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RESEARCH

Histone deacetylase inhibitory and antioxidant activities of some ethanolic plant extracts: A novel therapeutic approach for cancer

Bazı etanolik bitki ekstrelerinin histon deasetilaz inhibitör ve antioksidan aktiviteleri: Kanser için yeni bir terapötik yaklaşım

Eda Dagsuyu[1], Refiye Yanardag[1]

[1]Istanbul University-Cerrahpaşa, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey.

Abstract

Purpose: The aim of this study was to evaluate histone deacetylase inhibitory and antioxidant activities of some ethanolic plant extracts.
Materials and Methods: In this study, the inhibitory effects of ethyl alcohol extracts prepared from various plants and some chemical compounds on histone deacetylase activity were investigated. In addition, the antioxidant activity of plant-derived active substances, which have had an important value in alternative medicine in recent years, has been determined to replace chemicals.
Results: All the plant extracts and chemical substances used in the study showed histone deacetylase inhibitory effect (with IC_{50} range of 0.000078-319.0 μg/mL) and antioxidant activity. The results indicated that the percentage of histone deacetylase inhibition and antioxidant activities increased with increasing concentrations of the plant extracts and chemical compounds.
Conclusion: These plant extracts which are potential sources of histone deacetylase inhibitors may be appropriately used as an alternative support to drug treatment rather than the use of toxic chemicals.
Keywords:. Antioxidant activity, cancer, enzyme inhibition, histone deacetylase, plant extract.

Öz

Amaç: Bu çalışmanın amacı, bazı etanolik bitki ekstraktlarının histon deasetilaz inhibitörü ve antioksidan aktivitelerini değerlendirmektir.
Gereç ve Yöntem: Bu çalışmada, çeşitli bitkilerden hazırlanan etil alkollü ekstrelerin ve bazı kimyasal bileşiklerin histon deasetilaz aktivitesi üzerindeki inhibitör etkileri araştırıldı. Ayrıca son yıllarda alternatif tıpta önemli bir değere sahip olan bitki kaynaklı aktif maddelerin, kimyasalların yerini alması için antioksidan aktivitesi de tayin edildi.
Bulgular: Çalışmada kullanılan tüm bitki ekstreleri ve kimyasal maddeler histon deasetilazı inhibe edici etki (IC_{50} 0.000078-319.0 μg/mL aralığında) ve antioksidan aktivite göstermiştir. Sonuçlar, histon deasetilaz inhibisyon yüzdesinin ve antioksidan aktivitelerinin, bitki ekstrelerinin ve kimyasal bileşiklerin artan konsantrasyonu ile arttığını göstermiştir.
Sonuç: Histon deasetilaz inhibitörlerinin potansiyel kaynakları olan bu bitki ekstrelerinin, toksik kimyasalların kullanımı yerine ilaç tedavisine alternatif bir destek olarak uygun şekilde kullanılabileceği önerilebilir.

Anahtar kelimeler: Antioksidan aktivite, kanser, enzim inhibisyonu, histon deasetilaz, bitki ektresi.

INTRODUCTION

Cancer development is also dependent on epigenetic alterations due to covalent modification of deoxyribonucleic acid (DNA) (such as DNA methylation and post-translational histone acetylations), that can alter DNA accessibilities, gene expression and chromatin structures without alterations in the DNA sequence[1]. The acetylation and deacetylation of histones are regulated by histone acetyltransferases (HATs) and deacetylases (HDACs), respectively[2]. Histone modification is vital in the regulation of chromatin structure and gene expression. Gene expression can be regulated by these modifications which can cause many diseases[3]. HATs transfer acetyl groups to N-ε-lysine in

Address for Correspondence: Eda Dagsuyu, Istanbul University-Cerrahpaşa, Faculty of Engineering, Department of Chemistry, Istanbul, Turkey E-mail: eda.dagsuyu@iuc.edu.tr
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histones, which results in local expansion of chromatin and increases the accessibility of regulatory proteins to DNA and transcriptional activation. Whereas, HDACs remove the acetyl groups of lysine residues of histones as well as many other non-histone proteins, leading to chromatin condensation, silencing of genes and transcriptional repression[4,5].

Inhibition of HDAC plays an important role in apoptosis, proliferation, differentiation, migration and angiogenesis of many cancer cells[6]. Mammalian HDACs can be subdivided into two families according to the cofactor (zinc/Zn^{2+} dependent for Classes I, II and IV or nicotinamide adenine dinucleotide/NAD^{+} dependent for Class III) required for activity. These are further classified into 18 isoforms depending on their primary homology to Saccharomyces cerevisiae HDACs (RPD3, HDA1 and SIR2)[7,8]. Class I HDACs (HDAC1, HDAC2, HDAC3, HDAC8 and HDAC11) are located mostly within the nucleus and are expressed ubiquitously. Class II HDAC enzymes (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9 and HDAC10) generally shuttle between the nucleus and the cytoplasm, with the exception of HDAC6, which are located only in the cytoplasm. Class III members are named sirtuins (SIRTs) which consist of 7 isoforms in humans. SIRTs 1, 2, 6, and 7 are found in the nucleus, whereas SIRTs 3, 4, and 5 are found in the mitochondria[7,9,10].

