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ANTIOXIDANT PROPERTIES OF CUMIN (*BUNIUM PERSICUM* BOISS.) EXTRACT AND ITS PROTECTIVE ROLE AGAINST ULTRASOUND-INDUCED OXIDATIVE STRESS TESTED BY MICRORNA BASED MARKERS

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ABSTRACT
Bunium persicum Boiss. seeds have been used for medicinal and nutritional properties such as antioxidant, antihelmetic and antimicrobial activity. The aim of this study was to to tested protective role of cumin extract against abiotic stress by microRNA markers. Secondary also was to evaluate antioxidant activity as well as total polyphenol, flavonoid and phenolic acid content of cumin extract. We observed that cumin DNA itself has not been damaged by sonication teratment. This protective impact indicates that cumin antioxidant properties can efficiently quench free radicals induced by sonication. On the other side, ultrasound-mediated formation of reactive oxygen species did induce the DNA polymorphism of lettuce samples which was detected by miRNAs-based markers. The range of sonication impact was time-dependent. Markers based of miRNA-DNA sequences has proven to be an effective tool. We have confirmed statistically significant differences ($p \leq 0.01$) in miRNAs markers ability to detect the polymorphism due to sonication treatment. The antioxidant activity was determined by a method using DPPH radical and phosphomolybdenum method, total polyphenol content with Folin – Ciocalteu reagent, total flavonoid with aluminium-chloride mehod and total phenolic acid with Arnova reagent. Results showed that cumin is rich for biologically active substances and can be used more in different kind of industry as a cheap source of these substances. Antioxidant activity with DPPH method was 1.18 mg TEAC.g^{-1} (TEAC – Trolox equivalent antioxidant capacity per g of sample) and by phosphomolybdenum method 45.23 mg TEAC.g^{-1}. Total polyphenol content achieved value 4.22 mg GAE.g^{-1} (GAE – gallic acid equivalent per g of sample), total flavonoid content value 10.91 mg QE.g^{-1} (QE – quercetin equivalent per g of sample) and total phenolic acid content value 5.07 mg CAE.g^{-1} (CAE – caffeic acid equivalent per g of sample).

Keywords: cumin; antioxidant; ultrasound; microRNA marker

INTRODUCTION
Changing environmental conditions are giving rise to a variety of free radicals, which plants have to deal with them in order to survive. Besides that, free radicals and other reactive oxygen species (ROS) are produced in our body as byproducts of various biological processes of metabolism (**Dua et al., 2012**). Reactive oxygen species, such as single oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism (**Saeed et al., 2012**). However, overproduction of ROS is also harmful to the body because they cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury (**Dua et al., 2012**). In addition to those aspects, the technological methods of food processing and preservation include such approaches that themselves represent the source of free radicals and reactive oxygen species which can ultimately cause damage of the products. The different applications of ultrasound in the food industry include ultrasound and microbial inactivation, ultrasound in filtration, ultrasound-assisted extraction and ultrasound in enhancing fermentation (**Bhattacharya, 2015**).

Ultrasound can result in cavitation and acoustic microstreaming, which modifies cellular ultrastructure, enzyme stability and cell growth. It can also cause breaks in extracellular polymers, release DNA from the nucleus, decrease cell stability, alter cell membrane permeablity and modify charges on the surfaces of cells (**Rokhina et al., 2009**). In biological applications, sonication may be sufficient to disrupt or deactivate a biological material. Cells being sonicated are subjected to various stimulation including mechanical, thermal, and oxidative stress (**Riesz and Kondo, 1992**). A cell may sense this stimulation by chemical changes and special movements of molecules

within the cell, and then convey the information to the other parts of the cell through certain signal transduction networks. Receiving such information, the cell may undergo fundamental changes to respond and adapt itself to the stimulations. By doing so, the cell may be able to protect itself from the stimulation, repair any damage and so on **(Suslick, 1990)**. Ultrasound irradiation may lead to various kinds of interactions with the living organism depending upon the sonication conditions. Under certain sonication conditions, it was reported that ultrasound induced gene expression changes in cells **(Abdollahi et al., 2004; Wei et al., 2012)**. Ultrasound (10 – 60 kHz) have been used to modify growth and developmental processes in plants **(Rokhina et al., 2009)**. Sonication also affects the endogenous hormonal balance of treated plant tissue or cells and thereby promotes growth and development **(da Silva and Dobranszki, 2014)**.

Plants contain a wide variety of antioxidant phytochemicals or bioactive molecules, which can neutralize the free radicals and thus retard the progress of many chronic diseases associated with oxidative stress **(Ani et al., 2006)**. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals **(Saeed et al., 2012)**. Antioxidants are the compounds that can delay, inhibit or prevent the oxidation of biomolecules like lipids, proteins or nucleic acids. Antioxidants may scavenge the free radicals or break the chain reaction due to their redox properties **(Schafer et al., 2003)**. In general, there are two categories of antioxidants-natural and synthetic. These days, interest has increased in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are restricted due to their carcinogenicity and toxicity **(Barlow, 1990; Klimešová et al., 2015)**. Naturally occurring antioxidants in leafy vegetables, fruits and seeds such as ascorbic acid, vitamin E and phenolic compounds have abiliy to reduce oxidative damage associated with many diseases including cancer, cardiovascular diseases, cataract, arthiritis, diabetes **(Saphira et al., 1989)**.

