides[15], flavonoids[16], phenolic compounds[17], monoterpenoids and triterpenoids[18]. In addition, caffeoyl phenylethanoid glycosides and iridoids glycosides which constitute the most characteristic classes of compounds showed good correlation in their chemotaxonomy significance[19–21]. *Plantago albicans* L. (*P. albicans*) is an annual plant wide distributed in Egypt (North Sinai, Isthmic Desert, Galala Desert and Mountainous Southern Sinai). Apigenin and two phenylpropanoids polumoside and

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verbascoside were isolated from *P. albicans*, which showed moderate cytotoxic activity against both normal and the viral transformed mouse fibroblast cell lines^[22]. Flavonoid profile of *P. albicans* indicated presence of apigenin-7-O-glucoside, luteolin-7-O-glucoside, chrysoeriol-7-O-gentiobioside, apigenin, luteolin and chrysoeriol^[23]. In this study, we assessed antimutagenic activity of the defatted ethanol and polysaccharides extracts of *P. albicans* as well as identification of their chemical composition.

2. Material and methods

2.1. General experiment

Ultra-violet spectra were recorded on UV visible spectrophotometer (Labomed Inc., USA). NMR measurements were carried out using Jeol EX-500 spectroscopy: 500 MHz (¹H-NMR) and 125 MHz (¹³C-NMR). Electron ionization mass spectrometry was performed using Finnigan-MatSSQ 7000 spectrometer, high resolution mass spectrometer for galloyl compounds, JMS HX 110 at Center for Instrumental Analysis Hokkaido University Japan. and electrospray ionization mass spectrometry using LCQ Advantage Thermo Finnigan spectrometer. For micronucleus and chromosomal aberrations, sample examination was carried out with light microscope (Olympus, Saitama, Japan). For fractionation of defatted ethanol extract, column chromatography (CC) was performed using Diaion® HP-20, (Supelco-13606, Sigma-Aldrich, Bellefonte, PA, USA) methanol/water. CC was carried out on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, D-30926 Seelze Hanver, Germany) using methanol/H₂O as eluent. Paper chromatography (PC) (descending) Whatman No.1 and 3 mm papers, using solvent systems: (1) water, (2) 15% HOAc (water: acetic acid 85:15), (3) 50% HOAc, (4) BAW (butanol: acetic acid: water 4:1:5, upper layer) and (5) BBWP (benzene: butanol: water: pyridine 1:5:3:3, upper layer). BAW and BBWP were used for sugar identification. The purification was performed in Sephadex LH-20 (Pharmazia, Sweden). Solvents used for plant extraction were from SDFCL (Industrial Estate, 248 Worli Road, Mumbai-30, India).

2.2. Plant material

A fresh sample of *P. albicans* was collected from Borg El-Arab, near Bremly Cave, Egypt in March 2007 and identified by Dr. Mona M. Marzouk. A voucher specimen (No. 810) was deposited in the National Research Centre herbarium (CAIRC).

2.3. Extraction and isolation

The dried powdered aerial parts of *P. albicans* (350 g) were exhaustively extracted with 70% aqueous ethanol at room temperature till negative Molisch's test^[24]. The extract was filtered and dried under reduced pressure then defatted with dichloromethane to give defatted aqueous ethanol extract (47 g) which was subjected to CC equipped with diaion HP-20 (120×5 cm), starting elution with water then increasing percentage of methanol gradually till 100% methanol. Similar fractions were combined to give five main fractions (A–E). Fraction A was chromatographed on PC using BAW then H₂O

several times, leading to the isolation of compounds 1–3. Fraction B was also chromatographed on PC eluted with BAW and H₂O, leading to the isolation of compounds 4 and 5. Fractions C and D were chromatographed on PC using H₂O as eluent to obtain compounds 6–8. Fraction E was subjected to PC fractionation using 50% acetic acid to obtain compounds 9–11. All compounds were further purified on a Sephadex LH-20 column using methanol as eluent^[25,26]. The remaining powder after extraction was percolated with hot water till complete exhaustion^[24]. The aqueous extract was concentrated to small volume (100 mL), absolute ethanol (300 mL) was added drop wise till complete precipitation. The precipitate was separated by centrifugation, washed several times with absolute ethanol then stirred with acetone, filtered, dried in a vacuum desiccator and weighed, then finally 3 g polysaccharides were obtained.

