JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2021 April 15; 41(2): 293-300
ISSN 0255-2922
© 2021 JTCM. All rights reserved.

RESEARCH ARTICLE

Efficacy of methanol-water extract of Inula helenium root against oxidative DNA damage

Önder Aybastıer

Önder Aybastıer, Bursa Uludag University, Faculty of Science and Arts, Department of Chemistry, Bursa 16059, Turkey
Supported by a Grant from the Bursa Uludag University Research Foundation (Isolation of Phenolic Materials from Some Natural Specimens and Investigation of Their Effects on DNA Oxidative Damage; No. OUAP (F)-2013/13)
Correspondence: Dr. Önder Aybastıer, Bursa Uludag University, Faculty of Science and Arts, Department of Chemistry, Bursa 16059, Turkey. aybastier@uludag.edu.tr
Telephone: +90-224 29 41738
DOI: 10.19852/j.cnki.jtcm.20210209.008
Accepted: July 3, 2020

Abstract

OBJECTIVE: To investigate the efficacy of methanol-water extract of Inula helenium root against oxidative DNA damage.

METHODS: Antioxidant properties of methanol-water extract of Inula helenium root were revealed by 2,2'-azinobis (3-ethylbenzothiazoline-6-suphonic acid) di-ammonium salt (ABTS), Folin-Ciocalteu and high-performance liquid chromatography (HPLC) methods. The effect against oxidative DNA base damage was determined analyzing oxidative DNA base damage products by GC-MS/MS.

RESULTS: The methanol-water extract of Inula helenium root showed good antioxidant capacity [32.20 mg trolox equivalents (TE) /g dried weight (dw) by ABTS method, 54.53 mg TE/g dw by chromium reducing antioxidant capacity method] and total phenolic content [29.83 mg gallic acid equivalent (GAE) / g dw]. The chlorogenic acid, ferulic acid, rosmarinic acid and caffeic acid were determined in the extract by HPLC. The chlorogenic acid, ferulic acid and Inula helenium root extract were revealed to inhibit the oxidative DNA base damage by GC-MS/MS.

CONCLUSION: The studies showed a crucial decrease in the amount of the DNA base damage products when antioxidants were used. The results showed that ferulic acid has better inhibition than chlorogenic acid for DNA oxidation. The methanol-water extract of Inula helenium root was determined to inhibit the oxidative DNA damage.

© 2021 JTCM. All rights reserved.

Keywords: Inula; DNA damage; Antioxidants; Phytochemicals; Gas chromatography-mass spectrometry

INTRODUCTION

Free radicals are regularly produced as by product of aerobic organism. Furthermore, they can also form with various external effects.[1] The hydroxyl radical is the most reactive one among free radicals. It also reacts with all biomolecules and DNA at or near diffusion-controlled rates.[2] Oxidative stress occurs when non-enzymatic and enzymatic defense systems are exposed to high levels of oxidizing agents.[1] Oxidative stress can also drive the beginning of ill effects, which produces reactive oxygen species and is a hallmark of carcinoma, predisposing individuals to different types of carcinomas.[3,4] Unless repaired before DNA replication, oxidative DNA damage may lead to genomic instability and mutagenesis, therefore contributing to disease processes including cancer.[4,5] Cancer cell lines or cancerous and precancerous tissues or have been shown

to contain oxidative DNA damages at high amounts when compared to normal cell lines or to surrounding healthy tissues.[4,6]

Natural antioxidants have attracted particular interest because they can recuperate the human body from harmful oxidizing agents.[7] Antioxidant compounds of natural origin are substances that may protect living cells against the harmful effects of oxidative stress.[8] As such, the dietary route is an important factor in modulating endogenous antioxidant protection.[8,9] Antioxidants counter the production and harmful effects of and especially free radicals, preventing oxidative stress.[4] Antioxidants might have beneficial effects on protecting DNA and other biomolecules against oxidative damage. Therefore, the supplementation of antioxidants has become an effective therapeutic strategy for reduce the harmful effect of oxidants.[10,11]

Phenolic acids also have a wide distribution naturally among plants. They are divided into two classes: hydroxybenzoic acids and hydroxycinnamic acids.[12] Hydroxycinnamic acids and hydroxycinnamic acid derivatives are generally considered to be among the most valuable antioxidants in plants and fruits. The presence of antioxidant compounds confers their free radical scavenging properties. The chemical structure of hydroxycinnamic acid derivatives was evaluated to find out their ability to act as free radical scavengers.[10]

Chlorogenic acid (5-O-caffeicquinic acid) and ferulic acid (4-hydroxy-3-methoxycinnamic acid) are a hydroxycinnamic acid. Chlorogenic acid is the ester of 3, 4-dihydroxycinnamic acid and quinic acid that functions as an intermediate in the biosynthesis of lignin in plants.[13] Chlorogenic acid is a naturally occurring phenolic acid which is an inhibitor for formation of cancer. It has also been shown to prevent oxidative stress in rats.[14] Ferulic acid is a ubiquitous phenolic compound in plant tissues; consequently, it constitutes a bioactive component of many plants. It is generally found as ester cross-links with polysaccharides in the cell wall. Ferulic acid is found in many plants and occurs in particularly high amounts in bamboo shoots and popcorn.[15] Ferulic acid possesses antioxidant, anti-cancer, anti-inflammatory, antibacterial, anti-thrombosis activities. It is rapidly absorbed from the digestive system and excreted through the urine.[16]