Cumin (*Bunium persicum* L.) is a flowering and annual herb from *Apiaceae* family which might have originated in the area between Central Asia and Northern India. Cumin is a high value herbaceous spice widely used for culinary, flowering, perfumery and carminative purposes. One of the commonly used spices for their special aromatic effect in food preparations that are prevalent in Central Asia, Caucasus, Crimea. It is also used in herbal medicine as a stimulant, carminative and astringent. Cumin seeds have been reportedly used for traditional treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice **(Sultana et al., 2010)**. The plant type of *Bunium persicum* L. varies from dwarf (30 cm) to tall (80 cm) compact or spreading, moderately to highly branched, tuberous and perennial herb **(Panwar, 2000)**. The study of antioxidant properties and antioxidant compounds of cumin **(Dua et al., 2012)** indicate that polyphenol rich methanolic extract of cumin had efficient free radical scavenging and metal chelating activity to protect biomolecules like proteins, lipids and DNA against oxidative stress. A significant protection role of bitter cumin (*Cuminum nigrum* L.) extract againts DNA damage induced by hydroxyl radicals recorded **Ani et al. (2005)**. They also tested the antibacterial activity of cumin extract finding out that the growth of *Bacillus subtilis*, *Bacilus cereus* and *Staphylococcus aureus* was significantly inhibited. Due to contamination of cumin seeds by microorganisms, **Fatemi et al. (2011)** tested the effect of decontamination treatment by gamma-irradiation on the chemical composition and antioxidant properties of cumin extracts. The research showed that total percentage of ten essential oil components was not affected and the changes in flavonoid content were nonsignificant. The antioxidant activities of cumin extract were not altered by gamma-irradiation treatment.

Emerging evidence has suggested that a class of small regulatory RNAs, called microRNAs (miRNAs) play a critical role in regulation of DNA damage response **(Wan et al., 2011)** as a response to various biotic and abiotic stress. Certain miRNAs are either under or over-expressed or new miRNAs are synthesized under stress. Mostly miRNA target genes which encodes various transcriptional factors or functional enzymes having important roles in abiotic stress response **(Bej and Basak, 2014; Kantar et al., 2010)**. Emerging evidence has suggested that miRNAs molecules regulate the DNA damage response by a mechanism based on the nature and intensity of damage **(Simon et al., 2009)**. Although several DNA damage responsive miRNAs and their targets have been identified, there is a need to establish the complex interconnections between miRNAs and their DNA damage response targets **(Wan et al., 2011)**. **Zhang et al. (2011)** found that DNA damage led to increased levels of some pre-miRNAs and mature miRNAs suggesting functional connection between DNA damage response and miRNA processing and maturation.

The aim of this work was to study the protective role of seed cumin extracts and its antioxidant properties on plant DNA from lettuce as a model plant under conditions of sonication induced oxidative stress. An oxidative stress has been recorded on molecular level by microRNA-based markers. Secondary also was to evaluate antioxidant activity as well as total polyphenol, flavonoid and phenolic acid content of cumin extract.

Scientific hypothesis

We have tested the protective role of antioxidant properties of seed cumin extract in conbination with abiotic oxidative stress, on DNA polymorphism. We hypothesized that the effect of sonication-induced oxidative stress can be recorded on molecular level by microRNAs-based markers.

MATERIAL AND METHODOLOGY

Biological material

Cumin (*Bunium persicum* L.*)* seeds were grown in the mountain area of Uzbekistan. They were procured from the local market, identified and authenticated at Department of Botany, National University of Uzbekistan.

Lettuce (*Lactuca sativa* L.) grown in climatic chamber with phytotron system (Pol-Eko Aparatura KK350 Top +Fit) of the AgroBioTech, Research Centre of the Slovak University of Agriculture in Nitra. The plants were grown under photoperiod 16/8 hours (day/night), temperature 21/18 °C, humidity 50% and light intensity 15,000 lx.

Chemicals

All chemicals for antioxidant analyses were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA). Chemicals for molecular analyses were analytical grade and were purchased from ThermoFisher Scientific (USA), Promega (USA), Biotium (Canada) and Microsynth (Switzerland).

Sample preparation

An amount of 0.2 g of cumin sample was extracted with 20 mL of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction as well as measurements were carried out in triplicates.

Radical scavenging activity

Radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sánchéz-Moreno et al., 1998)**. The extract (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). After 10 minutes in darkness, absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10 – 100 $mg.L^{-1}$; $R^2 = 0.9881$) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Phosphomolybdenum method

Phosphomolybdenum method was realized according to method **Prieto et al. (1999)** with slight modifications. The mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10 – 1000 $mg.L^{-1}$; $R^2 = 0.998$) was used as the standard and the results were expressed in $mg.g^{-1}$ Trolox equivalents.

Total polyphenol content

Total polyphenol content extracts was measured by the method of **Singleton and Rossi (1965)** using Folin-Ciocalteu reagent. 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25 – 300 $mg.L^{-1}$; $R^2 = 0.998$) was used as the standard and the results were expressed in $mg.g^{-1}$ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of **Willett (2002)**. 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5 – 20 $mg.L^{-1}$; $R^2 = 0.989$) was used as the standard and the results were expressed in $\mu g.g^{-1}$ quercetin equivalents.

Total phenolic acid content

Total phenolic acids content was determined using method of **Farmakopea Polska (1999)**. A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ +10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 $mg.L^{-1}$, $R^2 = 0.999$) was used as a standard and the results were expressed in $mg.g^{-1}$ caffeic acid equivalents.

Genomic DNA extraction and sonication treatment

The genomic DNA (3 independent samples) was isolated from leaves of model plant *Lactuca sativa* L. based on method by **Padmalatha a Prasad (2007)**. The methodology of this experiment was based on **Dua et al. (2012)**. Genomic DNA extracted from cumin seeds (3 independent samples) was isolated by **Padmalatha a Prasad (2007)** and used as a control. DNA was quantified by NanoPhotometer™ (IMPLEN). The reaction mixture (9 µL) contained 2.70 µg of DNA in 20.0 $mmol.dm^{-3}$ phosphate buffer saline (PBS) (pH 7.4) and different concentrations of cumin extract (0.5%; 1.0% and 1.5%). The samples were pre-incubated for 15 min at ambient temperature. The oxidation stress was induced by sonication of DNAs during different time periods (5, 10 and 15 minutes) in laboratory ultrasonic bath (type K-5LE, KRAINTEK CZECH, s.r.o., Czech Republic) with the ultrasound frequency 38 KHz and power 150 W. Each treatment was performed in duplicates. The reaction was terminated by the addition of loading buffer (Blue/Orange 6x, Promega) and the mixture was subjected to gel electrophoresis in 1.5% agarose/TBE buffer run at 60V. DNA was visualized and photographed by UV-transilluminator (G-Box, Syngene, United Kingdom) system to assess the DNA damage.

