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NITRITE SCAVENGING ACTIVITIES AND REDUCING POWER CAPACITIES OF ETHANOLIC EXTRACTS OF SOME FRUITS AND VEGETABLES

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Abstract

The use of fruits and vegetables for therapeutic purposes has been continuing for centuries. Now-a-days, fruits and vegetables are increasingly used as alternative medicines. The antioxidant activities of some fruits and vegetables used in the present study were determined by nitrite scavenging and reduction power experiments. Antioxidant capacity increased with increasing concentration. The highest nitrite scavenging activity and inhibitory power were observed in garlic extract. The least nitrite scavenging activity and reducing power were found in white grape and kiwi extracts. It was suggested that garlic extract which had the highest nitrite scavenging capacity and reducing power may be an appropriate additive in drugs and other pharmaceuticals intended for medical purposes.

Introduction

Inflammation is a biological process that occurs in response to inflammation, infection, injury, or irritation (Wang *et al*. 2013). The three main goals of inflammation in the organism are to eliminate the cause of disease, to separate/isolate it from the body, and to remove damaged tissues if they cannot be repaired (Kar 2018). Several diseases such as cancer, arthritis, allergies and atherosclerosis appear to be associated with chronic inflammation (Devi *et al.* 2015). The sequential release of pro-inflammatory cytokines is a complex immune response associated with inflammatory process. Inhibiting the overproduction of pro-inflammatory cytokines can prevent or suppress various inflammatory diseases (Lin and Tang 2008). The main symptoms of inflammation include temperature rise, redness, swelling, pain and loss of organ function (Calhelha *et al*. 2023), with nitric oxide (NO) implicated to be among the major inflammatory mediators.

Since ancient times, mankind has been treated for inflammation-related conditions using plants or herbal formulations. Many antioxidants rich natural products show protective effects against inflammation. Mueller *et al*. (2010) reported that some plant and isolated compounds exhibited anti-inflammatory activities. The ability of these compounds to suppress some cancers *in vitro* and *in vivo* is naturally attributed to the anti-inflammatory properties of phytochemicals found in the structure of the plants (Kang *et al.* 2005). In the present study, the antioxidant activity/effects of some plants extracts was evaluated through nitrite scavenging activity and reducing power analysis.

Materials and Method

Plant materials used in the experiments were obtained from local market in Istanbul, Turkey. The supplied plants were dried in the shade after passing through distilled water for decontamination. The edible parts of plant materials were collected for use in extract preparation using 80% ethyl alcohol solution as solvent.

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The plant part (5 g) was weighed into a 250 ml flask, followed by addition of 50 mL of 80% ethyl alcohol solution. The flask is sealed and closed, allowed to stay in the dark for 7 days at room temperature. The resulting mixture was then filtered. The filtrate was placed in the previously tared balloon flask and placed in a rotary evaporator to remove ethyl alcohol under low pressure. The amount of extract obtained was weighed and recorded. It was stored in the Eppendorf tube at -20 °C.

Nitrite scavenging activity assay is based on the nitric acid diazotization with a primary aromatic amine (sulfanilamide) in an acidic environment and forming a purple azo product with N-(1-naphthyl)ethylenediamine hydrochloride. The nitrite scavenging activity experiment was performed according to the method of Kim *et al.* (2014). Griess reagent was prepared according to the method of Choi *et al*. (2008).

Determination of reducing power can give information about antioxidant capacity depending on concentration. The reducing power is determined by reduction of $Fe[(CN)_6]^{+3}$ to F $[(CN)6]^{+2}$. With the addition of Fe^{+3} to the reduced product, $Fe_4[Fe(CN)_6]$ complex is formed, which is read at 700 nm. The reduction in power was determined according to the method of Oyaizu (1986).