Inula helenium L. (family: Asteraceae), also known as elecampane, is a source of phenolic compounds and it is a perennial herb which is distributed in East Asia, North America and Europe.[17] Food and Drug Administration (FDA) permits the use of alcoholic beverages obtained from Inula helenium roots and rhizomes as natural adjuvants and natural flavouring substances in foods and The Council of Europe (CE) lists Inula helenium as a natural food flavouring.[18] The roots of Inula helenium are collected in autumn after 2 to 3 years of growth.[19] They are used in traditional medicine to treat a variety of diseases including bronchitis, tuberculosis, cough, lung disorder, intestinal ulcers, chronic enterogastritis, indigestion, diabetes, infectious, a variety of dermatitis, and pediatric disease.[17] Anti-bacterial, anti-tumor and insecticidal properties were also reported.[20,21] Previous studies have explained most of these biological activities by the presence of polysaccharides, isoalantolactone, alantolactone and phenolic compounds such as ferulic, chlorogenic, rosmarinic and caffeic acids.[22,23]

In this study, we aimed to investigate the protective efficacy of methanol-water extract of Inula helenium root on oxidative DNA base damage.

METHODS

Chemicals

Formic acid, acetonitrile, phosphoric acid, trimethylchlorosilane (TMCS) and methanol were purchased from Merck (Darmstadt, Germany). Ethanol, sodium hydroxide, potassium dichromate, copper sulphate, sodium carbonate, Folin-Ciocalteu reagent, sodium potassium tartarate, 1,5-diphenyl carbazide, Trolox ([±]-6-hydroxy-2,5,7,8-tetramthylchroman-2-carboxylic acid) and DNA from calf thymus were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide, N,O-bis (trimethylsilyl) triflouroacetamid (BSTFA) and 2,2'-azinobis (3-ethylbenzothiazoline-6-suphonic acid) di-ammonium salt (ABTS) were purchased from Fluka (Buchs, Switzerland). Thymine glycol, 5-hydroxyuracil, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 8-hydroxyadenine and 5-hydroxycytosine were obtained from NIST (Gaithersburg, MD, USA). All other chemicals were of analytical grade.

Extraction methods

The ultrasound-assisted extraction of Inula helenium root was carried out in an ultrasonic bath. Dried Inula helenium root was purchased from a local market in Bursa-Turkey. The dried Inula helenium root was grinded into a uniform dry powder and stored at 4 °C. In each extraction, 1 g of powdered plant material was used with 20 mL of methanol∶pure water (70∶30) (v/v). The ultrasonic-assisted extraction was carried out at 50 ℃ for 90min. using an ultrasonic bath set to 40 kHz. The extract was filtered and stored in glass bottles at 4 ℃.

Determination of total phenolic content

The total phenolic content of the extract was determined used to Folin-Ciocalteu method as described in our previous paper.[24] Lowry A, B, C solutions were prepared fresh. Lowry A solution was prepared by dissolving 2 g of Na_2CO_3 and 0.4 g of NaOH in 100 mL water. Lowry B solution was also prepared by dissolving 0.5 g of $CuSO_4$ and 1 g of $NaKC_4H_4O_6$ in 100 mL water. Folin-Ciocalteu reagent was prepared by diluting the stock solution in a ratio of 1∶3 using water as the

dilution solvent. Lowry C solution was prepared by mixing 50 mL Lowry A and 1 mL Lowry B. The extract (0.10 mL), 1.90 mL pure water and 2.50 mL of Lowry C solution were mixed and finally 0.25 mL of Folin-Ciocalteu reagent was added to the mixture. The absorbance was recorded by spectrophotometer at 750 nm after 30 min. Total phenolic content were expressed as mg of gallic acid equivalent (GAE) / g dried weight (DW) of the plant.

ABTS assay

The ABTS assay was presented to determine the antioxidant capacity of extract, as described by Aybastıer *et al*.[24] $ABTS^{+}$ radical cation was produced by reacting 2.45 mM potassium persulfate solution with 20 mM ABTS solution and the mixture was kept in darkness for 18 hours at room temperature. The prepared solution was diluted with pure water at a ratio of 1∶10 before use. 0.20 mL extract, 3.80 mL ethanol and 1.00 mL $ABTS^{+}$ diluted solution. It was mixed and the absorbance was measured at 734 nm after 6 min. The results were expressed as mg of trolox equivalents (TE) / g dried weight of the plant.