In the second type of experiments, the fresh lettuce leaves (100 mg) of were incubated in 1.5 mL of 1×PBS and diferent concentrations (0.5%; 1% and 1.5%) of ethanolic cumin extract, for 15 minutes and consequently the samples were affected by sonication in duration 1, 5, 10 and 15 minutes. Each treatment was performed in duplicates. DNA was extracted using the modified method according to **Padmalantha and Prasad (2006)**. In total 30 samples od DNA were extracted from leaf tissues.The DNA concentration was quantified by the ImplenNano Photometer® (Germany), measuring the absorbance at 260nm. The purity and integrity was assessed by the absorbance 260/280nm ratio. And diluted then into 50 $ng.\mu L^{-1}$ with nuclease-free water for PCR amplification.

microRNA molecular markers assay

Genomic DNAs isolated from lettuce leaves were analyzed by molecular markers based on conservative families of microRNA (miR156 and miR168). The miRNA based markers PCR amplified in 20 µl reaction

mixture that contained 70 ng of genomic DNA, 1x DreamTaq Buffer (KCl, $(NH_4)_2SO_4$, 20mmol.dm^{-3} $MgCl_2$), 2 units DreamTaq DNA polymerase, 0.8 mmol.dm^{-3} dNTPs, 10 mmol.dm^{-3} of each primer and nuclease-free water for PCR amplification. The PCR amplification program used 'touchdown' method as follows: initial denaturation at 94 °C for 5 min; 5 cycles of 30 s at 94 °C, 45 s at 64 °C with a 1 °C decrease in annealing temperature per cycle and 60 s at 72 °C; 30 cycles of 30 s at 94 °C, 45 s at 60 °C and 60 s at 72 °C; and a final extension at 72 °C for 10 min.

The primers for the miRNA-based markers were designed according to the mature miRNA sequences, originated from the miRNA database (http:www.mirbase.org/). Two miRNA-based forward primers, miRNA156 (5`-GTGCAGGGTCCGAGGT-3`) and miR168 (5`-CACGCATCGCTTGGTGCAGGT-3`) and one universal miRNA reverse primer (5`-CCAGT GCAGGGTCCGAGGTA-3`) were used and combined to perform a marker assay.

PCR product were separated on 3 % agarose and 10% TBE-Urea gels (miRNA assay) in 1 x TBE Running Buffer at a constant power of 90 V , 120mA for 60min, respectively at 180 V, 15 mA for 90 min. The polyacrylamide gels were stained with GelRed™ (0.5 µg.mL^{-1}) and were visualized under UV by the G-Box electrophoresis documentation system. The DNA fragment size was compared to 100bp Gene Ruler and in the case of polyacrylamide gels by 10bp DNA ladder.

The DNA amplification profile by both markers was analyzed by GeneTool software (Syngene, Germany). Each fragment is characterized by quantity and volume of profile in pixels. Profiles are recorded on the basis of the set threshold value in which the analysis is carried out.

Statisic analysis

The statistical analyses were performed with the statistical program Statgraphics (version 5.0) and the SAS program (the SAS system v 9.2.). Results of microRNA-based markers assay were evaluated by One-Way ANOVA. Differences were considered significant at $p \leq 0.01$. To test differences was used Tuckey test. Results of antioxidant activity as well as total polyphenols, flavonoids and phenolic acids are presented as mean value with standard deviation.

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidant activity (Table 1) of tested cumin extract was 1.18 mg TEAC.g^{-1} evaluated with DPPH method and 45.23 mg TEAC.g^{-1} by phosphomolybdenum method. **Sharififar et al. (2010)** tested methanolic extract of cumin and reported strong activity with DPPH method – 45.7 µg.mL^{-1}. This authors also reported that in general, the cumin essential oil showed higher activity than solvent extracts from the cumin **(Sharififar et al., 2010)**. Similarly **Chizzola et al. (2014)** tested antioxidant activity of methanolic extract of cumin and their value varied from 9.2 to 14.4 mg Trolox equivalents in the DPPH test and from 6.1 to 10.9 mg.g^{-1} in the Fe-reduction test. In our study we tested activity of ethanolic extract, but many researchers also detected strong antioxidant activity of black cumin essential oil. **Shahsauari et al. (2008)** reported strong activity evaluated with DPPH methos of this oil with value 0.88 mg.mL^{-1} (EC50). These authors also showed that *Bunium persicum* essential oil is able to reduce the oxidation rate of the soybean oil in the accelerated condition at 60 degrees C (oven test). Study of **Kareshk et al. (2015)** showed that *B. persicum* essential oil as a natural source can be used for production of new prophylactic agent for use in toxoplasmosis. *Bunium persicum* Boiss. is an economically important medicinal plant growing wild in the dry temperature regions in Iran, Kazakhstan and Uzbekistan with the strong potential of widely using in different kind of industry.

Total polyphenol, flavonoid and phenolic acid content

Total polyphenol content in evaluated cumin extract achieved value 4.22 mg GAE.g^{-1}, total flavonoid value 10.91 mg QE.g^{-1} and total phenolic acid value 5.07 mg CAE.g^{-1} (Table 1). Our results are comparable with findings of **Chizzola et al. (2014)** which tested these compounds in methanolic extract of cumin and total polyphenol content in their study was between 8.7 – 14.7 mg GAE.g^{-1}, total flavonoid content between 4.3 – 9.5 mg CE.g^{-1} (catechin equivalent). **Nickavar and Abolhasani (2009)** reported a total flavonoid content of cumin extract in value 20.2 mg.g^{-1} as rutin-equivalents. **Seri et al. (2017)** measured total polyphenol conten in hydroalcoholic extract of cumin and found value 122.41 mg GAE.g^{-1}. These authors also published that this hydroalcoholic extract might have therapeutic potentials in the prevention of glycation-mediated diabetic complications. **Souri et al. (2008)** published total polyphenol content in cumin in amount 214.03 ±4.10 mg GAE.100 g^{-1}.

MicroRNA-based markers assay

The ultrasound is widely used tool not only in medicine,

Table 1 Antioxidant activity, total polyphenol, flavonoid and phenolic acid content in cumin (*Bunium persicum* Boiss.) ethanolic extract.