2.4. High performance liquid chromatography (HPLC) analysis of defatted aqueous ethanol extract

Two grams of Defatted aqueous ethanol extract were dissolved in 5 mL methanol of HPLC grade and analyzed by an Agilent HPLC 1200 series equipped with diode array detector (Agilent Technologies, Waldbronn, Germany). Chromatographic separations were performed using a waters column C18. The binary mobile phase consisted of acetonitrile and 0.1% acidified water with formic acid. The elution profile was: 0–1 min 100% 0.1% acidified water with formic acid (isocratic), 1–30 min 100%–70% 0.1% acidified water with formic acid (linear gradient), 30–35 min 70%–20% 0.1% acidified water with formic acid (linear gradient). The flow rate was 0.3 mL/min and the injection volume was 5 µL. Chromatograms were recorded at 278 nm. This analysis enabled the characterization of phenolic compounds on the basis of their retention time and UV spectra. The isolated compounds were used as reference standards to measure them relatively in the extract. Separated peaks were identified by direct comparison of their retention times with those of standards.

2.5. Acid hydrolysis of the polysaccharide

Polysaccharide extract (0.1 g) was acid hydrolyzed^[27], part of the hydrolyzate (0.5 mL) was subjected to silylation^[28].

2.6. Gas liquid chromatography (GLC) analysis of the polysaccharide hydrolyzate

The silylated sugars (1 µL) was subjected to GLC analysis using ZB-1701 column (30 m×0.25 m×0.25 µm) containing 14% cyanopropyl phenyl methyl as stationary phase. Carrier gas was helium with flow rate at 1.2 mL/min, 10.6 pounds per square inch. Initial temperature was 150 °C for 2 min, increasing by 7 °C/min; final temperature was 200 °C for 20 min. Injector chamber temperature was 250 °C. Flame ionization detector was used as a detector.

2.7. Animals

Male white Swiss mice (*Mus musculus*) aged 9–12 weeks with an average weight of (25.0±2.5) g obtained from the National Research Center, Cairo, Egypt,

were used. Animals were maintained under standard conditions of temperature, humidity and light. The animals were given standard food and water.

2.8. Ethics

Anesthetic procedures and handling of animals were complied with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt and performed ensuring that the animals do not suffer at any stage of the experiment.

2.9. Antimutagenic assay

The antigenotoxic activity of defatted aqueous ethanol extract and polysaccharide extract was performed using micronucleus and chromosomal aberrations test, a sensitive protocol for detection of DNA damage^[29,30]. Animals were divided into ten groups (5 animals each). Group A was used as negative control, Group B (positive control) was treated by injection (*i.p.*) of cyclophosphamide (CP) 20 mg/kg body weight for 24 h. Groups C and D, and E and F were orally treated by gavage, for 7 d, with 10 and 15 mg/kg body weight defatted aqueous ethanol extract and polysaccharide extract of *P. albicans*, respectively. Groups G and H, and I and J were orally treated with 10 and 15 mg/kg body weight of defatted aqueous ethanol extract and polysaccharide extract, respectively, for 7 d before treatment with CP (*i.p.*, 20 mg/kg body weight for 24h).

2.10. Micronucleus test

The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with fetal calf serum^[31]. The cell suspension was centrifuged at 1 000 r/min for 10 min and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread onto clean glass slides and air-dried. The bone marrow smears were made in five replicates and fixed in absolute methanol for 10 min and stained with May–Grünwald/Giemsa at pH 6.8^[32]. Scoring was performed by detecting the number of micronuclei in 1 000 polychromatic erythrocyte cells per animal under magnification 1 000× with a light microscope.

2.11. Chromosomal aberrations test

Mice were injected (*i.p.*) with colchicine 2–3 h before sacrifice. Bone marrow preparations were made according to the technique described by Yosida and Amano^[33]. A total of 100 well-spread metaphases were analyzed per mouse. Metaphases with different types of chromosomal aberrations were recorded under magnification 2 500×^[33].

2.12. Statistical analysis

The significance of the results from the negative control data, and defatted aqueous ethanol extract and polysaccharide extract with CP comparing to CP alone was calculated using student's *t*-test^[34].