Histone deacetylase inhibitors (HDACi) have the potential to arrest growth and differentiation, and can induce apoptosis in a variety of cancer cell lines[11]. These molecules cause cell death through multiple mechanisms including upregulation of death receptors, induction of oxidative injury, disruption of DNA repair and also have immunomodulatory activity[12,13]. HDAC inhibitors can be categorized into varied groupings named according to the chemical structures: hydroxamates (such as trichostatin A; TSA, suberoylanilide hydroxamic acid; SAHA), cyclic peptides (such as depsipeptide, apicidin), short-chain fatty acids (such as butyrates, valproic acid) and benzamides (such as MS-275)[14,15]. Many natural and synthetic inhibitors have been identified to inhibit HDACs by blocking their active site. Various HDACi have been approved for hematological malignancies by the Food and Drug Administration (FDA). Valproic acid induces tumor differentiation and apoptosis. It also suppresses tumor growth and metastatic processes[5]. Vorinostat (SAHA) and romidepsin are approved for the treatment of cutaneous T cell lymphoma. Belinostat and panobinostat are approved for the clinical treatment of cutaneous T-cell lymphoma, peripheral T-cell lymphoma or multiple myeloma[16].

In this study, HDAC inhibition activity and free radical scavenging activity (such as 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity and ferric-reducing antioxidant power) of some chemicals and ethanol extracts of various plants *in vitro* were investigated.

In our study, we argue that plant extracts may have therapeutic potential against oxidative stress-related diseases. Thus, this study will provide some preliminary evidence that these plant extracts may be effective therapeutic agents in controlling cancer and their anticarcinogenic effects. These plant extracts could perhaps provide an alternative in the future when it comes to creating novel therapeutic targets and alternative therapeutic approaches for the treatment of cancer.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade and purchased from Sigma, Merck and Fluka Chemical Co. Histone Deacetylase Assay Kit, Fluorometric (CS1010) was purchased from Sigma-Aldrich.

Plant materials

Plant materials and fruits were either purchased from a market or spice store. Seeds of black sesame and flaxseed, flower of broccoli, fruit of lemon, pineapple, and pomegranate; the root of licorice, and leaves of mistletoe, oleander, stinging nettle, tree of heaven, wormwood, and white cabbage were used.

Preparation of the plant extracts

Fresh plant samples were washed with water after collection. Air dried at room temperature for about 10 days. Fruits were cut into small pieces and dried in an oven at +37°C for 48 h. All plant materials were stored at +4°C until extraction. For the 70% ethanol extracts preparation, 20 grams of plant material were extracted by Soxhlet apparatus with 200 mL 70% ethanol for 8 h until fully colorless. Ethanol was removed by rotary evaporation (Bibby, USA) under

reduced pressure at 50°C[17]. Extracts were concentrated, dried and kept in the dark at -20°C for further experiments. This study was carried out at Istanbul University-Cerrahpaşa, Faculty of Engineering, Department of Chemistry. As study was conducted on cell lines, there is no requirement for ethics committee approval for this study.

In vitro HDAC inhibition activity screening

Inhibition of whole nuclear HDAC activity was measured using the Histone Deacetylase Assay Kit, Fluorometric (Sigma-Aldrich, St. Louis, USA) according to the manufacturer's instructions. Briefly, HeLa cell nuclear extracts (which contain a variety of HDAC enzymes, supplied with kit) were incubated for 30 min at 30°C in an assay buffer with HDAC fluorometric substrate and various concentrations of inhibitor samples. The reaction was stopped by addition of developer solution and incubation at room temperature for 10 min. The assay was performed in 96-well test plates. Fluorescence signal of the wells was detected using a fluorometric plate reader (BioTek FLx800) with excitation set at 350-380 nm and emission detection set at 440-480 nm. The % inhibition was calculated from the fluorescence readings of inhibited wells relative to those of control wells. The assay is automated and performed by a Gen 5 Software work station. All compounds were screened in triplicate. Trichostatin A (TSA) and valproic acid were used as standards.

$$\text{HDAC inhibition activity (\%)} = \left[\left(\frac{\text{fluorescence value as control} - \text{fluorescence value as sample}}{\text{fluorescence value as control}}\right) \times 100\right]$$

Determination of antioxidant capacity

DPPH radical scavenging activity

Radical scavenging activity of the plant extracts and some chemicals against stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) was determined by the method of Brand-Williams et al.[18]. DPPH radical reacts with a compound which can donate hydrogen (called as antioxidant) to reduce DPPH. The reaction mixture was shaken and kept in the dark for 5 and 30 minutes at room temperature, the decrease in absorbance was thereafter measured at 517 nm using a UV visible light spectrophotometer (Shimadzu UVmini-1240). The experiment was carried out three times. BHA and BHT were used as standards. The percentage of DPPH radical scavenging activity was calculated according to the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left[\frac{(\text{Absorbance as control} - \text{Absorbance as sample})}{\text{Absorbance as control}}\right] \times 100$$

ABTS·+radical scavenging activity

Radical scavenging activities against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS·+) of the plant extracts and chemicals were determined by the method of Arnao et al.[19]. The absorbance was measured at 734 nm. The experiment was carried out three times. BHA and BHT were used as standards. The percentage of ABTS·+ radical scavenging activity was calculated according to the following formula:

$$\text{ABTS}^{\cdot+}\text{ radical scavenging activity (\%)} = \left[\frac{(\text{Absorbance as control} - \text{Absorbance as sample})}{\text{Absorbance as control}}\right] \times 100$$

Ferric-reducing antioxidant power

Ferric ion reducing ability (FRAP assay) of the extracts and chemicals was performed as described by Oyaizu with some modifications[20]. The absorbance was measured at 700 nm using a UV visible light spectrophotometer (Shimadzu UVmini-1240). The experiment was carried out three times. The increased absorbance indicates stronger reducing power. BHA and BHT were used as standards. Results were expressed as the mean ± standard deviation of three replicates.