Sample	DPPH (mg TEAC.g^{-1})	PM (mg TEAC.g^{-1})	TPC (mg GAE.g^{-1})	TFC (mg QE.g^{-1})	TPA (mg CAE.g^{-1})
Cumin (*Bunium persicum* Boiss.)	1.18 ±0.11	45.23 ±2.38	4.22 ±0.78	10.91 ±1.63	5.07 ±0.42

Note: DPPH - radical scavenging activity; PM – phosphomolybdenum method; TPC – total polyphenol content; TFC – total flavonoid content; TPA – total phenolic acid content; TEAC – trolox equivalent antioxidant capacity; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent; ±standard deviation.

but its applications in food industry and agriculture are more than significant. In addition, the thermal and chemical effects of ultrasound could be considered as a kind of serious environmental abiotic stress factor equal to the impacts of the current advanced telecommunication technologies on human beings. Therefore the research on the impact of sonication treatment on biological subjects is necessary. Ultrasound induces thermal, mechanical and chemical changes on morphological, cytological and molecular level. The ultrasound bioeffects include formation of free radicals and reactive oxygen species **(Milowska and Gabryelak, 2007)**. The use of genomic DNA as a template for fragmentation by sonication is one of the necessary approaches prior to library construction or subcloning for DNA sequencing **(Sambrook and Russell, 2006; Lee and Abdullah, 2014)**. Antioxidant properties of spices and herbs are known since ancient times and their pharmaceutical and medicinal potential is inexhaustible and still brings new possibilities of their use.

The protective role of cumin seed extract has been tested both on isolated genomic DNA treated by ultrasound and on plant tissue material effected by ultrasound during various time points. Non-treated genomic DNA isolated from lettuce and cumin seeds represented the control samples. Additional control was the cumin DNA treated with ultrasound in duration of 5, 10 and 15 minutes as the rest of tested samples. From the Figure 1 can be seen the effect of sonication on DNA degradation in the presence of different concentration of cumin extract. In comparison to non-treated control samples (C0 and C1) and sonicated samples of cumin DNA (C25, C210 and C215), dagradation proces of DNA due to the ultrasound treatment is visible, having increasing tendency with the time of action. It seems that the pretreatment of DNA with the cumin extract of various concentrations (0.5%; 1.0% and 1.5%), does not have any impact on DNA protection againts the damage, probably due to unsifficient of cumin extract in these solutions. But on the other hand, it can be observed that the cumin DNA, itself (Figure 1; $C2_5$, $C2_{10}$ and $C2_{15}$) has not been disrupt by sonication at any time point. This protective impact of the extract indicates that antioxidant properties of cumin seeds can efficiently quench free radicals induced by sonication. This corresponds to the results of Dua et al. (2012). As the authors stated, the presence of extract equivalent to 0.5 µg and 1.0 µg cumin in the incubation mixture could prevent damage of DNA.

In order to analyze the impact of ultrasound-mediated ROS formation at the molecular level more closely, we applied the markers based on microRNA sequences, due to

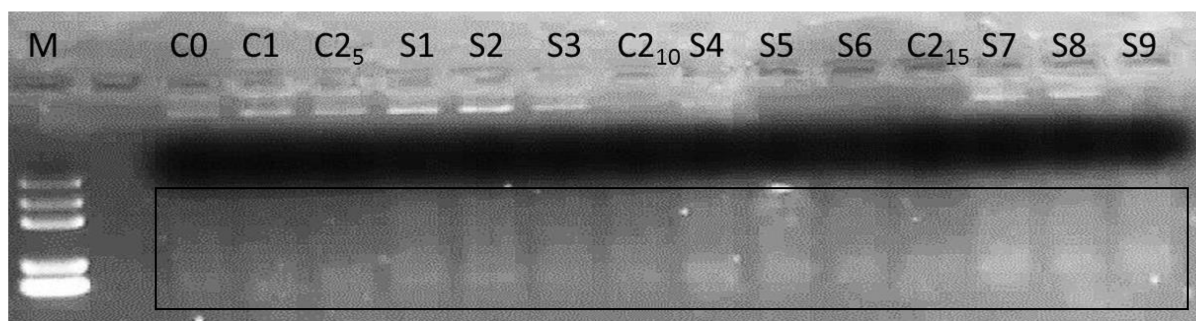

Figure 1 The effect of cumin seeds extract during the sonication treatment of lettuce DNA vizualized on 1.5% agarose gel. Note: M – DNA marker; control samples C0 – lettuce DNA non-treated, C1 – cumin DNA non-treated, C2 – cumin DNA treated with ultrasound in duration of 5, 10 and 15 minutes; samples S1, S2 and S3 - lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 5 min; S4, S5 and S6 – lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 10 min; S7, S8 and S9 – lettuce DNA in 0.5%, 1.0% and 1.5% cumin extract, treated by sonication 15 min. The highlighted area shows the degree of DNA degradation.

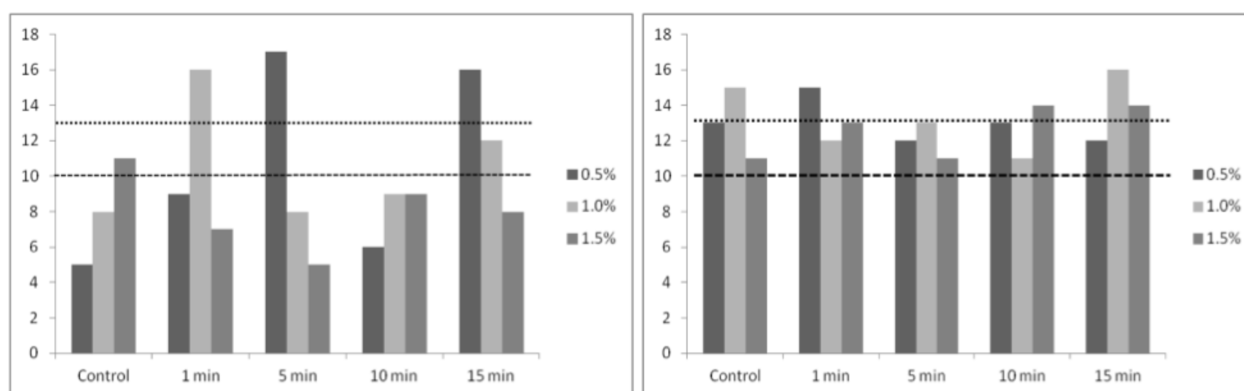

Figure 2 Number of DNA fragments of lettuce (*Lactuca sativa* L.) samples after ultrasound treatment, preceded by cumin extracts pre-incubation, detected by marker miRNA156 (left) and miR168 (right). Linear lines show numbers of DNA fragments in cotrol samples of cumin (interrupted line) and lettuce (starred line).

their role in regulation of DNA damage response (**Zhou et al., 2007; Kruszka et al., 2012**). For this reason were pieces (approximately 100 mg) of fresh leaf tissue material taken and prior to ultrasound treatment, they were incubated 15 minutes in PBS buffer together with various concentrations of cumin seed extract.