3. Results

3.1. Genotoxic effect

The results presented in Tables 1 and 2 showed that the defatted aqueous ethanol extract and polysaccharide extract of *P. albicans* have safe effect on genetic material in both tests examined comparing to the negative control. In addition the two extracts possessed antimutagenic activity. In comparing the positive control with CP-treated mice administered with defatted aqueous ethanol extract or polysaccharide extract, a statistically significant decrease was found in the percentage of micronucleated polychromatic erythrocytes and chromosomal aberrations in bone marrow cells. The rate of protection was proportionally associated to the dose of the extracts. Moreover the defatted ethanol extract gave more effective protection in both tests compared to polysaccharide extract.

Table 1

Frequency of chromosomal aberrations in bone marrow induced after treatments.

Groups	No. of metaphases with different types of aberrations				Abnormal metaphases	
	Chromatid or chromosome gaps	Fragment or break	More than one aberration	Endo-mitosis	No.	%±SE
A	5	6	–	1	12	2.40±0.50
B	5	10	–	–	15	3.00±0.51
C	6	6	–	1	13	2.60±0.24
D	7	11	–	–	18	3.60±0.40
E	7	9	–	–	16	3.20±0.20
F	15	55	32	1	103	20.60±0.40**
G	15	21	3	2	41	8.20±0.37**
H	10	15	7	4	36	7.20±0.74**
I	8	44	13	4	69	13.80±0.86**
J	10	44	3	3	60	12.00±0.83**

There are 5 mice in each group. **: Highly significant ($P<0.01$); SE: stander error. NO. of examined metaphases=500.

Table 2

Percentage of micronuclei in polychromatic erythrocyte induced after treatments.

Groups	No. of micronuclei	% of micronuclei±SE
A	55	1.10±0.57
B	56	1.12±0.61
C	53	1.06±0.51
D	55	1.10±0.54
E	50	1.00±0.42
F	585	11.70±0.54**
G	376	7.52±0.43**
H	337	6.74±5.67**
I	448	8.96±0.50*
J	431	8.62±0.45*

There are 5 mice in each group. No. of polychromatic erythrocytes=5000. *: Significant ($P<0.05$); **: Highly significant ($P<0.01$).

3.2. Phytochemical constituents of defatted aqueous ethanol extract and polysaccharide extract

Eleven phenolic compounds were isolated from defatted aqueous ethanol extract of the aerial parts of *P. albicans*. They were identified as gallic acid, methyl gallate, tri-O-galloyl-β-glucopyranoside, tetra-O-galloyl-β-

glucopyranoside, penta-O-galloyl- β -glucopyranoside (PGG), kaempferol 3-O- β -sophropryanoside, quercetin-3-O- β -glucopyranoside-7-O- α -rhamnopyranoside, luteolin 7-O- β -glucopyranoside, luteolin, kaempferol and quercetin. The structure elucidation of the isolated compounds was carried out through chemical and spectral analysis^[25,26], and co-chromatography with reference samples spectral data coincided well with those previously published^[25,35,36]. HPLC analysis of defatted aqueous ethanol extract allows quantification of the isolated compounds relative to their peak area. Galloyl glucosides including PGG (52.31%), tri-O-galloyl- β -glucopyranoside (12.02%), methyl gallate (5.29%) and tetra-O-galloyl- β -glucopyranoside (4.37%) constitute 75% of the isolated compounds whereas flavonoids represent 6.25% of the total identified compounds (Table 3). GLC of the polysaccharides revealed the presence of xylose (50.40%), arabinose (18.80%), rhamnose (17.00%), galactose (7.27%) and glucose (5.48%) as the major sugars of polysaccharide extract while mannose (0.86%) and glucouronic acid (0.05%) were the minor sugars (Table 4).

Table 3

HPLC analysis of phenolic compounds from *P. albicans* and their relative percentage in defatted aqueous ethanol extract.