The results of HDAC inhibitory activity and antioxidant activities (for DPPH and ABTS radical scavenging activities) are given as half maximal inhibitory concentrations (IC_{50}) calculated by regression prepared from the concentrations of samples. Lower IC_{50} values indicate greater enzyme inhibition effect, or antioxidant activities of samples.

Statistical analysis

The IC_{50} was determined as the concentration of plant extracts and chemical compounds required to inhibit HDAC activity and DPPH and $ABTS^{\cdot+}$ radical scavenging activity by 50%. The percentage enzyme inhibition activities of the inhibitors were used to calculate half maximum inhibitors (IC_{50}) for individual enzymes via regression analysis data. Data are presented as the mean of three replicates ± standard deviation.

RESULTS

In vitro HDAC inhibition activity screening

The inhibitory effects of some chemical compounds and ethyl alcohol extracts prepared from various plants on HDAC enzyme were examined. The % inhibition and the half maximal inhibitory concentration (IC_{50}) values are shown in Table 1-2.

The HDAC inhibitory activities and the half maximal inhibitory concentration (IC_{50}) values of some chemical compounds and plant extracts were investigated *in vitro* in comparison with well-known HDAC inhibitors, i.e. TSA and valproic acid. Table 1-2 show that % inhibition values of plant extracts and chemical compounds on the HDAC are increased with increasing concentration. Lower (IC_{50}) values indicate greater HDAC inhibitory activity.

The results indicate that all the plant extracts exhibited HDAC inhibitory effects. The lowest IC_{50} value for HDAC inhibitor activity among the plant extracts and the chemicals was exhibited by the pomegranate extract. This means that pomegranate extract had the highest HDAC inhibitory activity. The HDAC inhibitory activities of plant extracts decreased in the order of: pomegranate> wormwood> black sesame> pineapple> mistletoe> white cabbage> flaxseed> tree of heaven> broccoli> licorice root> oleander> stinging nettle> lemon. The HDAC inhibitory activities of chemical compounds decreased in the order of: TSA> boric acid> vitamin B_6> valproic acid> vitamin U> tocopherol acetate.

The extracts showed moderate to strong inhibitions against HDAC activity, ranging from 6.39 ± 1.00 to 71.89 ± 4.38 % at a concentration of 0.1 µg/mL. The extracts of pomegranate, wormwood, black sesame, pineapple and mistletoe showed good inhibitions in comparison with the reference standards TSA (IC_{50}= 0.0695 ± 0.018 µg/mL) and valproic acid (IC_{50}= 82.27 ± 20.70 µg/mL).

DPPH radical scavenging activity

Basically, a higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. The plant extracts exhibited higher DPPH radical scavenging activity at the 30th minute than at the 5th minute as seen in Tables 3-4.

All the extracts, tocopherol acetate, vitamin B_6, vitamin U and standards (BHA and BHT) exhibited a significant, time dependent and dose-dependent radical scavenging activity. According to this study, the DPPH radical scavenging activities of plant extracts decreased in the order of: tree of heaven> oleander> wormwood> licorice root> mistletoe> black sesame> stinging nettle> broccoli> flaxseed> pomegranate> white cabbage> lemon> pineapple. The DPPH radical scavenging activities of chemicals decreased in the order of: BHA> BHT> tocopherol acetate> vitamin B_6> vitamin U. These results indicate that all the plant extracts and chemical compounds except boric acid function as DPPH radical scavengers/activities.

The tree of heaven extracts showed radical scavenging activity with IC_{50} almost similar to BHA (0.015 ± 0.000 mg/mL for minute 5 and 0.008 ± 0.001 mg/mL at minute 30) which is a well known antioxidant. The oleander extract (IC_{50}= 0.052 ± 0.0006 mg/mL) and wormwood extract (IC_{50}= 0.056 ± 0.001 mg/mL) exhibited significantly higher DPPH radical scavenging activity than BHT (IC_{50}= 0.09 ± 0.06 mg/mL) at minute 5. Tree of heaven had

the highest ability to scavenge DPPH radical, followed by oleander and wormwood among the plant extracts. Vitamin U presented the lowest DPPH radical scavenging activity.

Table 1. Inhibitory effects of various plant extracts on HDAC enzyme and IC_{50} values.