Consequently, the total genomic DNA was extracted and loaded on 1.5% agarose gel (picture not shown). Along with this proccesure was DNA analyzed by miRNA-based markers in touchdown PCR method. For these purposes two types of miRNA families have been chosen, conservative miR156 and miR168. Both types of miRNAs are considered as biomarkers of abiotic stress (**Bej and Basak, 2014; Kruszka et al., 2012; Zhou et al., 2007**). The family miR156 targets squamosa promoter binding protein (SBP), transcription factor which is involved in

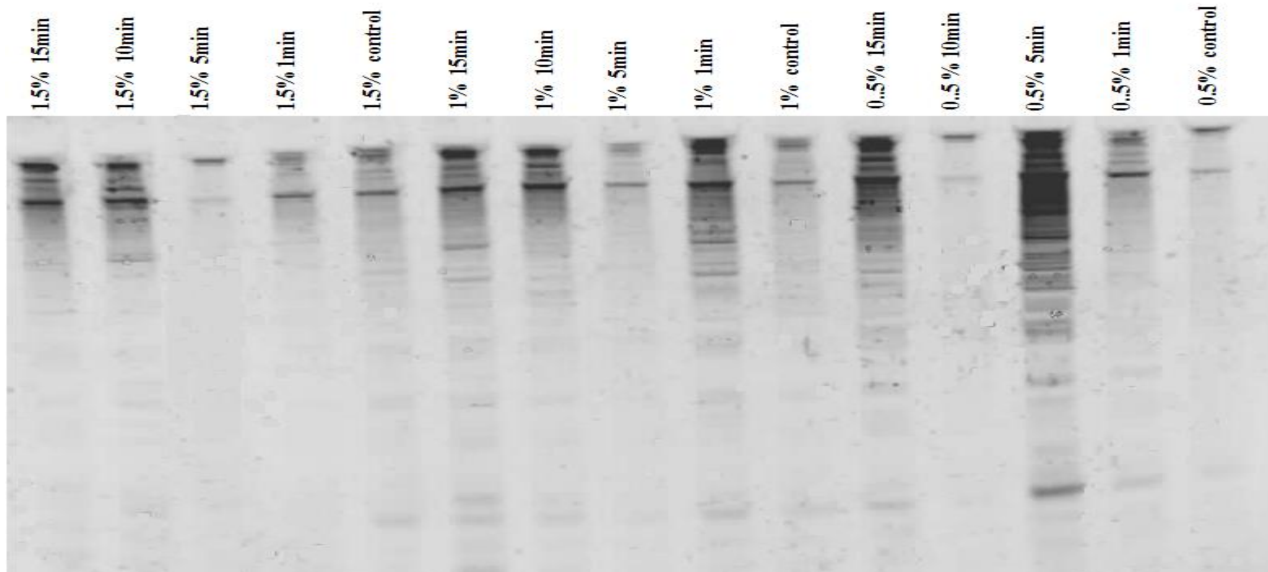

Figure 3 An example of the the gel with the PCR products of lettuce generated by miR156 marker.