Chromium reducing antioxidant capacity (CHROMAC) assay

CHROMAC assay was implemented according to the method described by Işık *et al*.[25] Two mL of pH 2.8 phosphate buffer solution, 0.25 mL of $K_2Cr_2O_7$ solution (100 mg/L), 0.25 mL of extract was mixed in a test tube. $K_2Cr_2O_7$ is reacted with the antioxidants, after 1min. it was mixed with 0.5 mL 1,5-diphenylcarbazide solution (3.4×10^{-4} mol/L), and 2.0 mL water was added. The absorbance was measured at 540 nm after 50 min. The results were expressed as mg of trolox equivalents (TE) / g dried weight of the plant.

High-performance liquid chromatography-diode array detection (HPLC-DAD) analysis

An Agilent 1200 HPLC system, consisting of a diode-array detector was used for the quantitative determination of phenolic compounds in the extract. The separation was performed by the XBridge C18 column (4.6 × 250 mm, 3.5 μm). A mobile phase consisting of acetonitrile (A) and 1% (v/v) formic acid in water (B); where a gradient elution program of 90% A (0 min), 87% A (10 min), 58.5% A (20 min), 30% A (35 min), and 90% A (37 min). The injection volume of sample was 10 μL and the flow rate of mobile phase was 0.5 mL/min. The monitoring wavelengths of interest were 320 nm for chlorogenic acid (Figure 1A), ferulic acid (Figure 1B), caffeic acid and rosmarinic acid. Peaks were identified on the basis of comparison of UV spectra and retention times with standards of phenolic compounds. (Table 1)

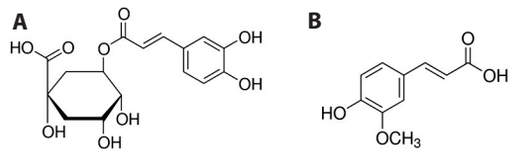

Figure 1 Chemical structure of two phenolic compounds determined by HPLC

A: chlorogenic acid; B: ferulic acid. HPLC: high performance liquid chromatography.

Preparation of calf thymus DNA

Lyophilized powder of DNA from calf thymus (5 mg) was dissolved in 100 mL ultra-pure water and incubated at 4 ºC for 24 h.[11] The concentration of DNA was measured at 260 nm by UV-Vis spectrophotometer (Varian Cary 50, Australia). The DNA concentration was calculated using the following equation (1).

$$\mu g\ DNA = A_{260} \times 50 \quad (1)$$

Effects of Inula helenium antioxidants on DNA

The oxidative stress medium was formed using Fenton reaction. The hydroxyl radical is produced by the reaction of hydrogen peroxide with iron (Ⅱ) ion.[11] Hydrogen peroxide, Fe (Ⅱ), chlorogenic acid and ferulic acid stock solutions were prepared. Each sample volume was 2.50 mL and contained 0.10 mg DNA in addition to the other compounds described as follows: (a) only 0.10 mg DNA, (b) 20 μM chlorogenic acid, (c) 20 μM ferulic acid, (d) extract including 20 μM chlorogenic acid and 65 μM ferulic acid, (e) Fenton (100 μM Fe^{+2} and 200 μM H_2O_2), (f) 5 μM chlorogenic acid and Fenton, (g) 20 μM chlorogenic acid and Fenton, (h) 50 μM chlorogenic acid and Fenton, (i) 5 μM ferulic acid and Fenton, (j) 20 μM ferulic acid Fenton, (k) 50 μM ferulic acid and Fenton, (l) extract containing 20 μM chlorogenic acid and 6.1 μM ferulic acid and Fenton. Each sample preparation begins with adding 0.10 mg of calf thymus DNA, pure water, antioxidant solution, H_2O_2 and Fe (Ⅱ) consecutively. After, they were incubated at 37 ℃ for 20 min. The samples then were frozen at − 24 ℃, and then was lyophilized using freeze dryer at a vacuum of 0.1 mbar and − 85 ℃.

Table 1 Calibration data of phenolic compounds for HPLC-DAD analysis

Phenolic compound	Retention time (min)	Calibration equation	Regression coefficient (R^2)
Chlorogenic acid	15.62	y=49.950x − 29.053	0.9982
Caffeic acid	19.96	y=155.860x − 7.955	0.9972
Ferulic acid	24.13	y=112.780x − 180.750	0.9965
Rosmarinic acid	25.37	y=42.728x + 43.959	0.9976

Note: HPLC-DAD: high-performance liquid chromatography-diode array detection.

The analysis of DNA base damage products by GC-MS/MS

The analysis of DNA base damage products was carried out according to the procedure reported in our previous studies.[6,11,26] The DNA was hydrolyzed by the addition of 1 mL 60% (v/v) formic acid solution. It was heated at 130 ℃ for 30 min. in an oven. DNA base damage products were released after hydrolysis process. Finally, the samples were frozen at − 24 ℃ and lyophilized at same conditions before derivatization for GC-MS/MS analysis.