Plant Name (Botanical Name)	Part Used	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Black sesame *(Nigella sativa)*	Seed	1 0.1 0.01	80.73 ± 8.42 71.24 ± 10.71 47.01 ± 3.97	0.0105 ± 0.0007
Broccoli (*Brassica oleracea italica)*	Flower	1 0.1 0.01	61.16 ± 6.73 40.17 ± 7.68 38.56 ± 12.53	0.485 ± 0.370
Flaxseed (*Linum usitatissimum)*	Seed	0.15 0.1 0.001	57.69 ± 2.38 44.11 ± 11.94 19.69 ± 7.56	0.13 ± 0.06
Lemon (*Citrus limon)*	Fruit	20 10 0.1	15.68 ± 4.71 11.01 ± 2.85 6.39 ± 1.00	102.05 ± 42.91
Licorice root (*Glycyrrhize glabra)*	Root	1 0.5 0.1	60.11 ± 2.62 39.06 ± 0.97 35.03 ± 0.66	0.64 ± 0.07
Mistletoe *(Viscum album L.)*	Leaves	0.1 0.01 0.001	61.96 ± 14.95 42.64 ± 6.39 22.61 ± 6.85	0.065 ± 0.037
Oleander *(Nerium oleander)*	Leaves	25 10 0.1	63.65 ± 0.30 58.63 ± 4.47 45.69 ± 14.38	8.55 ± 0.65
Pineapple (*Ananas comasus)*	Fruit	0.1 0.05 0.01	54.95 ± 0.34 50.51 ± 5.83 33.98 ± 9.89	0.0497 ± 0.0057
Pomegranate (*Punica granatum)*	Fruit	0.01 0.001 0.0001	71.89 ± 4.38 68.14 ± 3.85 64.3 ± 9.09	0.000078 ± 0.000011
Stinging nettle *(Urtica dioica)*	Leaves	20 10 0.1	45.84 ± 3.87 34.56 ± 4.42 12.81 ± 3.06	21.62 ± 1.71
Tree of heaven *(Ailanthus altissima)*	Leaves	0.2 0.1 0.01	66.67 ± 2.65 40.25 ± 8.94 24.55 ± 18.16	0.15 ± 0.03
White cabbage (*Brassica oleracea* var. *Capitata)*	Leaves	1 0.1 0.05	78.15 ± 9.34 68.65 ± 4.67 34.48 ± 2.67	0.072 ± 0.005
Wormwood *(Artemisia Absinthium)*	Leaves	1 0.1 0.001	60.94 ± 10.86 58.19 ± 7.78 56.75 ± 8.20	0.00088 ± 0.00013

*Data are presented as the mean of three replicates ± standard deviation.

Table 2. Inhibitory effects of some chemical compounds on HDAC enzyme and IC_{50} values.

Chemical Name	Concentration (µg/mL)	Inhibition (%)*	IC_{50} (µg/mL)*
Boric acid	1 0.75 0.005	66.71 ± 8.80 49.34 ± 0.20 31.42 ± 15.59	0.76 ± 0.099
Tocopherol acetate	100 10 1	22.23 ± 8.36 15.66 ± 0.12 12.16 ± 4.92	319.29 ± 2.59
Trichostatin A (TSA)**	7.5 0.75 0.075	93.54 ± 2.14 81.42 ± 0.47 55.47 ± 13.84	0.0695 ±0.018
Valproic acid**	50 20 5	34.74 ± 6.04 13.82 ± 1.40 10.32 ± 2.02	82.27 ± 20.70
Vitamin B_6	20 10 1	36.32 ± 0.47 26.21 ± 1.38 13.78 ± 0.58	27.53 ± 3.76
Vitamin U	100 10 1	30.83 ± 13.08 23.59 ± 13.08 21.31 ± 1.43	178.22 ± 75.61

*Data are presented as the mean of three replicates ± standard deviation.
** It means standard.

Table 3. DPPH radical scavenging activity of plant extracts.

Plant Name (Botanical Name)	Part Used	Concentration (mg/mL)	DPPH radical scavenging activity (%)* (min. 5)	IC_{50} (mg/mL)* (min.5)	DPPH radical scavenging activity (%)* (min. 30)	IC_{50} (mg/mL)* (min.30)
Black sesame *(Nigella sativa)*	Seed	0.5 0.25 0.1 0.05	68.11 ± 1.89 40.80 ± 0.45 20.77 ± 0.67 12.41 ± 0.27	0.35 ± 0.004	84.38 ± 0.12 54.35 ± 3.45 23.76 ± 0.71 14.40 ± 0.37	0.230 ± 0.015
Broccoli (*Brassica oleracea italica)*	Flower	1 0.75 0.5 0.25	78.10 ± 0.91 65.79 ± 0.20 48.23 ± 0.93 30.57 ± 0.22	0.55 ± 0.006	90.30 ± 0.18 83.18 ± 1.97 61.61 ± 0.54 36.12 ± 0.10	0.43 ± 0.006
Flaxseed (*Linum usitatissimum)*	Seed	1 0.5 0.1 0.01	65.17 ± 0.57 47.55 ± 0.18 17.56 ± 1.08 5.63 ± 0.29	0.675 ± 0.006	79.27 ± 0.14 61.01 ± 0.78 22.56 ± 0.29 3.87 ± 0.006	0.410 ± 0.005
Lemon (*Citrus limon)*	Fruit	3 2 1 0.1	62.19 ± 0.75 47.54 ± 0.38 30.58 ± 1.71 5.42 ± 2.23	2.24 ± 0.02	78.39 ± 0.16 63.25 ± 0.26 39.72 ± 0.61 7.44 ± 0.30	1.64 ± 0.01
Licorice root (*Glycyrrhize glabra)*	Root	0.5 0.25 0.1 0.01	87.10 ± 0.63 61.13 ± 2.56 26.85 ± 0.22 2.65 ± 0.45	0.24 ± 0.01	91.75 ± 0.26 59.84 ± 0.00 29.52 ± 0.00 2.66 ± 0.26	0.24 ± 0.01
Mistletoe *(Viscum album L.)*	Leaves	0.5 0.25 0.1 0.05	75.63 ± 1.75 42.98 ± 0.99 24.83 ± 1.05 13.95 ± 0.65	0.305 ± 0.0005	91.60 ± 0.16 71.42 ± 4.37 34.61 ± 0.57 22.00 ± 1.03	0.19 ± 0.0069
Oleander *(Nerium oleander)*	Leaves	0.1 0.05 0.025	90.32 ± 0.42 49.04 ± 0.82 32.60 ± 0.28	0.052 ± 0.0006	92.74 ±0.00 65.86 ± 0.84 42.42 ± 1.50	0.042 ± 0.001