Results and Discussion

Results showed the nitrite scavenging activities of the extracts increased with increasing concentration (Table 1). Garlic with the lowest IC_{50} value was found to show the highest nitrite scavenging activity (2.04 ± 0.09 mg/ml). After garlic, black radish (6.13 ± 0.55 mg/ml), quince (9.89 ± 0.63 mg/ml), lemon (10.28 ± 0.45 mg/ml) and onion (12.22 ± 0.30 mg/ml) showed the highest nitrite scavenging effect. Anti-nitrite activity in plant extracts decreases as follows: garlic > black radish > quince > lemon > onion > grapefruit > green pepper > cabbage > pomegranate > pear > green apple > black grape > kiwi > carrot > pineapple extract (Table 1).

Table 1. Nitrite scavenging inhibitory activity of some selected ethanol plant extracts.

Plant extract/standard	IC_{50} (mg/ml)*
Black grape	32.14 ± 3.76
Black radish	6.13 ± 0.55
Cabbage	15.48 ± 0.37
Carrot	35.67 ± 3.60
Garlic	2.04 ± 0.09
Grape furit	13.43 ± 0.16
Green apple	27.55 ± 0.66
Green paper	14.15 ± 0.51
Kiwi	33.84 ± 3.14
Lemon	10.28 ± 0.45
Onion	12.22 ± 0.30
Pear	26.54 ± 1.10
Pinapple	45.02 ± 5.53
Pomegranate	22.28 ± 1.01
Red apple	29.75 ± 1.40
Red radish	14.37 ± 0.60
Rutin (Standard)	1.37 ± 0.18
Quince	9.89 ± 0.63
White grape	45.61 ± 6.04

*Mean ± SD.

The nitrite scavenging activity of the extract solutions increased with increasing concentration (Table 2). Epicatechin had the lowest IC_{50} value, which corresponds to the highest nitrite scavenging activity (0.082 ± 0.004 mg/ml). Epigallocatechin (0.087 ± 0.003 mg/ml), catechin (0.093 ± 0.003 mg/ml), gallic acid (0.111 ± 0.002 mg/ml) and quercetin (0.195 ± 0.003 mg/ml) showed the highest nitrite scavenging activity after epicatechin (Table 2).

Table 2. Nitrite scavenging inhibitory activity of some chemical compounds.

Chemical compounds/standard	IC_{50} (mg/ml)*
Ascorbic acid	0.550 ± 0.030
Catechin	0.093 ± 0.003
Chlorogenic acid	0.280 ± 0.010
Epicatechin	0.082 ± 0.004
Epigallocatechin	0.087 ± 0.003
Gallic acid	0.111 ± 0.002
Kojic acid	0.240 ± 0.010
Rutin (Standard)	1.370 ± 0.180
Quercetin	0.195 ± 0.003

*Mean ± SD

The nitrite scavenging activity of the chemicals is in the order epicatechin >epigallocatechin > catechin > gallic acid > quercetin > kojic acid > chlorogenic acid > ascorbic acid > rutin (Table 2).

The reducing power values of the prepared plant extracts are presented in Table 3. The absorbance value increases with the increase in concentration. With higher absorbance signifying higher reducing power and antioxidant action. Ascorbic acid, which was used as a standard at a concentration of 1 mg/ml, showed the highest reducing power. Among the extracts, garlic extract had the highest reducing power (0.697 ± 0.001 mg/ml) at similar concentration. This is closely followed by cabbage (0.684 ± 0.047 mg/ml) and green pepper extracts (0.576 ± 0.049 mg/ml). The order of the reducing power capacities of the plant extracts at a concentration of 1 mg/ml are as follows: Ascorbic acid > garlic > cabbage > green pepper > red radish > pomegranate > onion > grapefruit > lemon > black grape > pineapple > green apple > quince > white grape > pear > red apple > kiwi.

The World Health Organization (WHO) stated that a significant part of the world population depend on plants under the name “traditional medicine” for treatment and protection against diseases. WHO also estimates that about 70,000 and 21,000 are used for medicinal and pharmaceutical purposes, respectively (Atalay and Erge 2018). Today, extracts from parts of medicinal plants such as roots, leaves, flowers and fruits are the main ingredient of many medicinal drugs (Kurt and Karaoğul 2018).