RESULTS

Total phenolic content

The total phenolic content of the Inula helenium root was determined to (29.8 ± 0.7) mg gallic acid equivalent/g dried weight using Folin-Ciocalteu method. The total phenolic content result of Inula helenium was higher than the results of Petkova *et al*.[22] This may be due to the extraction time, solvent and the area where the plant grows. The total phenolic content showed in this study were compared with those obtained by Spiridon *et al*.[27] Where TPC of the methanolic extract in our study was found to be quite similar to that of the extracted fraction coded as F2 where a multistep extraction process including methanol, water and chloroform as solvents was implemented. The high polarity of the phenolic compounds makes them extractable by polar solvents and for this reason, the phenolic content of the extract increased with increasing the polarity of the extraction solvent using HCl. Furthermore, hydrolysis of glycoside bonds is often used in the extraction procedure by acid, and thereby essential information of total phenolic compounds as aglycones can be obtained.[27] Furthermore, the different plant varieties used, the different conditions of extraction and preparation of the plant material contribute to the different results.

Antioxidant capacity

The antioxidant capacity of the extract of Inula helenium root was evaluated by CHROMAC and ABTS methods. CHROMAC [(54.5 ± 1.7) mg trolox equivalent/g dried weight] showed a slightly higher antioxidant capacity value than ABTS [(32.2 ± 0.5) mg trolox equivalent/g dried weight] for Inula helenium root extract. These findings in addition to the TPC results suggest that the extract has an antioxidant property. The antioxidant capacity correlates with the total phenolic content. The antioxidant capacities of fruits and plants have been mainly attributed to phenolic compounds. Santoyo *et al* [28] reported that statistical analysis showed a correlation between the amount of phenolic compounds of various plants and the antioxidant capacity. Generally, the antioxidant capacity increases as the phenolic content increases.

HPLC-DAD analysis results

The extract of Inula helenium root was investigated for their phenolic content using HPLC-DAD system. The chromatogram indicates four phenolic compounds that chlorogenic acid, ferulic acid, caffeic acid and rosmarinic acid in the Figure 2A-B. Some small peaks appeared prior to ferulic acid and after caffeic acid indicating the presence of other compounds. These compounds can be ignored because they are at lower concentrations than those of the targeted ones in this study. These peaks may belong to other phenolic compounds mentioned in the literature. Petkova *et al* [22] determined that the highest amount of phenolic acid is chlorogenic acid and ferulic acid in the Inula helenium. Mekinic *et al* [23] reported the rosmarinic acid is one of the main compounds identified in Inula helenium. The methanol-water extract of Inula helenium roots contains chlorogenic acid [(3.90 ± 0.19) mg/g dried weight], ferulic acid [(1.20 ± 0.07) mg/g dried weight], rosmarinic acid [(0.96 ± 0.03) mg/g dried weight] and caffeic acid [(0.13 ± 0.01) mg/g dried weight]. Spiridon *et al* [27] reported the presence of caffeic and chlorogenic acids in the extract of I. helenium which comply with the results obtained by our study. They also reported that derivatives of ferulic acids including its O-glucoside form were found in the extracts.

Antioxidant effects of extracts against DNA base damage by GC-MS/MS

The ability of the chlorogenic acid, ferulic acid and extract of Inula helenium root to inhibit DNA base oxidation was investigated by determining the DNA base

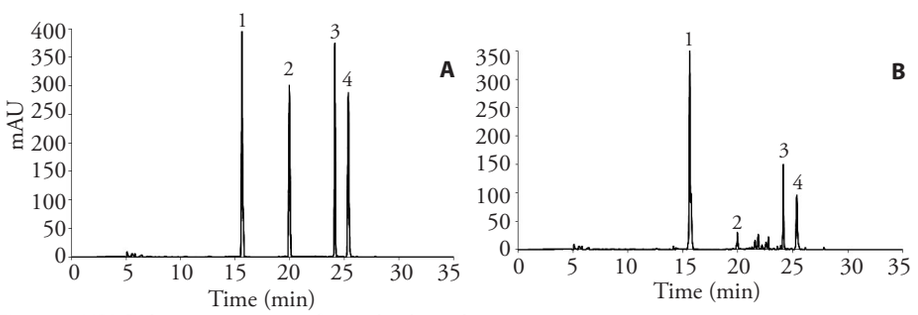

Figure 2 HPLC chromatograms of standards and extract at 320 nm

A: phenolic standards each of 100 mg/L concentration; B: extract of Inula helenium root. Peak 1: chlorogenic acid, Peak 2: caffeic acid, Peak 3: ferulic acid, peak 4: rosmarinic acid. HPLC: high performance liquid chromatography.

damage products by GC-MS/MS. Eighteen DNA base damage products were sensitively determined by selected reaction monitoring (SRM) mode based on product ions and evaluated quantitatively. The results of oxidative DNA base damage products in DNA control, Fenton control, chlorogenic acid, ferulic acid and Inula helenium root extract with and without Fenton reaction are shown in Tables 2 and 3. The data of Table 2 shows the amounts of the oxidative DNA base damage products without the use of Fenton reaction. The effects of hydroxyl radical on the DNA bases are shown in Table 3. More detailed information about the analysis of DNA base damage products can be found in our other works.[6,11,26]