		0.01	9.23 ± 0.42		12.56 ± 0.02	
Pineapple (*Ananas comasus)*	Fruit	10 5 2.5 1	90.03 ± 0.12 69.78 ± 0.46 41.35 ± 0.85 18.07 ± 0.76	3.99 ± 0.07	94.09 ± 0.02 86.78 ± 0.04 55.31 ± 0.52 25.97 ± 2.54	2.40 ± 0.01
Pomegranate (*Punica granatum)*	Fruit	2.5 1 0.5 0.1	75.94 ± 1.12 44.48 ± 0.18 25.21 ± 0.36 7.70 ± 0.35	1.45 ± 0.017	88.78 ± 0.55 50.75 ± 0.49 28.42 ± 0.49 7.58 ± 0.37	0.98 ± 0.0096
Stinging nettle *(Urtica dioica)*	Leaves	1 0.5 0.1 0.05	86.00 ± 0.36 49.08 ± 1.23 13.29 ± 0.16 8.86 ± 0.69	0.54 ± 0.005	89.93 ± 0.44 61.62 ± 0.56 15.99 ± 0.00 10.30 ± 0.32	0.47 ± 0.005
Tree of heaven *(Ailanthus altissima)*	Leaves	0.04 0.02 0.01 0.005	94.19 ± 0.001 68.46 ± 0.46 38.01 ± 0.99 20.60 ± 0.26	0.017 ± 0.001	94.34 ± 0.16 73.05 ± 0.57 41.81 ± 0.91 21.87 ± 0.44	0.016±0.0005
White cabbage (*Brassica oleracea* var. *Capitata)*	Leaves	2.5 1 0.5 0.1	75.31 ± 0.33 34.84 ± 0.68 18.63 ± 0.36 4.19 ± 0.19	1.60 ± 0.006	88.32 ± 0.31 45.94 ± 1.33 25.58 ± 0.52 5.67 ± 0.32	1.28 ± 0.01
Wormwood *(Artemisia Absinthium)*	Leaves	0.1 0.05 0.025 0.01	82.78 ± 0.20 49.72 ± 0.50 25.05 ± 0.11 12.43 ± 0.96	0.056 ± 0.001	92.72 ± 0.32 56.69 ± 0.48 34.58 ± 1.29 15.38 ± 0.59	0.047±0.0006

*Data are presented as the mean of three replicates ± standard deviation.

Table 4. DPPH radical scavenging activity of some chemical compounds and standard antioxidants.

Chemical Name	**Concentration (mg/mL)**	**DPPH radical scavenging activity (%)* (min. 5)**	**IC_{50} (mg/mL)* (min.5)**	**DPPH radical scavenging activity (%)* (min. 30)**	**IC_{50} (mg/mL)* (min.30)**
BHA**	0.025 0.01 0.005 0.001	77.58 ± 0.93 39.42 ± 1.33 26.53 ± 0.90 5.99 ± 0.33	0.015 ± 0.000	91.70 ± 0.02 70.32 ± 0.23 49.71 ± 0.57 14.69 ± 0.68	0.008 ± 0.001
BHT**	0.1 0.05 0.025 0.01	54.18 ± 0.73 35.30 ± 1.53 25.05 ± 1.83 11.86 ± 1.05	0.09 ± 0.06	93.44 ± 0.75 83.36 ± 0.34 63.11 ± 0.53 33.75 ± 0.77	0.015 ± 0.001
Boric acid	200 10 1 0.005	N.D.	N.D.	N.D.	N.D.
Tocopherol acetate	10 7.5 2 1	72.31 ± 0.08 57.91 ± 0.27 16.25 ± 0.36 9.31 ± 0.52	6.67 ± 0.02	75.42 ± 0.48 58.77 ± 0.19 17.36 ± 0.24 9.71 ± 0.39	6.44 ± 0.02
Vitamin B_6	10 5 1 0.1	12.78 ± 0.144 5.58 ± 0.23 3.03 ± 0.16 2.06 ± 0.68	44.76 ± 1.05	29.66 ± 0.21 14.33 ± 0.19 6.23 ± 0.90 1.91 ± 0.17	17.44 ± 0.05
Vitamin U	200 100 50 10	32.68 ± 1.11 22.75 ± 0.65 8.25 ± 0.33 3.86 ± 1.02	302.91 ± 5.63	56.41 ± 0.56 40.66 ± 1.01 15.37 ± 0.39 10.69 ± 1.15	162.58 ± 0.26

*Data are presented as the mean of three replicates ± standard deviation. ; ** It means standard. N.D. means not detected.

Table 5. ABTS$^{\cdot+}$ radical scavenging activity of plant extracts.