Compounds	Retention time	Relative
	(min)	(%)
Gallic acid	11.200	1.03
Methyl gallate	29.703	5.29
Tri-O-galloyl- β -glucopyranoside	40.259	12.02
Tetra-O-galloyl- β -glucopyranoside	40.856	4.37
PGG	41.853	52.31
Kaempferol 3-O- β -sophropryanoside	18.117	0.71
Quercetin 3-O- β -glucopyranoside-7-O- α -rhamnopyranoside	16.698	0.85
Luteolin 7-O- β -glucopyranoside	23.945	0.92
Luteolin	31.624	0.95
Kaempferol	37.262	1.47
Quercetin	3.670	1.34

Table 4

GLC results of silylated polysaccharide hydrolyzate.

Retention time (min)	Compounds	Relative %
8.19	Arabinose	18.80
8.27	Xylose	50.40
9.32	Rhamnose	17.00
12.17	Galactose	7.27
12.21	Mannose	0.86
12.39	Glucose	5.48
15.95	Glucuronic acid	0.05
	Total identified	99.86

4. Discussion

CP is an alkylating agent widely used in cancer chemotherapy drugs in spite of its toxic side effects. Its cytotoxic effects result from chemically reactive metabolites that alkylate DNA and protein, producing cross-links. The injury of normal tissues is the major limitation of using CP, which gives rise to numerous side effects. It has been reported that oxidative stress mediated disruption of redox balance after CP exposure generates biochemical and

physiological disturbances^[37]. Several studies suggest that antioxidant supplementation can influence the response to chemotherapy as well as the development of adverse side effects that result from treatment with antineoplastic agents^[38]. Nowadays, the diets play an important role in our life and this necessitates the ongoing search for natural antimutagens of promising anticancer therapeutics. The objective of this study is to evaluate the antimutagenic activity of defatted aqueous ethanol extract and polysaccharide extract of *P. albicans* as well as correlate this activity with chemical composition of these extracts. The results demonstrated that, while the extracts are non-genotoxic, they have in fact been found to possess antimutagenic influences. The selected anticancer drug CP induced significant percentage of chromosomal aberrations and micronucleated polychromatic erythrocytes in bone marrow cells. Oral pretreatment of CP-treated mice with *P. albicans* L. extracts showed antimutagenic effects. The results revealed that defatted aqueous ethanol extract gave the most effective protection with the highest dose which represents a significant rate of inhibition reaching about 65% for chromosomal aberration and 45% for micronuclei. Our findings concerning the significant antimutagenic effect of defatted aqueous ethanol extract of *P. albicans* are consistent with the reports of others who demonstrated high correlation between scavenging potency and the total phenolic content of extracts from several *Plantago* species^[39]. It was reported that diets rich in phenolic components play a role in reducing risk of chronic diseases including cancer^[14]. Damage to DNA may cause mutations that potentially lead to cancer. Therefore, protection against DNA damage and induction of DNA repair enzymes represent important mechanisms of anticarcinogenic activity of natural compounds. The ability of defatted aqueous ethanol extract and polysaccharide extract to reduce the DNA damage induced by CP may be due to scavengers of active oxygen species and electrophiles released from the CP^[40].

Bioactivity guided isolation and characterization of phytoconstituents from the aerial part of *P. albicans* were performed in the present study to give a new insight into the usage of *Plantago* species in traditional medicine. Eleven phenolic compounds were isolated from defatted aqueous ethanol extract of the aerial parts of *P. albicans*. Such compounds possess wide biological activities. The antimutagenic properties of PGG were reported in several studies. A study reported that PGG exerted remarkably strong inhibition of mutagenicity of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-b] indole on *Salmonella typhimurium*; PGG was more potent than gallic acid. Other study revealed that PGG completely blocked the genotoxicity induced by the known mutagens nifuroxazide and aflatoxin B1 using SOS chromotes. Interestingly, gallic acid was more effective than PGG in the case of nifuroxazide. Furthermore PGG was demonstrated to have promising *in vitro* inhibitory effects against cancer cells from different organs through multiple mechanisms^[41]. PGG had a cytoprotective effect on immune cells exposed to normally damaging amount of radiation^[42]. Luteolin exhibits potent antimutagenic and anticarcinogenic effect against dietary carcinogens^[43,44]. Also, quercetin has

the ability to inhibit the DNA damage induced in human lymphocytes and liver cells^[45,46]. Methyl gallate and quercetin isolated from plant origin were demonstrated to inhibit human lung cancer cells^[47].