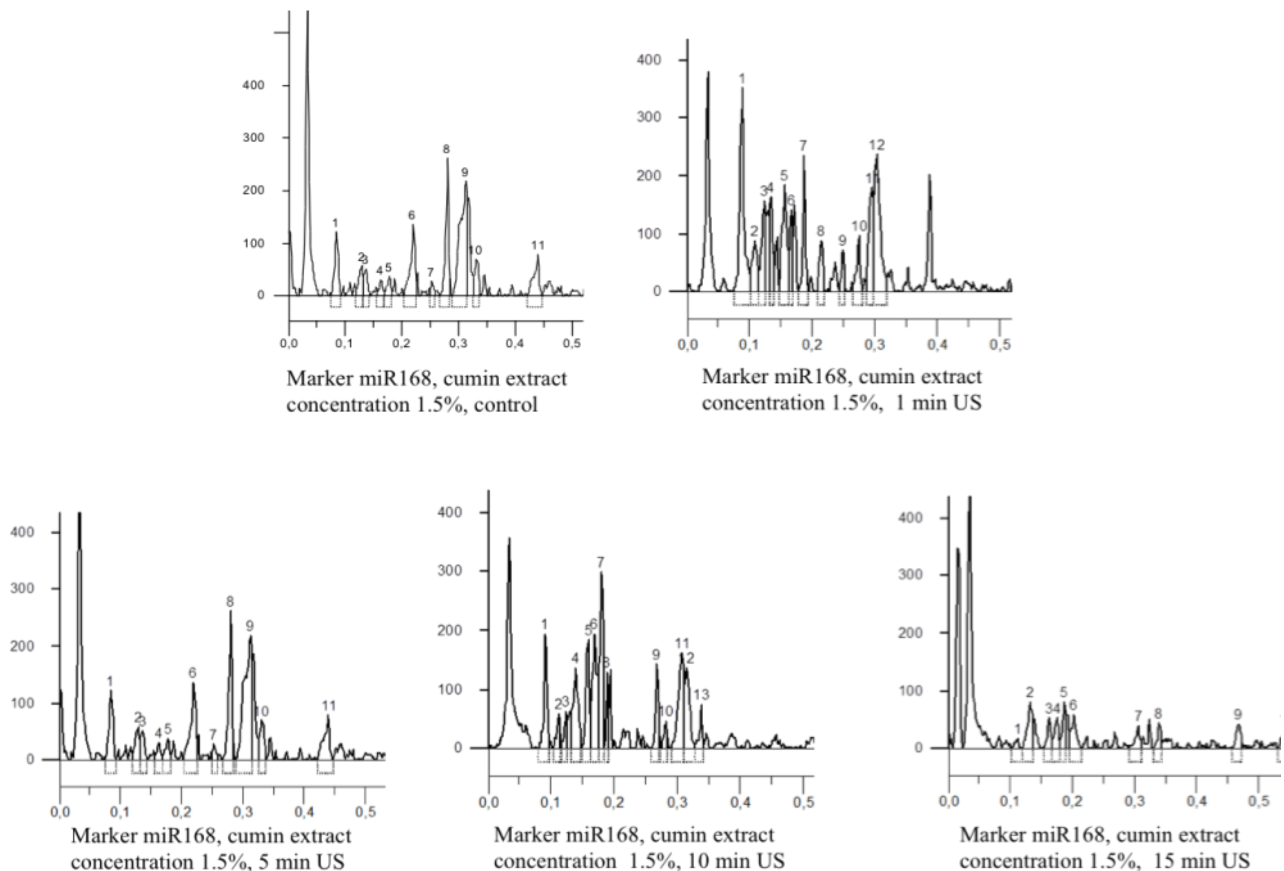

Figure 4 An example of the profiles of miRNA-based DNA fragments in control and treated samples by the ultrasound (US) generated by miR168 marker. Before treatment were samples incubated in solution of 1.5% cumin seed extract.

many biologically important processes **(Barvkar et al., 2013)**. One of the target sequences of miR168 family are sequences of cytochrome P450 which is involved in a wide range of biosynthetic reactions, which include ROS inactivation **(Kruszka et al., 2012)**. Based on the number and profile of individual peaks representing DNA fragments, we could observe more sensitive response to applied ultrasound treatment in miR168 marker in comparison to the marker miR156 (Figure 2) regardless to cumin extract pre-treatment of the samples. An example of the the gel with the PCR products of lettuce generated by miR156 marker shows figure 3.

The number of amplified fragments by miR168 was doubled in comparison to miR156. This difference was statistically significant (significance level 0.0056 at $p \leq 0.01$). More interestingly, the profile of peaks generated by marker miR168 has been distinguished (Figure 4) and the increasement of the marker activity has been recorded depending on the length of the ultrasound treatment. The extent of DNA damage may lead to activation of miRNAs which suggest that these molecules regulate the DNA damage response by a mechanism based on the intensity of DNA damage **(Simon et al., 2009)**.

In contrary, the profiles of DNA fragments generated by marker miR156 was quite balanced (Figure 3a, b and c). Our results correspond to observations of **Kruszka et al., (2012)**, **Xin et al. (2010)** and **Lu et al. (2005)**. As it has been recorded in our previous studies **(Ražná et al., 2015)** the polymorphism and expression of miR156 and miR168 markers was not only species- but also tissue- and developmentally-specific.

Molecular effects of ultrasound can be observed on the level physical, chemical and stress-induced changes which arise as a result of thermal, mechanical and acoustic axposure **(Rokhina et al., 2009)**. With the duration of treatment the temperature is increasing which may lead to denaturation of proteins and this consequently interferes with cellular machineries to repair proteins and membranes **(Bej and Basak, 2014)**. In response to heat stress the miR156 and miR168 in wheat were found to be up regulated **(Xin et al., 2010)**. Differential expression profile of miR156 and miR168 was studied in different plant species under various abiotic and biotic stress factors **(Kruszka et al., 2012)**. In most of tested abiotic stress conditions was marker miR168 up regulated.

However, in the study of *Populus trichocarpa* **(Lu et al., 2005)** was observed that miR156 was found to be down regulated during the mechanical stress and miR168 was up regulated in tension-stressed tissues. As **Bej and Basak (2014)** has stated in the context of response of UV-B radiation stress-responsive miRNAs families, that their reaction to the stress factor may be species-specific.

The results demonstrated that ultrasound at the used intensity and frequency is inducing the polymorphic variability at molecular level what was detected by the miRNA-based DNA markers. This effect of ultrasound was dependent on the duration of the treatment. Our results are in agreement with the observation of **Milowska and Gabryelak, (2007)**. **Juhaimi et al. (2017)** studied the effect of preultrasonic process on oil content and fatty acid composition of black cumin seeds and found out the major decrease in linoleic acid content after sonication for 30 minutes.

At the same time, we conducted the same type of experiments with the incense (*Boswellia sacra* FLUECKIGER) (results not shown). The antioxidant properties as well as the DNA degradation profile due to the ultrasound treatment have shown similar behavior. However, significant differences in the number and profile of individual peaks representing miRNA-based DNA fragments, with respect to tested concentrations of cumin extract has not been noticed. The reason for the observed phenomenon may be caused by low level of antioxidants in the particular sample of cumin seeds and subsequently with an insufficient concentration of the extracts in the solutions applied during the samples pre-treatment. The antioxidant properties of cumin seed extract are dependent of the total radical scavenging activity (DPPH) reflecting the total phenolic and flavonoid content. **Abdelhaliem and Al-Huqail, (2016)** observed distinct variations in DPPH and the content of phenols and flavonoids among and between 11 cumin accessions. Observed variations could be due to high variability in the substances with antioxidant characteristics which may be induced by diverse ecogeographical areas of cumin accessions cultivation.

CONCLUSION

Cumin (Bunium persicum Boiss.) is very interesting medicinal plant which contains various bioactive compounds. This plant is not typically for Slovak repuplic, but nowadays start be very popular mainly in gastronomy due to the specifical flavour. Results in this study showed that cumin had good antioxidant activity as well as polyphenol composition. Nevertheless, it is clear that reactive oxygen species, induced by sonication treatment did cause the polymorphism at the molecular level detected by miRNAs-stress markers, despite the co-operation of lettuce samples and the cumin extract. MiRNAs-based molecular markers effectively dettected the variability at the molecular level caused by abiotic stress factors. Specifically, the response of miR168 marker was statistically more sensitive in comparison to miR156 marker. The results, at the same time, pointed out the fact that the changes that took place within the molecules of biological objects exposed to ultrasonic waves, might be significant for food technological processes.