Plant Name (Botanical Name)	Part Used	Concentration (mg/mL)	ABTS radical scavenging activity (%)*	IC_{50} (mg/mL)*
Black sesame *(Nigella sativa)*	Seed	2 1 0.5 0.25	72.09 ± 1.34 49.62 ± 1.50 29.76 ± 1.31 17.70 ± 0.68	1.21 ± 0.01
Broccoli (*Brassica oleracea italica)*	Flower	10 2.5 1 0.75	99.45 ± 0.07 76.43 ± 0.62 36.74 ± 1.11 29.42 ± 1.02	1.63 ± 0.015
Flaxseed (*Linum usitatissimum)*	Seed	5 2.5 1 0.5	98.76 ± 0.18 75.36 ± 1.39 41.89 ± 0.39 23.43 ± 0.31	1.89 ± 0.01
Lemon (*Citrus limon)*	Fruit	10 7.5 5 3	86.60 ± 1.02 74.34 ± 0.08 49.57 ± 3.12 35.38 ± 0.17	5.20 ± 0.09
Licorice root (*Glycyrrhize glabra)*	Root	2 1 0.5 0.1	96.67 ± 0.31 81.76 ± 0.39 58.25 ± 0.33 13.89 ± 0.83	0.423 ± 0.005
Mistletoe *(Viscum album L.)*	Leaves	5 1 0.5 0.25	100.00 ± 0.00 60.18 ± 1.33 35.20 ± 0.48 18.68 ± 0.46	0.83 ± 0.017
Oleander *(Nerium oleander)*	Leaves	1 0.5 0.1 0.05	63.79 ± 0.48 39.57 ± 0.27 10.97 ± 0.43 5.39 ± 0.59	0.74 ± 0.006
Pineapple (*Ananas comasus)*	Fruit	15 10 5 2.5	79.03 ± 1.12 63.35 ± 0.80 36.35 ± 0.16 20.40 ± 0.38	8.41 ± 0.03
Pomegranate (*Punica granatum)*	Fruit	20 10 5 2.5	95.93 ± 1.49 53.13 ± 0.45 31.21 ± 1.39 11.78 ± 1.29	9.80 ± 0.09
Stinging nettle *(Urtica dioica)*	Leaves	4 2 1 0.5	96.63 ± 0.42 63.09 ± 0.78 37.42 ± 0.18 18.21 ± 0.69	1.77 ± 0.01
Tree of heaven *(Ailanthus altissima)*	Leaves	0.5 0.25 0.1 0.02	99.89 ± 0.00 78.10 ± 0.54 40.77 ± 0.75 7.64 ± 0.59	0.19 ± 0.005
White cabbage (*Brassica oleracea* var. *Capitata)*	Leaves	10 5 2.5 1	97.80 ± 0.52 66.57 ± 2.07 29.62 ± 0.66 14.27 ± 1.37	4.49 ± 0.04
Wormwood *(Artemisia Absinthium)*	Leaves	2.5 0.5 0.1 0.05	100.00 ± 0.00 58.02 ± 0.73 13.13 ± 0.99 7.98 ± 0.56	0.45 ± 0.026

*Data are presented as the mean of three replicates ± standard deviation.

Table 6. ABTS·+ radical scavenging activity of some chemicals and standard antioxidants.

Chemical Name	Concentration (mg/mL)	ABTS radical scavenging activity (%)*	IC_{50} (mg/mL)*
BHA**	0.1 0.05 0.025 0.01	89.33 ± 0.77 57.66 ± 1.04 26.53 ± 0.28 11.41 ± 1.89	0.043 ± 0.001
BHT**	1 0.5 0.1 0.05	99.92 ± 0.07 89.93 ± 0.13 81.26 ± 0.86 51.43 ± 0.03	0.04856 ± 0.00006
Boric acid	250 40 1 0.01	17.30 ± 0.33 7.67 ± 0.42 5.46 ± 0.91 4.96 ± 0.36	722.71 ± 60.95
Vitamin B_6	0.1 0.05 0.01 0.001	74.06 ± 0.70 41.67 ± 0.35 29.25 ± 4.09 3.21 ± 0.93	0.060 ± 0.002
Vitamin U	600 400 200 100	16.46 ± 0.28 10.77 ± 0.08 5.60 ± 0.94 0.75 ± 0.42	1822.21 ± 317.09
Tocopherol acetate	20 10 7.5 2	20.53 ± 1.49 10.02 ± 0.38 7.49 ± 0.94 2.77 ± 0.81	50.87 ± 3.60

*Data are presented as the mean of three replicates ± standard deviation. ** It means standard.

ABTS·+ radical scavenging activity

The ability of the plant extracts, chemicals and standards to scavenge ABTS cation is expressed in Tables 5-6. All the plant extracts, vitamin B_6 and tocopherol acetate exhibited dose-dependent $ABTS^+$ radical scavenging activities. The best ABTS·+ radical scavenging activities were exhibited by ethanolic extracts obtained from the tree of heaven and licorice root.The result showed that all plant extracts are likely to have excellent ABTS free radicals scavenging effects.

All tested plant extracts had a strong capacity to scavenge ABTS·+ The extract tree of heaven had the highest activity (IC_{50}= 0.19 ± 0.005 mg/mL) among the plant extracts. The antioxidant capacities are in the following manner; tree of heaven> licorice root> wormwood> oleander> mistletoe> black sesame> broccoli> stinging nettle> flaxseed> white cabbage> lemon> pineapple> pomegranate. The ABTS radical scavenging activities of chemicals decreased in the order of: BHA> BHT> vitamin B_6> tocopherol acetate> boric acid> vitamin U.