Our results about the antimutagenic effect of polysaccharide are in agreement with the finding of Chen *et al.*, who observed that the crude polysaccharide extracted from *Gracilaria lemaneiformis* had antioxidant activity and inhibitory effect on mouse bone marrow micronucleus and abnormal sperm induced by CP^[48]. The antimutagenic activity of a novel salt-soluble polysaccharide from *Auricularia polytricha* against the *in vivo* DNA-damaging effect of the indirectly acting alkylating agent CP was reported^[49].

To the best of our knowledge, this is the first study that has investigated the possible genotoxicity and/or DNA damage protection effects of *P. albicans* extracts. It therefore evaluated the genotoxic and antigenotoxic activity of defatted aqueous ethanol extract and polysaccharide extract from *P. albicans* in bone marrow cells of mice *in vivo*, using the micronucleus and chromosomal aberrations assays. The results demonstrated the potential role of *P. albicans* component as natural protective antimutagenic agents. This describes direction for future research to establish the activity and utility of medicinal plant *P. albicans* as human cancer preventive and therapeutic drug. Furthermore, except for luteolin 7-O- β -glucopyranoside and luteolin, all compounds were isolated for the first time from this species.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; **15**: 7313–7352.
- [2] Willis J. *A dictionary of the flowering plants and ferns*. 8th ed. Cambridge: Cambridge University Press; 1980.
- [3] World Health Organization. WHO monographs on selected medicinal plants, vol 1. Geneva: World Health Organization; 1999. [Online] Available from: <http://apps.who.int/medicinedocs/en/d/Js2200e/> [Accessed on 14th December, 2013]
- [4] Huang DF, Xie MY, Yin JY, Nie SP, Tang YF, Xie XM, et al. Immunomodulatory activity of the seeds of *Plantago asiatica* L. *J Ethnopharmacol* 2009; **124**: 493–498.
- [5] Türel I, Ozbek H, Erten R, Oner AC, Cengiz N, Yilmaz O. Hepatoprotective and anti-inflammatory activities of *Plantago major* L. *Indian J Pharmacol* 2009; **41**(3): 120–124.
- [6] Beara IN, Orčić DZ, Lesjak MM, Mimica-Dukić NM, Peković BA, Popović MR. Liquid chromatography/tandem mass spectrometry study of anti-inflammatory activity of plantain (*Plantago* L.) species. *J Pharm Biomed Anal* 2010; **52**(5): 701–706.
- [7] Chiang LC, Ng LT, Chiang W, Chang MY, Lin CC. Immunomodulatory activities of flavonoids, monoterpeneoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago* species. *Planta Med* 2003; **69**(7): 600–604.
- [8] Tinkov AA, Nemereshina ON, Popova EV, Polyakova VS, Gritsenko VA, Nikonov AA. *Plantago maxima* leaves extract inhibits adipogenic action of a high-fat diet in female Wistar rats. *Eur J Nutr* 2014; **53**(3): 831–842.
- [9] Sarić-Kundalić B, Dobes C, Klatté-Asselmeyer V, Saukel J. Ethnobotanical study on medicinal use of wild and cultivated plants in middle, south and west Bosnia and Herzegovina. *J Ethnopharmacol* 2010; **131**(1): 33–55.
- [10] Mahmood T, Saeed S, Naveed I, Munir F, Raja GK. Assessment of antioxidative activities of extracts from selected *Plantago* species. *J Med Plants Res* 2010; **5**(20): 5172–5176.
- [11] Kobeasy MI, Fatah OMA, Salam SMA, Mohamed ZEM. Biochemical studies on *Plantago major* L. and *Cyamopsis tetragonoloba* L. *Int J Biodivers Conserv* 2011; **3**: 83–91.
- [12] Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used against cancer—an extension of the work of Jonathan Hartwell. *J Ethnopharmacol* 2000; **73**: 347–377.
- [13] Gálvez M, Martín-Cordero C, López-Lázaro M, Cortés F, Ayuso MJ. Cytotoxic effect of *Plantago* spp. on cancer cell lines. *J Ethnopharmacol* 2003; **88**: 125–130.
- [14] Heimler D, Isolani L, Vignolini P, Tombelli S, Romani A. Polyphenol content and antioxidative activity in some species of freshly consumed salads. *J Agric Food Chem* 2007; **55**: 1724–1729.
- [15] Radu N, Ghita I, Rau I. Therapeutic effect of polysaccharides from *Plantago* species. *Mol Cryst Liq Cryst* 2010; **523**(1): 236–246.
- [16] Radu N, Ghita I, Coman O, Rau I. Therapeutic effect of flavonoids derived from *Plantago* species. *Mol Cryst Liq Cryst* 2010; **523**(1): 273–281.
- [17] Dalar A, Türker M, Konczak I. Antioxidant capacity and phenolic constituents of *Malva neglecta* Wallr. and *Plantago lanceolata* L. from eastern Anatolia region of Turkey. *J Herb Med* 2012; **2**(2): 42–51.
- [18] Afifi MS, Zaghlol MG, Hassan MA. Phytochemical investigation of the aerial part of *Plantago ovate*. *Mansouri J Pharm Sci* 2000; **16**(2): 178–190.
- [19] Janković T, Menković N, Zdunić G, Beara I, Balog K, Šavikin K,