REFERENCES

Abdollahi, A., Domhan, S., Jenne, J. W., Hallaj, M., Dell´Aqua, G., Mueckenthaler, M., Richter, A., Martin, H., Debus, J., Ansorqe, W., Hynynen, K., Huber, P. E. 2004. Apoptosis signals in lymphoblasts induced by focused ultrasound. *The FASEB Journal*, vol. 18, no. 12, p. 1413-1414. https://doi.org/10.1096/fj.04-1601fje

Ani, V., Varadaraj, M. C., Naidu Akhilender, K. 2006. Antioxidant and antibacterial activiries of polyphenolic compounds from bitter cumin (*Cuminum nigrum* L.). *European Food Research and Technology*, vol. 224, p. 109-115. https://doi.org/10.1007/s00217-006-0295-z

Barvkar, V. T., Pardeshi, V. C., Kale, S. M., Qiu, S., Rollins, M., Datla, R., Kadoo, N. Y. 2013. Genome-wide identification and characterization of microRNA genes and their targets in flax (*Linum usitatissimum*): Characterization of flax miRNA genes. *Planta*, vol. 237, no. 4, p. 1149-161. https://doi.org/10.1007/s00425-012-1833-5

Barlow, S. M. 1990. Toxicological Aspects of Antioxidants Used as Food Additives. In: Hundson, B. J. *Food Antioxidants*. Amsterdam, Netherlands : Springer Netherlands, p. 253-307. ISBN 978-94-010-6824-6. https://doi.org/10.1007/978-94-009-0753-9_7

Bej, S., Basak, J. 2014. MicroRNAs: The potential biomarkers in plant stress response. American Journal of Plant Sciences, vol. 5, p. 748-759. https://doi.org/10.4236/ajps.2014.55089

Bhattacharya, S. 2015. Conventional and Advanced Food Processing Technologies. Chapter 21. In Jambrak, A. R. et al. *Application of Ultrasonics in Food Preservation and Processing*. Hoboken, New Jersey, USA : John Wiley and Sons, Ltd. p. 515-536. ISBN 9781118406281.

da Silva, J. A. T, Dobranszki, J. 2014. Sonication and ultrasound: impact on plant growth and development. *Plant Cell, Tissue and Organ Culture*, vol. 117, no. 2, p. 131-143. https://doi.org/10.1007/s11240-014-0429-0

Chizzola, R., Saeidnejad, A. H., Azizi, M., Oroojalian, F., Mardan, H. 2014. Bunium persicum: variability in essential oil and antioxidant acitivty of fruits different Iranian wild populations. *Genetic Resources and Crop Evolution*, vol. 61, no. 8, p. 1621-1631. https://doi.org/10.1007/s10722-014-0158-6

Dua, A., Gupta, S. K., Mittal, A., Mahajan, R. 2012. A study of antioxidant properties and antioxidant compounds of cumin. *International Journal of Pharmaceutical and Biological Archives*, vol. 3, no. 5, p. 1110-1116.

Farmakopea Polska. 1999. 5th ed. Warszawa, Poland : PTFarm. 664 p.

Fatemi, F., Dadkhah, A., Rezaei, M. B., Dini, S. 2013. Effect of ɣ-irradiation on the chemical composition and antioxidant properties of cumin extracts. *Journal of Food Biochemistry*, vol. 37, no. 4, p. 432-439. https://doi.org/10.1111/j.1745-4514.2011.00641.x

Juhaimi, F. A., Uslu, N., Özcan, M. M. 2017. The effect of preultrasonic process on oil content and fatty acid composition of hazelnut, peanut and black cumin seeds. *Journal of Food Processing and Preservation*, vol. 42, no. 1, p. 1-4.

Kantar, M., Unver, T., Budak, H. 2010. Regulation of Barley miRNAs upon dehydration stress correlated with target gene expression. *Functional and Integrative Genomics*, vol. 10, no. 4, p. 493-507. https://doi.org/10.1007/s10142-010-0181-4
PMid:20676715

Kareshk, A. T., Keyhani, A., Mahmouduand, H., Oliaei, R. T., Asadi, A., Andishmand, M., Azzizian, H., Babaei, Z., Ziaali, N. 2015. Efficacy of the Bunium persicum Boiss. essential oil against Toxoplasmosis in mice model. *Iran Journal of Parasitology*, vol. 10, no. 4, p. 625-631.

Klimešová, M., Horáček, J., Ondřej, M., Manga, I., Koláčková, L., Nejeschlebová, Ľ., Ponížil, A. 2015. Microbial contamination of spice sused in production of meat products. *Potravinárstvo*, vol. 9, no. 1, p. 154-159. https://doi.org/10.5219/440

Kruszka, K., Pieczynski, M., Windels, D., Bielewicz, D., Jarmolowski, A., Kulinska-Szweykowska, Z., Vazquez, F. 2012. Role of microRNAs and other sRNAs of plants in their changing environments. *Journal of Plant Physiology*, vol. 169, no. 16, p. 1664-1672. https://doi.org/10.1016/j.jplph.2012.03.009
PMid:22647959

Lee, L. J., Abdullah, M. 2014. Optimization of genomic DNA shearing by sonication for next-generation sequencing library preparation. *Asia-Pacific Journal of Molecular Biology and Biotechnology*, vol. 22, no. 3, p. 200-208.

Lu, S., Sun, Y., Shi, R., Clark, C., Li, L., Chiang, V. 2005. Novel and mechanical stress-responsive MicroRNAs in Populus trichocarpa that are absent from Arabidopsis. *Plant Cell*, vol. 17, no. 8, p. 2186-2203. https://doi.org/10.1105/tpc.105.033456
PMid:15994906

Milowska, K., Gabryelak, T. 2007. Reactive oxygen species and DNA damage after ultrasound exposure. *Biomolecular Engineering*, vol. 24, no. 2, p. 263-267. https://doi.org/10.1016/j.bioeng.2007.02.001
PMid:17353145

Padmalatha, K, Prasad, M. N. V. 2005. Optimization of DNA isolationand PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal Biotechnology*, vol. 5, no. 3, p. 230-234.