Some DNA base damage products were represented as under the limit of detection (< LOD) in Tables 2 and 3 for they were not detected by GC-MS/MS. Comparing the samples subjected to oxidative stress (Table 3) to those without oxidative stress medium (Table 2), as expected the amounts of DNA base damage products are more in the samples of Table 5 than those of Table 2. The amounts of base damage products show obvious increases with oxidative stress. The effect of the oxidative medium induced by the use of Fenton reaction is the main cause for the high levels of DNA base damage. Three different concentrations of the chlorogenic acid and ferulic acid were tested for their ability to inhibit oxidative DNA base damage. In comparison to the Fenton control sample without antioxidant, the ferulic acid and chlorogenic acid were inhibited the damage in proportion to the concentration.

When the data of total DNA base damage in Table 3 were analyzed, the antioxidant power of ferulic acid was observed to be better than for chlorogenic acid. There is a clear commensurate correlation between the concentration of chlorogenic acid and ferulic acid with the prevention of oxidative DNA base damage (Figure 3). The concentration of 50 μM of ferulic acid decreases by about 84% of the total DNA base damage products caused by Fenton reaction, while 50 μM of chlorogenic acid could only decrease the base damage products by about 66% . The effect of concentration was shown to be significant where a 10 times increase of ferulic acid concentration contributed to about 70% decrease of base damage products, whereas the same increase in chlorogenic acid concentration resulted in an about 33% decrease of base damage products.

Inula helenium root extract was revealed to inhibit the oxidative DNA base damage. The extract decreased about 64% of base damage products caused by the Fenton control sample. The chlorogenic acid is the phenolic compound of the highest amount in the extract ac-

Table 2 Oxidatively induced DNA damage products (ng/mg DNA) in control, standard antioxidant samples and extract without Fenton reaction medium.

Item	DNA control	20 μM Chl	20 μM Fer	20 μM Chl/ 20 μM Fer	Ext
56DHT	<LOD	<LOD	<LOD	<LOD	<LOD
56DHU	0.24	<LOD	<LOD	<LOD	<LOD
5H5MH	0.58	0.88	0.15	2.29	5.34
5HH	5.42	6.60	2.50	9.93	9.17
5FU	<LOD	11.95	12.33	12.06	7.36
5HU	9.45	8.79	2.12	2.03	2.36
5HMU	3.06	4.68	5.37	7.61	5.21
Alx	1.95	1.93	1.82	0.48	<LOD
5HC	3.85	5.69	2.31	4.97	6.28
46D5NP	1.53	1.07	1.16	1.07	2.76
TG	<LOD	<LOD	<LOD	<LOD	0.59
5HMC	0.93	5.80	1.33	6.54	7.07
FapyAde	0.26	<LOD	<LOD	<LOD	2.91
8HA	0.28	3.88	1.90	0.98	3.61
2HA	<LOD	0.25	2.05	5.28	5.54
FapyGua	<LOD	0.01	3.22	0.13	4.54
28DHA	0.42	0.42	0.42	1.23	2.96
8OHG	2.51	2.47	3.20	1.72	1.98
Total	30.48	54.42	39.88	56.32	67.68

Notes: Chl: chlorogenic acid; Fer: ferulic acid; Ext: contains 20 μM chlorogenic acid and 6.1 μM ferulic acid; LOD: limit of detection; 56DHT: 5;6-Dihydrothymine; 56DHU: 5;6-Dihydrouracil; 5H5MH: 5-Hydroxy-5-methylhydantoin; 5HH: 5-Hydroxy hydantoin; 5FU: 5-Formyluracil; 5HU: 5-Hydroxyuracil; 5HMU: 5-(Hydroxymethyl)uracil; Alx: Alloxane; 5HC: 5-Hydroxycytosine; 46D5NP: 4, 6-Diamino-5-nitropyrimidine; TG: thymine glycol; 5HMC: 5-(Hydroxymethyl)cytosine; FapyAde: 4,6-Diamino-5-(formylamino)pyrimidine; 8HA: 8-Hydroxyadenine; 2HA: 2-Hydroxyadenin; FapyGua: 2,6-Diamino-4-hydroxy-5-formamidopyrimidine; 28DHA: 2,8-Dihydroxyadenine; 8OHG: 8-Hydroxyguanine.