- et al. Quantitative determination of aucubin in seven *Plantago* species using HPLC, HPTLC, and LC–ESI–MS methods. *Anal Lett* 2010; **43**: 2487–2495.
- [20] Rùnsted N, Gobel E, Franzyk H, Jensen SR, Olsen CE. Chemotaxonomy of *Plantago*. Iridoid glucosides and caffeoyl phenylethanoid glycosides. *Phytochemistry* 2000; **55**: 337–348.
- [21] Taskovaa R, Evstatiieva L, Handjievab N, Popovb S. Iridoid patterns of genus *Plantago* L. and their systematic significance. *Z Naturforsch C* 2002; **57**: 42–50.
- [22] Afifi MS, Salama OM, Hassan MA, Naeim ZEM, Mohammed KF. Phenolic constituents of *Plantago albicans* L. *Mansouri J Pharm Sci* 2000; **16**(2): 204–212.
- [23] Kawashty SA, Gamal–el–din E, Abdalla MF, Saleh NAM. Flavonoids of *Plantago* species in Egypt. *Biochem Syst Ecol* 1994; **22**: 729–733.
- [24] Campbell JA, Goheen SC, Donald P. Carbohydrates from soybean plant tissues. In: Dora Krezhova, editor. *Recent trends for enhancing the diversity and quality of soybean products*. Rijeka: InTech; 2011, p. 421–446.
- [25] Markham KR. *Techniques of flavonoid identification*. London: Academic Press; 1982.
- [26] Andersen OM, Markham KR. *Flavonoids: chemistry, biochemistry and applications*. Boca Raton: CRC Press; 2006.
- [27] Emaga TH, Rabetafika N, Blecker CS, Paquot M. Kinetics of the hydrolysis of polysaccharide galacturonic acid and neutral sugars chains from flaxseed mucilage. *Biotechnol Agron Soc Environ* 2012; **16**(2): 139–147.
- [28] Orata F. Derivatization reactions and reagents for gas chromatography analysis. In: Mohd MA, editor. *Advanced gas chromatography – progress in agricultural, biomedical and industrial applications*. Rijeka: InTech; 2012, p. 83–108.
- [29] Shelby MD, Witt KL. Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ Mol Mutagen* 1995; **25**: 302–313.
- [30] Scolatici C, Alves de Lima RO, Barbisan LF, Ferreira AL, Ribeiro DA, Salvadori DM. Antigenotoxicity and antimutagenicity of lycopene in HepG2 cell line evaluated by the comet assay and micronucleus test. *Toxicol In Vitro* 2008; **22**: 510–514.
- [31] Valette H, Dolle F, Bottlaender M, Hinnen F, Marzin D. Fluro–A–85380 demonstrated no mutagenic properties in *in vivo* rat micronucleus and Ames tests. *Nucl Med Biol* 2002; **29**: 849–853.
- [32] D’Souza UJA, Zain A, Raju S. Genotoxic and cytotoxic effects bone marrow of rats exposed to low dose of paquat via the dermal route. *Mutat Res* 2002; **581**: 187–190.
- [33] Yosida TH, Amano K. Autosomal polymorphism in laboratory bred and wild Norway rats, *Rattus norvegicus*, found in Misima. *Chromosoma* 1965; **16**: 658–667.
- [34] Sendecor WG, Cochran WG. *Statistical methods*. 10th ed. Ames: Iowa State University Press; 1982.
- [35] Tan HP, Ling SK, Chuah CH. Characterisation of galloylated cyanogenic glucosides and hydrolysable tannins from leaves of *Phyllagathis rotundifolia* by LC–ESI–MS/MS. *Phytochem Anal* 2011; **22**: 516–525.