Ferric-reducing antioxidant power

The reducing power of plant extracts and some chemicals are represented in Tables 7-8. The reducing power of the plant extracts was arranged in the following manner; tree of heaven> oleander > wormwood > mistletoe > licorice root > black seed> stinging nettle > flaxseed > broccoli > lemon > white cabbage> pineapple > pomegranate. The reducing power of the chemicals decreased in the order of: BHA> BHT> tocopherol acetate> vitamin B_6> boric acid> vitamin U.

The ethanol extracts of the tree of heaven, oleander, wormwood and mistletoe showed a marked ferric-reducing power. The extracts of licorice root, black seed, stinging nettle, flaxseed, broccoli, lemon, white cabbage, pineapple and pomegranate were also found to exhibit a more effective ferric-reducing power in comparison to the reference standards (BHA and BHT). In this assay, tree of heaven extract (1.407 ± 0.028) had the highest reducing activity among plant extracts followed by oleander extract (0.466 ± 0.012), wormwood extract (0.229 ± 0.020), and mistletoe (0.192 ± 0.008) for 0.5 mg/mL concentration, respectively.

Table 7. Ferric-reducing power of plant extracts.

Plant Name (Botanical Name)	Part Used	Concentration (mg/mL)	Reducing Power (Absorbance, 700 nm)*
Black sesame *(Nigella sativa)*	Seed	5	1.432 ± 0.016
		2.5	0.774 ± 0.017
		1	0.358 ± 0.015
		0.5	0.186 ± 0.005
Broccoli (*Brassica oleracea italica)*	Flower	5	1.213 ± 0.083
		2.5	0.539 ± 0.003
		1	0.267 ± 0.008
		0.5	0.143 ± 0.005
Flaxseed (*Linum usitatissimum)*	Seed	10	1.602 ± 0.046
		7.5	1.384 ± 0.073
		5	1.107 ± 0.026
		2.5	0.620 ± 0.007
Lemon (*Citrus limon)*	Fruit	10	1.513 ± 0.077
		7.5	1.247 ± 0.036
		5	0.905 ± 0.030
		2.5	0.492 ± 0.017
Licorice root (*Glycyrrhize glabra)*	Root	5	1.412 ± 0.020
		2.5	0.784 ± 0.050
		1	0.344 ± 0.010
		0.5	0.189 ± 0.004
Mistletoe *(Viscum album L.)*	Leaves	5	1.760 ± 0.020
		2.5	0.929 ± 0.020
		1	0.358 ± 0.001
		0.5	0.192 ± 0.008
Oleander *(Nerium oleander)*	Leaves	2.5	1.662 ± 0.025
		1	0.782 ± 0.023
		0.5	0.466 ± 0.012
		0.25	0.205 ± 0.003
Pineapple (*Ananas comasus)*	Fruit	10	0.875 ± 0.020
		7.5	0.652 ± 0.030
		5	0.441 ± 0.002
		2.5	0.251 ± 0.006
Pomegranate (*Punica granatum)*	Fruit	10	0.866 ± 0.018
		7.5	0.641 ± 0.004
		5	0.445 ± 0.006
		2.5	0.234 ± 0.001
Stinging nettle *(Urtica dioica)*	Leaves	2.5	0.650 ± 0.018
		1	0.360 ± 0.006
		0.5	0.183 ± 0.008
		0.1	0.049 ± 0.004
Tree of heaven *(Ailanthus altissima)*	Leaves	0.5	1.407 ± 0.028
		0.25	0.810 ± 0.042
		0.1	0.301 ± 0.024
		0.05	0.172 ± 0.021
White cabbage (*Brassica oleracea* var. *Capitata)*	Leaves	10	1.233 ± 0.042
		7.5	1.017 ± 0.036
		5	0.674 ± 0.014
		2.5	0.356 ± 0.004
Wormwood *(Artemisia Absinthium)*	Leaves	1	0.359 ±0.020
		0.5	0.229 ± 0.020
		0.1	0.054 ± 0.001
		0.05	0.039 ± 0.002

*Data are presented as the mean of three replicates ± standard deviation.

Table 8: Ferric-reducing power of some chemical compounds.

Chemical Name	Concentration (mg/mL)	Reducing Power (Absorbance, 700 nm)*
BHA**	0.1	0.814 ± 0.017
	0.05	0.455 ± 0.007
	0.01	0.101 ± 0.001
	0.005	0.040 ± 0.002
BHT**	0.25	1.249 ± 0.006
	0.1	0.703 ± 0.009
	0.05	0.407 ± 0.001
	0.01	0.186 ± 0.004
Boric acid	100	0.035 ± 0.008
	40	0.025 ± 0.002
	10	0.020 ± 0.002
	5	0.010 ± 0.002
Tocopherol acetate	10	0.119 ± 0.060
	7.5	0.066 ± 0.002
	5	0.052 ± 0.002
	2.5	0.031 ± 0.010
Vitamin B_6	10	0.056 ± 0.002
	7.5	0.048 ± 0.001
	5	0.041 ± 0.001
	2.5	0.038 ± 0.002
Vitamin U	400	0.097 ± 0.007
	200	0.018 ± 0.001
	100	0.016 ± 0.006
	10	0.013 ± 0.001

*Data are presented as the mean of three replicates ± standard deviation.
** It means standard.