Reactive oxygen species (ROS) and free radicals occur within the scope of metabolic events that develop throughout life. Oxidative stress disrupts the balance between ROS production and antioxidant defence, thereby causing oxidative damage. This may arise due to enzymatic (catalase, superoxide dismutase, glutathione peroxidase, etc.) or nonenzymatic (A, C and E vitamin, glutathione, ubiquinone, flavonoid, etc.) imbalance, as well as a compromised antioxidant defence mechanism, or increase in ROS and excessive activation. Reactive oxygen species have been shown to play significant role in the onset of many diseases including cardiological and neurological diseases, diabetes, asthma and rheumatological diseases (Altan *et al.* 2006).

Table 3. Reducing power of some selected ethanol plant extracts.

Plant extract/ standard	Concentration (mg/ml)	Reducing power (absorbance)*	Plant sxtract/ standard	Concentration (mg/ml)	Reducing power (absorbance)*
Ascorbic acid	0.01	0.391 ± 0.018	Onion	0.1	0.325 ± 0.036
(Standard)	0.1	1.132 ± 0.001		0.5	0.387 ± 0.009
	0.5	1.395 ± 0.032		1	0.522 ± 0.026
	1	1.475 ± 0.021		10	1.372 ± 0.006
Black grape	0.1	0.295 ± 0.009	Pear	0.1	0.225 ± 0.009
	0.5	0.333 ± 0.002		0.5	0.262 ± 0.001
	1	0.391 ± 0.002		1	0.301 ± 0.032
	10	1.083 ± 0.001		10	0.856 ± 0.063
Cabbage	0.1	0.283 ± 0.006	Pinapple	0.1	0.266 ± 0.016
	0.5	0.546 ± 0.069		0.5	0.289 ± 0.004
	1	0.684 ± 0.047		1	0.338 ± 0.026
	10	1.392 ± 0.012		10	0.954 ± 0.005
Garlic	0.1	0.277 ± 0.021	Pomegranate	0.1	0.225 ± 0.008
	0.5	0.459 ± 0.012		0.5	0.367 ± 0.021
	1	0.697 ± 0.001		1	0.537 ± 0.051
	10	1.381 ± 0.023		10	1.203 ± 0.021
Grapefurit	0.1	0.326 ± 0.019	Red apple	0.1	0.228 ± 0.005
	0.5	0.347 ± 0.034		0.5	0.262 ± 0.005
	1	0.430 ± 0.035		1	0.300 ± 0.006
	10	1.377 ± 0.016		10	0.849 ± 0.013
Green apple	0.1	0.275 ± 0.007	Red radish	0.1	0.260 ± 0.004
	0.5	0.297 ± 0.007		0.5	0.395 ± 0.021
	1	0.328 ± 0.016		1	0.560 ± 0.013
	10	0.954 ± 0.028		10	1.261 ± 0.007
Green paper	0.1	0.333 ± 0.053	Quince	0.1	0.251 ± 0.009
	0.5	0.476 ± 0.083		0.5	0.279 ± 0.012
	1	0.576 ± 0.049		1	0.325 ± 0.033
	10	1.335 ± 0.004		10	0.941 ± 0.004
Kiwi	0.1	0.223 ± 0.004	White grape	0.1	0.220 ± 0.001
	0.5	0.252 ± 0.011		0.5	0.243 ± 0.008
	1	0.283 ± 0.018		1	0.305 ± 0.059
	10	0.794 ± 0.011		10	0.786 ± 0.011
Lemon	0.1	0.233 ± 0.009			
	0.5	0.333 ± 0.013			
	1	0.428 ± 0.004			
	10	1.330 ± 0.021			

*Mean ± SD

The substance that prevents or delays oxidation is referred to as an antioxidant (Santos-Sánchez *et al.* 2019). In addition to the biological antioxidant system, ROS are rendered harmless by phenolic compounds, carotenoids and vitamins present found in fruits and vegetables. Several studies suggest that these compounds found in fruits and vegetables reduce the risk and even prevent the onset/occurrence of certain types of cancer, cardiovascular and cerebrovascular diseases, stroke, cataracts, Alzheimer's and Parkinson's disease (Santos-Sánchez *et al.* 2019).