Table 3 Oxidatively induced DNA damage products (ng/mg DNA) in samples under oxidative conditions by Fenton reaction: 200 μM H_2O_2 and 100 μM Fe^{2+}

Item	Fenton control	5 μM Chl	20 μM Chl	50 μM Chl	5 μM Fer	20 μM Fer	50 μM Fer	Ext
56DHT	2.26	<LOD	<LOD	0.19	0.23	<LOD	0.35	<LOD
56DHU	2.16	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5H5MH	66.92	53.06	52.33	46.62	19.27	68.63	31.76	10.01
5HH	43.60	26.25	18.46	14.28	16.08	22.39	11.35	36.71
5FU	49.16	15.47	27.93	30.41	<LOD	<LOD	<LOD	51.62
5HU	359.56	19.35	25.38	18.76	12.73	17.01	11.03	30.90
5HMU	40.35	22.02	33.17	26.64	18.15	21.22	16.47	56.76
Alx	10.86	3.91	2.66	1.07	1.12	0.89	0.34	13.80
5HC	43.30	25.95	20.72	25.81	19.35	24.27	18.08	36.09
46D5NP	2.12	1.21	1.22	0.97	1.12	1.21	1.07	1.76
TG	5.73	3.60	3.47	3.54	1.77	8.85	3.10	10.23
5HMC	18.24	10.30	8.97	5.41	6.62	11.63	5.39	21.49
FapyAde	196.90	178.39	141.73	121.28	247.93	15.53	11.15	19.11
8HA	77.96	20.22	29.52	19.16	18.49	27.28	13.33	3.01
2HA	113.11	80.06	71.16	18.42	23.13	42.94	21.31	59.18
FapyGua	3.25	3.26	3.20	3.16	3.12	3.14	3.12	3.49
28DHA	2.24	1.93	1.64	1.48	1.06	1.19	0.96	8.71
8OHG	130.46	135.85	69.26	63.91	51.30	94.99	42.21	51.99
Total	1168.18	600.83	510.82	401.11	441.47	361.17	191.02	414.86

Notes: Chl: chlorogenic acid; Fer: ferulic acid; Ext: contains 20 μM chlorogenic acid and 6.1 μM ferulic acid; LOD: limit of detection; 56DHT: 5;6-Dihydrothymine; 56DHU: 5;6-Dihydrouracil; 5H5MH: 5-Hydroxy-5-methylhydantoin; 5HH: 5-Hydroxy hydantoin; 5FU: 5-Formyluracil; 5HU: 5-Hydroxyuracil; 5HMU: 5-(Hydroxymethyl)uracil; Alx: Alloxane; 5HC: 5-Hydroxycytosine; 46D5NP: 4, 6-Diamino-5-nitropyrimidine; TG: thymine glycol; 5HMC: 5-(Hydroxymethyl)cytosine; FapyAde: 4,6-Diamino-5-(formylamino)pyrimidine; 8HA: 8-Hydroxyadenine; 2HA: 2-Hydroxyadenin; FapyGua: 2,6-Diamino-4-hydroxy-5-formamidopyrimidine; 28DHA: 2,8-Dihydroxyadenine; 8OHG: 8-Hydroxyguanine.

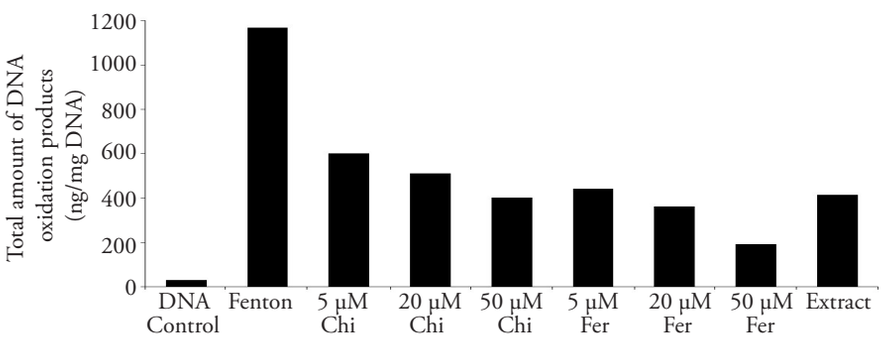

Figure 3 Effect of chlorogenic acid and ferulic acid and extract to reduce oxidatively induced DNA damage products
Chl: chlorogenic acid; Fer: ferulic acid; Extract: Inula helenium root extract contains 20 μM chlorogenic acid and 6.1 μM ferulic acid.

cording to the HPLC results. The protective efficacy of the extract that contains 20 μM chlorogenic acid was better than those of 20 μM chlorogenic acid standard. In many previous studies, the beneficial and antioxidant effects of ferulic acid and chlorogenic acid have been mentioned. Chlorogenic acid is one of the most abundant phenolic compounds in the human diet with vegetables, fruits and coffee as its main sources. It acts as an antioxidant in plants and protects against degenerative diseases in living creatures when supplied in their diet. Chlorogenic acid has been reported to exert inhibitory effects on various types of cancer in the large and a protective action on oxidative stress *in vivo*. Other research has examined the protective effects of chlorogenic acid derivatives against oxidative damage. Chlorogenic acid is phenolic antioxidant as protective functional factor and beneficial in oxidative stress-related diseases.[29] Ferulic acid is found in most of the food plants and cell walls of cereal grains. Ferulic acid possesses several protective activities such as, antioxidative, antidiabetic, anti-carcinogenic, anti-inflammatory, hypotensive and hypolipidemic effects. Ferulic acid supplementation increases cellular antioxidant activities, thereby neutralizing reactive oxygen species under pathophysiological condition as well as reduces the risk of oxidative-induced diseases by scavenging free radicals. It was shown that ferulic acid can potentiate the cell growth inhibitory activity in a wide range of cancer

cells, including breast, pancreatic and prostate cancer cells.[30]