The significant ferric-reducing power of these extracts can be attributed to their antioxidant potential as reducing agents. Vitamin U and boric acid showed a weak ferric-reducing power. Tocopherol acetate exhibited a ferric-reducing power of 0.052 ± 0.002 at 5 mg/mL, while vitamin B_6, pineapple extract, pomegranate extract, white cabbage extract, lemon extract, flaxseed extract, broccoli extract, licorice root extract, black sesame extract and mistletoe extract showed 0.041 ± 0.001, 0.441 ± 0.002, 0.445 ± 0.006, 0.674 ± 0.014, 0.905 ± 0.030, 1.107 ± 0.026, 1.213 ± 0.083, 1.412 ± 0.020, 1.432 ± 0.016 and 1.760 ± 0.020, respectively, at the same concentration.

DISCUSSION

Growing evidence suggests that dietary phytochemicals possessing HDAC inhibitory activity can be used to repair mechanisms in cancer cells[21]. An imbalance between acetylation and deacetylation changes the structure and the activity of histone proteins, influencing tumor cell functions such as gene expression, cell growth and/or survival, and cell death pathways[22].

Prostate cancer cells can interact with HDAC inhibitors. This interaction varies between cell lines and is inhibitor specific. There are a number of reports indicating the use of various HDAC inhibitors for the treatment of prostate cancer since HDAC1 levels are up-regulated in prostate cancer. HDAC plays a role in the inactivation of various critical genes by DNA-methylation-induced chromatin-remodeling[23]. Pomegranate has been proven to possess *in vitro* and *in vivo* beneficial effects for the prevention and/or treatment of prostate and cervical cancer[24,25]. It was reported that fermented pomegranate juice polyphenols and pericarp polyphenols induce cell death in prostate cancer cell lines by inhibiting proliferation, and inducing apoptosis in prostate cancer cell lines[26]. In a study by Malik et al., the antiproliferative and pro-apoptotic properties of pomegranate extract on human prostate cancer cells were demonstrated. Pomegranate extract treatment of aggressive human prostate cancer cells resulted in a dose-dependent inhibition of cell growth

or cell viability, and induction of apoptosis[27]. In the present study, pomegranate had the highest HDAC inhibitory among the tested extracts.

In a study by Wei et al., wormwood ethanol extract and its subfractions including petroleum ether and ethyl acetate extracts were investigated for antitumor effects on hepatocellular carcinoma both *in vitro* and *in vivo*. They inhibited the growth of hepatoma cells both *in vitro* and *in vivo* without any cytotoxic effects. These extracts could arrest the cell cycle at the G2/M phase and induce apoptosis through endoplasmic reticulum (ER) stress and the mitochondrial-dependent pathway in human hepatoma BEL-7404 cells and mouse hepatoma H22 cells[28]. Similarly, wormwood methanol extract induced the apoptosis of human breast cancer cells through the modulation of BCL-2 family proteins[29]. Therefore, the wormwood ethanol extract may be used as a safe and effective agent for the regulation of the acetylation and deacetylation of histones by HATs and HDACs as observed in the previous and present studies. Moreover, it presented high antioxidant effects against the DPPH and ABTS radicals and a strong ferric reducing power compared to the synthetic antioxidants analyzed in the present study.

Natural products are important sources for the isolation of HDAC inhibitors since they are a rich source of novel chemotypes and pharmacophores. Animals, plants, and marine organisms are sources of effective inhibitors of HDAC. TSA is a powerful HDAC inhibitor that inhibits cell invasion and metastasis, while also decreasing tumor angiogenesis *in vitro* and *in vivo*. TSA on the other hand, has been found to be toxic and induce some biological defects. This has limited its clinical use and development[30]. Therefore, discovering novel HDAC inhibitors is an important issue.

In the present study, the extracts of pomegranate, wormwood, black sesame, pineapple, mistletoe and white cabbage presented high HDAC inhibitory activities compared to synthetic inhibitors like TSA and valproic acid. Thus, suggesting that these extracts may have therapeutic potential against diseases associated with cancer.

Because synthetic antioxidants have some toxic effects, researchers have focused their studies on plant-derived natural antioxidants[31]. It has been reported that wormwood contains flavonoids[32-34], thymol, carvacrol and other phenolic compounds[35]. These pharmacophores have rich antioxidant and free-radical scavenging activity[36]. To investigate the antioxidant activity of chemicals and plant extracts, the ability to scavenge stable free radical DPPH and the cation ABTS, as well as ferric reducing antioxidant power of the samples were investigated.

DPPH radical scavenging activity is one of the most commonly used methods to test the ability of compounds to act as free radical scavengers, hydrogen donors and/or antioxidants[37]. The extracts of tree of heaven, oleander, wormwood, licorice root and mistletoe exhibited high antioxidant effects against the DPPH and ABTS radicals, and a strong ferric reducing power compared to the synthetic antioxidants analyzed. These outcomes suggest that the extracts may have therapeutic potential against diseases associated with oxidative stress. The limitation of the study is that the inhibition and antioxidant effects of the ethanolic plant extract on the HDAC enzyme are considered *in vitro*, and cancer disease models are required for the *in vivo* effect.

In conclusion, the present results showed that the tested plant extracts possessed potent antioxidant activities. Likewise, the extracts inhibited the HDAC enzyme. Thus, indicating that they may be effective therapeutic agents for controlling cancer and bringing about some preliminary proof for their anticarcinogenic effects. The extracts in general exhibited significant *in vitro* HDAC inhibitory activities as well as antioxidant activities. In the future, we think that these plant extracts may be an alternative for developing different therapeutic approaches and new therapeutic targets against cancer.

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