Inflammation is a complex, protective response of the body to harmful substances such as microorganisms, allergens or damaged cells. They are targeted by the biological system to eliminate harmful stimuli to the body and promote healing. Inflammatory diseases can be classified into acute and chronic. Acute inflammation condition is characterized by established cell activation, mainly resulting in neutrophils reaching damage site through immune response. This condition acts to support the major symptoms of inflammation, such as pain, oedema and temperature rise/heat. Chronic inflammation is a long-term condition, characterized by a gradual change in the type of cell in the inflamed area. It results to both permanent damage and tissue healing over time. Chronic inflammation is common cause of many clinical abnormalities such as arthritis, atherosclerosis, allergies and even cancer (Devi *et al.* 2015). In general, individuals with inflammatory diseases use glucocorticoids or non-steroidal anti-inflammatory drugs (NSAIDs). However, these treatments regimes are often abandoned by patients due to their critical side effects, such as gastrointestinal ulcers and bleeding. In this context, an attempt to find new bioactive molecules constitutes an area if scientific interest for many researchers (Barboza *et al.* 2018).

Recent data implicate oxidative stress and inflammation to play a role in onset of diseases of the nervous systems. In a cellular system in which excessive ROS and nitrogen species (RNS) production exceeds the capacity of the antioxidant defence system, oxidative stress sets in. These reactive species, also called free radicals, and are capable of damaging macro structures (such as lipid, cell membrane, protein, and DNA/RNA) in the biological system if not controlled (Ji *et al*. 2020).

It is reported that a diet with a high fruit content reduces/attenuates oxidative DNA damage. Thus it has an important role in preventing cancer (Kumar and Pandey 2013). Some researchers have demonstrated that the strong antioxidant potentials of flavonoids are responsible for their pharmacological action ranging from antimutagenicity to anti-aging (Nijveldt *et al.* 2001). These flavonoids exhibit antioxidant activities in a variety of ways, including direct capture and elimination of free radicals, reduced leukocyte immobilization, and the regulation of nitric oxide and xanthine oxidase activity (Tripoli *et al.* 2007). Nitric oxide which is among the most potent radicals has the ability to react with other radicals to produce the highly damaging peroxynitrite. Flavonoids on the other hand directly clear these nitric oxide molecules. Thus, nitric oxide removal is thought to play a role in the therapeutic effects of flavonoids (Nijveldt *et al.* 2001).

Garlic, a member of the Amaryllidaceae family, is a plant that grows all over the globe and is popular in cooking. There are many studies showing that garlic consumption helps with fat metabolism, lowers blood cholesterol and fat levels protects blood vessels and heart and can suppress many cancers such as blood, bladder, gastrointestinal tract, breast, lung (Ciric *et al.* 2020). Previous studies reported that this protective effect is caused primarily by the presence of vitamins, phenolic compounds, anthocyanins, flavonoids, and tannins (Berginc and Kristl 2012, Shivashankara *et al*. 2012). Belge-Kurutas (2016) reported that polyphenols commonly found in fruits and vegetables act as reducing agents, singlet oxygen scavengers, and metal chelators against free radicals and ROS activities (Belge-Kurutas 2016). The present study, it can be said that the reason why garlic extract shows better results in nitrite scavenging activity and reducing power trials is due to these bioactive molecules in its structure, and our data are compatible with these studies.

Findings of the present study suggest that the plant extracts used have high nitric oxide removal activity. They may be suitable nitric oxide inhibitor when supplemented into drug therapy of inflammatory diseases. Further studies are needed to prove the nitric oxide scavenging activities of these plant extracts by *in vivo* experiments.

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