DISCUSSION

Phytochemicals are effective scavengers of free radicals and are considered effective antioxidant compounds obtained from plants. It is proposed that polyphenolic compounds have inhibitory effects on carcinogenesis and mutagenesis in humans.[31] Biologically active components, such as phenolic compounds present antioxidant activity by giving hydrogen to active free radicals. This scavenging potential of phenolic compounds to inhibit radicals was elucidated by their phenolic hydroxyl groups. These phenolic compounds have been described as an effective antioxidant component against hydroxyl radical, superoxide anion and hydrogen peroxide.[11,24,31] H_2O_2 is not a free radical and can readily diffuse through cell membrane. Therefore, it is generally supposed to be the major forerunner of free radicals such as hydroxyl radical, which is very reactive and causes biomolecules oxidation such as lipids, proteins and DNA. Oxidative stress is an important factor in the pathogenesis of various diseases. H_2O_2-induced oxidative stress model is often used to study the antioxidative effect of phenolic compounds *in vitro*.[7,32,33]

The generation of ROS is well balanced under normally physiological conditions maintaining. Antioxidant enzymes in cells, such as catalase, superoxide dismutase, glutathione peroxidase, could scavenge ROS, which play an essential role in keeping the antioxidant capacity of organism from free radical assault. Oxidative stress occurs when the balance between ROS production and scavenging of antioxidants is disturbed.[7,9,33]

In this study, in the examination of the total DNA base damage results, the antioxidant property of the different amounts of chlorogenic acid, ferulic acid standards and extract of Inula helenium root is obviously proved for protection of DNA against oxidation. Formation of oxidative DNA base damage products were decreased by chlorogenic acid and ferulic acid, apparently with different hydroxyl radical scavenging powers. Therefore, other antioxidants may also prevent the formation of DNA base damage products.

In conclusion, the results of this study reveal that ferulic acid has better inhibition than chlorogenic acid for DNA oxidation, and that the methanol-water extract of Inula helenium root inhibit the oxidative DNA damage. These may open new possibilities of using well-known Inula helenium in the food and pharmaceutical industry as a source of naturally active ingredients. Further studies are recommended to be achieved to discover *in-vivo* activities of those plants' extracts.

REFERENCES

1 **Ceylan D**, Tuna G, Kirkali G, Tunca Z, Can G, Arat HE, Kant M, Dizdaroglu M, Özerdem A. Oxidatively-induced DNA damage and base excision repair in euthymic patients with bipolar disorder. DNA Repair 2018; 65: 64-72.

2 **Dizdaroglu M**, Coskun E, Jaruga P. Measurement of oxidatively induced DNA damage and its repair, by mass spectrometric techniques. Free Radic Res 2015; 49(5): 525-548.

3 **Olinski R**, Siomek A, Rozalski R, Gackowski D, Foksinski M, Guz J, Dziaman T, Szpila A, Tudek B. Oxidative damage to DNA and antioxidant status in aging and age-related diseases. Acta Biochim Pol 2007; 54(1): 11-26.

4 **Dizdaroglu M**. Oxidatively induced DNA damage and its repair in cancer. Mutat Res 2015; 763: 212-245.

5 **Friedberg EC**. DNA Damage and Repair. Nature 2003; 421: 436-440.

6 **Aybastıer Ö**, Dawbaa S, Demir C, Akgün O, Ulukaya E, Arı F. Quantification of DNA damage products by gas chromatography tandem mass spectrometry in lung cell lines and prevention effect of thyme antioxidants on oxidative induced DNA damage. Mutat Res Fund Mol M 2018; 808: 1-9.

7 **Amin F**, Bano B. Damage of cystatin due to ROS-generation and radical-scavenging activity of antioxidants and associated compounds. Int J Biol Macromol 2018; 119: 369-379.

8 **Yadav A**, Kumari R, Yadav A, Mishra JP, Srivatva S, Prabha S. Antioxidants and its functions in human body - a review. Res Environ Life Sci 2016; 9(11): 1328-1331.

9 **Cooke MS**, Evans, MD, Mistry N, Lunec J. Role of dietary antioxidants in the prevention of *in vivo* oxidative DNA damage. Nutr Res Rev 2002; 15: 19-41.

10 **Llorens E**, del Valle LJ, Puiggali J. Inhibition of radical-induced oxidative DNA damage by antioxidants loaded in electrospun polylactide nanofiber. Macromol Res 2014; 22 (4): 388-396.