Panwar, K. 2000. Black caraway. In Arya P. S. *Spice crops of India*. New Delhi : Kalyani Publishers, p. 172-178.

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, vol. 269, no. 2, p. 337-341. https://doi.org/10.1006/abio.1999.4019
PMid:10222007

Riesz, P., Kondo, T. 1992. Free radical formation induced by ultrasound and its biological implications. *Free Radical Biology and Medicine*, vol. 13, no. 3, p. 247-270. https://doi.org/10.1016/0891-5849(92)90021-8

Rogers, S. O., Bendich, A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. *Plant molecular biology Manual*, p. 183-190. https://doi.org/10.1007/978-94-011-0511-8_12

Rokhina, E. V., Lens, P., Virkutyte, J. 2009. Low-frequency ultrasound in biotechnology: state of the art. *Trends in Biotechnology*, vol. 27, no. 5, p. 298-299. https://doi.org/10.1016/j.tibtech.2009.02.001
PMid:19324441

Saeed, N., Khan, M. R., Shabbir, M. 2012. Antioxidant activity, totalphenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*, vol. 12, p. 221. https://doi.org/10.1186/1472-6882-12-221

Sambrook, J., Russell, D. W. 2006. Fragmentation of DNA by sonication. *CSH protocols*, vol. 2006, no. 4.

SAS 2009. Users guide version 9. 2. SAS/stat (r) SAS institute inc. cary, nc, USA.

Shapira, Z., Terkel, J., Egozi, Y., Nyska, A., Friedman, J. 1989. Abortifacient potential for the epigeal parts of Peganum harmala. *Journal of Ethnopharmacology*, vol. 27, no. 3, p. 319-325. https://doi.org/10.1016/0378-8741(89)90006-8

Sánchés-Moreno, C., Larrauri, A., Saura-Calixto, F. 1998. A procedure to measure the antioxidant efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, vol. 76, no. 2, p. 270-276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Seri, A., Khorsand, M., Rezaei, Z., Hamedi, A., Takhshid, M. A. 2017. Inhibitory effect of Bunium persicum hydroalcoholic extract on glucose-induced albumin glycation, oxidation and aggregation in vitro. *Iran Journal of Medical Sciences*, vol. 42, no. 4, p. 369-376. PMid:28761203

Shahsavari, N., Barzegar, M., Sahari, M. A., Naghdibadi, H. 2008. Antioxidant activity and chemical characterisation of essential oil of Bunium persicum. *Plant Foods for Human*

Nutrition, vol. 63, no. 4, p. 183-188. https://doi.org/10.1007/s11130-008-0091-y PMid:18810640

Sharififar, F., Yassa, N., Mozaffarian, V. 2010. Bioactivity of major components from the seeds of Bunium persicum Boiss. *Pakistan Journal of Pharmaceutical Sciences*, vol. 23, no. 3, p. 300-309. PMid:20566444

Schafer, F. Q., Kelly E. E., Buettner, G. R. 2003. Oxidative stress and antioxidant intervention. In: Cutler, R. G. et al. *Critical Reviews of Oxidative Stress and Aging: Advances in Basic Science, Diagnostics and Intervention*. New Jersey, London, Singapore, Hong Kong : World Scientific, p 849-869. ISBN 981-02-4636-6.

Simone, N. L., Soule, B. P, Ly, D., Saleh, A. D., Savage, J. E., Degraff, W., Cook, J., Harris, C. C., Gius, D., Mitchell, J. B. 2009. Ionizing radiation-induced oxidative stress alters miRNA expression. *PLoS One*, vol. 4, no. 7, p. e6377. https://doi.org/10.1371/journal.pone.0006377 PMid:19633716

Singleton, V. L., Rossi, J. A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Agricultural*, vol. 16, p. 144-158.

Souri, E., Amin, G., Farsam, H., Barazandeh, T. M. 2008. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU Journal of Pharmaceutical Sciences*, vol. 16, no. 2, p. 83-87.

Sultana, S., Ripa, F. A., Hamid, K. 2010. Comparative antioxidant activity study of some commonly used spices in Bangladesh. *Pakistan Journal of Biological Sciences*, vol. 13, no. 7, p. 340-343. https://doi.org/10.3923/pjbs.2010.340.343 PMid:20836290

Suslick, K. S. 1990. Sonochemistry. *Science*, vol. 247, no. 4949, p. 1439-1445. https://doi.org/10.1126/science.247.4949.1439 PMid:17791211

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., Sun, Q. 2010. Diverse set of MicroRNAs are responsive to powdery mildew infection and hest stress in wheat (*Triticum aestivum* L.). *BMC Plant Biology*, vol. 10, p. 123. https://doi.org/10.1186/1471-2229-10-123 PMid:20573268

Wan, G., Mathur, R., Hu, X., Zhang, X., Lu, X. 2011. MicroRNA response to DNA damage. *Trends in Biochemical Sciences*, vol. 36, no. 9, p. 478-484. https://doi.org/10.1016/j.tibs.2011.06.002 PMid:21741842

Wei, M., Yang, C. Y., Wei, S. H. 2012. Enhancement of the differentiation of protocorm-like bodies of Dendrobium officinale to shoots by ultrasound treatment. *Journal of Plant Physiology*, vol. 169, no. 8, p. 770-774. https://doi.org/10.1016/j.jplph.2012.01.018 PMid:22437146

Willett, W. C. 2002. Balancing life-style and genomics research for disease prevention. *Science*, vol. 292, no. 5568, p. 695-698. https://doi.org/10.1126/science.1071055 PMid:11976443

Zhang, X., Wan, G., Berger, F. G., He, X., Lu, X. 2011. The ATM kinase induces microRNA biogenesis in the DNA damage response. *Molecular Cell*, vol. 41, no. 4, p. 371-383. https://doi.org/10.1016/j.molcel.2011.01.020 PMid:21329876

Zhou, X., Wang, G., Zhang, W. 2007. UV-B responsive microRNA genes in Arabidopsis thaliana. *Molecular Systems Biology*, vol 3, no. 103. https://doi.org/10.1038/msb4100143

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