- [36] Markham KR, Geiger H. ¹H–NMR of flavonoids and their glycosides in DMSO–d₆. In: Harborne JB, editor. *The flavonoids: advances in research since 1986*. London: Chapman and Hall; 1994.
- [37] Chakraborty P, Hossain Sk U, Murmu N, Das JK, Pal S, Bhattacharya S. Modulation of cyclophosphamide–induced cellular toxicity by diphenylmethyl selenocyanate *in vivo*, an enzymatic study. *J Cancer Mol* 2009; **4**(6): 183–189.
- [38] Block K, Koch A, Mead M, Newman RA, Gyllenhaal C. Re: should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? *J Natl Cancer Inst* 2009; **101**: 124–125.
- [39] Harput US, Gene Y, Saracoglu I. Cytotoxic and antioxidative activities of *Plantago lagopus* L. and characterization of its bioactive compounds. *Food Chem Toxicol* 2012; **50**(5): 1554–1559.
- [40] Jain R, Jain SK. Effect of *Buchanania lanzan* Spreng bark extract on cyclophosphamide induced genotoxicity and oxidative stress in mice. *Asian Pac J Trop Med* 2012; **5**: 187–191.
- [41] Zhang J, Li L, Kim SH, Hagerman AE, Lü J. Anticancer, anti–diabetic and other pharmacologic and biological activities of penta–galloyl–glucose. *Pharm Res* 2009; **26**: 2066–2080.
- [42] Bing SJ, Kim MJ, Park E, Ahn G, Kim DS, Ko RK, et al. 1,2,3,4,6–penta–O–galloyl–beta–D–glucose protects splenocytes against radiation–induced apoptosis in murine splenocytes. *Biol Pharm Bull* 2010; **33**(7): 1122–1127.
- [43] Orhan F, Gulluce M, Ozkan H, Alpsoy L. Determination of the antigenotoxic potencies of some luteolin derivatives by using a eukaryotic cell system, *Saccharomyces cerevisiae*. *Food Chem* 2013; **141**(1): 366–372.
- [44] Seelinger G, Merfort I, Wölfle U, Schempp CM. Anti–carcinogenic effects of the flavonoid luteolin. *Molecules* 2008; **13**: 2628–2651.
- [45] Gupta C, Vikram A, Tripathi DN, Ramarao P, Jena GB. Antioxidant and antimutagenic effect of quercetin against DEN induced hepatotoxicity in rat. *Phytother Res* 2010; **24**(1): 119–128.
- [46] Chan ST, Lin YC, Chuang CH, Shiau RJ, Liao JW, Yeh SL. Oral and intraperitoneal administration of quercetin decreased lymphocyte DNA damage and plasma lipid peroxidation induced by TSA *in vivo*. *BioMed Res Int* 2014; doi: 10.1155/2014/580626.
- [47] Ludwiczuk A, Saha A, Kuzuhara T, Asakawa Y. Bioactivity guided isolation of anticancer constituents from leaves of *Alnus sieboldiana* (Betulaceae). *Phytomedicine* 2011; **18**(6): 491–498.
- [48] Chen MZ, Yu J, Long ZJ, Luo QB. Studies on antimutagenic and the free radical scavenging effect of polysaccharide from *Gracilaria lemaneiformis*. *Food Sci* 2005; **26**(7): 219–222.
- [49] Zhou J, Chen Y, Xin M, Luo Q, Gu J, Zhao M, et al. Structure analysis and antimutagenic activity of a novel salt–soluble polysaccharide from *Auricularia polytricha*. *J Sci Food Agric* 2013; **93**(13): 3225–3230.