11 **Aybastıer Ö**, Dawbaa S, Demir C. Investigation of Antioxidant ability of grape seeds extract to prevent oxidatively induced DNA damage by gas chromatography-tandem mass spectrometry. J Chromatogr B 2018; 1072: 328-335.

12 **D'Archivio M**, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C. Masella R. Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 2007; 43(4): 348- 361.

13 **Hwang SH**, Zuo G, Wang Z, Lim SS. Novel aldose reductase inhibitory and antioxidant chlorogenic acid derivatives obtained by heat treatment of chlorogenic acid and amino acids. Food Chem 2018; 266: 449-457.

14 **Shahrzad S**, Bitsch I. Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography. J Chromatogr A 1996; 741 (2): 223-231.

15 **Zhao Z**, Moghadasian MH. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: a review. Food Chem 2008; 109: 691-702.

16 **Dulong V**, Kouassi MC, Labat B, Cerf DL, Picton L. Antioxidant properties and bioactivity of carboxymethylpullulan grafted with ferulic acid and of their hydrogels obtained by enzymatic reaction. Food Chem 2018; 262: 21-29.

17 **Koç K**, Özdemir O, Doğru Ü, Türkez H. Antioxidant

and anticancer activities of extract of Inula Helenium (L.) in human U-87 MG glioblastoma cell line. J Cancer Res Ther 2018; 14: 658-661.

18 Food and Drug Administration. Food additives permitted for direct addition to food for human consumption. Code of Federal Regulations. 2014; Title 21, Vol. 3.

19 **Stojakowska A**, Malarz J, Kis, AK. Hydroxycinnamates from elecampane (Inula Helenium L.) callus culture. Acta Physiol Plant 2016; 38-41.

20 **Konishi T**, Shimada Y, Nagao T, Okabe H, Konoshima T. Antiproliferative sesquiterpene lactones from the roots of Inula Helenium. Biol Pharm Bull 2002; 25(10): 1370-1372.

21 **Seca AML**, Grigore A, Pinto DCGA, Silva AMS. The genus inula and their metabolites: from ethnopharmacological to medicinal uses. J Ethnopharmacol 2014; 154: 286-310.

22 **Petkova N**, Ivanov I, Vrancheva R, Denev, P. Pavlov, A. Ultrasound and microwave-assisted extraction of elecampane (Inula helenium) roots. Nat Prod Commun 2017; 12 (2): 171-174.

23 **Mekinic IG**, Burcul F, Blazevic I, Skroza D, Kerum D, Katalinic V. Antioxidative / acetylcholinesterase inhibitory activity of some asteraceae plants. Nat Prod Commun 2013; 8(4): 471-474.

24 **Aybastıer Ö**, Şahin S, Demir C. Response surface optimized ultrasonic-assisted extraction of quercetin and isolation of phenolic compounds from Hypericum perforatum L. by column chromatography. Sep Sci Technol 2013; 48 (11): 1665-1674.

25 **Işık E**, Şahin S, Demir C. Development of a new chromium reducing antioxidant capacity (CHROMAC) assay for plants and fruits. Talanta 2013; 111: 119-124.

26 **Dawbaa S**, Aybastıer Ö, Demir C. Ultrasensitive determination of DNA oxidation products by gas chromatography-tandem mass spectrometry and the role of antioxidants in the prevention of oxidative damage. J Chromatogr B Analyt Technol Biomed Life Sci 2017; 1051: 84-91.

27 **Spiridon I**, Nechita CB, Niculaua M, Silion M, Armatu A, Teaca CA, Bodirlau R. Antioxidant and chemical properties of Inula Helenium root extracts. Cent Eur J Chem 2013; 11(10): 1699-1709.

28 **Santoyo S**, Lloria R, Jaime L, Ibanez E, Senorans FJ, Reglero G. Supercritical fluid extraction of antioxidant and antimicrobial compounds from Laurus Nobilis L. chemical and functional characterization. Eur Food Res Technol 2006; 222: 565-571.

29 **Upadhyay R**, Rao LJM. An outlook on chlorogenic acids-occurrence, chemistry, technology, and biological activities. Crit Rev Food Sci Nutr 2013; 53(9): 968-984.

30 **Chowdhury S**, Ghosh S, Das AK, Sil PC. Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy. Front Pharmacol 2019; 10: 27.

31 **31**. Barakat H, Almundarij TI. Phenolic compounds and hepatoprotective potential of Anastatica hierochuntica ethanolic and aqueous extracts against CCl_4-induced hepatotoxicity in rats. J Tradit Chin Med 2020; 40(6): 974-955.

32 **Ighodaro OM**, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alex J Med 2018; 54 (4): 287-293.

33 **Liu C**, Lu D, You J, Lu Y, Sun J, Pan J, Wang Y, Wang A, Lan Y, Li Y, Liu T. Efficacy of water fraction from dioscorea cirrhosa on oxidative stress and apoptosis in H9c2 cardiomyocytes induced by H_2O_2. J Tradit Chin Med 2021; 41(1): 51-58.