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Antioxidant characterization of cinnamon, *Citrus aurantium* and green tea leaves

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Abstract

Background and objective: Plants are full of phenolic compounds and natural antioxidants able to scavenge free radicals in foods and human body. They are more popular than synthetic antioxidants because of lower concern about their mutagenicity and carcinogenicity in human. This study tried to examine the antioxidant properties of water extracts of cinnamon and green tea, and methanolic extract of *Citrus aurantium* compared to butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) as synthetic antioxidants.

Materials and methods: Cinnamon, *Citrus aurantium*, and green tea leaves were prepared from local markets in Iran and their antioxidant properties were examined individually and in combination. The extracts were analyzed by gas chromatograph coupled with mass spectrometer. Total phenol content and antioxidant potency against synthetic antioxidants of BHT and BHA were respectively determined by Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods.

Results and conclusion: E. cinnamaldehyde (32.41%), linalool (65.08%), and kaempferol-3-O-glucoside (84%) were the most important components of cinnamon, *Citrus aurantium*, and green tea leaves extracts, respectively. According to the results, the treatments with a higher percentage of green tea leaves and cinnamon extracts had the highest phenolic content (28.98 ±0.12 µg/g GA) and those with the highest amount of *Citrus aurantium* had the lowest phenolic content (15.56 ±0.06 g/g GA). DPPH test revealed that the lowest IC_{50} was related to the mixture of three extracts (307.62 µg/ml) where the highest belonged to *Citrus aurantium* extract treatment (2100.3 µg/ml). In comparison, for BHT and BHA in 200 mg/l concentration, radical scavenging capacities were 50.7% and 64%, respectively. The three extracts had significant radical scavenging capacity. However, their activity was lower than the synthetic antioxidants of BHT and BHA currently used in food industry.

Keywords: Cinnamon, *Citrus aurantium*, Gas chromatography, Green tea leave, DPPH, Total phenol content

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1. Introduction

Oxidation is an important factor causing food spoilage. It changes the smell, taste, and color of the fatty foods exposed to the environmental factors such as light, temperature, and oxygen; a process ultimately leading to food deterioration.

Natural or synthetic antioxidants containing phenol compounds are used to increase food quality and shelf life and prevent oxidation [1]. Antioxidants are molecules or compounds that deactivate free radicals by damaging their functions [1,2]. Nowadays, synthetic antioxidants are used because they are more effective and stronger than natural counterparts, but some studies revealed that synthetic antioxidants may have side-effect on human health [3,4]. Therefore, studies have been performed on the use of natural antioxidants existing in plants. Plants produce secondary metabolites with antioxidant characteristics such as phenolic compounds when they are exposed to free radicals. Phenols demonstrate their activity in different ways, such as scavenging free radicals and individual oxygen, giving hydrogen, and chelating ions. There is a direct relation between amount of phenol and antioxidant activity in plants [5].

Cinnamon (*Cinnamomum verum J. Presl*) belongs to the Lauraceae family [6], containing phenolic and other antioxidant compounds, including eugenol, camphene, coumarin, cinnamaldehyde, cinnacassiol gamma-terpinene, and cinnamic acid. These compounds can prevent oxidative reactions and are extracted from the cinnamon plant [7,8].

Citrus aurantium from the Rutaceae family is a tree of 4-5 m height with fragrant leaves. Blossoms of this tree are full of alcohol acetate, hydrocarbons, and phenols [9,10].

Green tea leaves are important natural sources of antioxidants, with some groups of polyphenols. Ethanol extract of green tea leaves has antioxidant effect in soy oil similar to 2% butylated hydroxyltoluene (BHT) at equal concentration [4,11-13]. Because of the antioxidant characteristic of plants, and consumers' interest in natural preservations, this study aimed to survey the antioxidant potency of cinnamon, *C. aurantium*, and green tea leaves by total phenol content and 2,2-diphenyl-1picryl-hydrazyl (DPPH) assays. Results of DPPH are compared to the antioxidant activity of two synthetic antioxidants of BHT and butylated hydroxylanisole (BHA).

2. Materials and methods

2.1. Apparatus

Clevenger Mirotek (India), gas chromatography/mass spectrometry (Agilent 6890, USA), refrigerator 4 °C (Iran), refrigerator (0-4 °C) (Iran), shaker (Ika, Germany), spectrophotometer (Model M:6100, Jenway, UK), digital scale (Model TE201, Sartorius, Germany), bain-marie (Wise, South Korea), homogenizer (Wise, South Korea), oven (Model Helios-28452C Elektro, Switzerland), vacuumed oven (Elektro, Switzerland), incubator (Memmert, Germany), and blender (Edmund Buhler, Germany) were used for analysis.

2.2. Materials

All chemicals of hydroxypropyl methylcellulose, glycerol, Turin 80 and 40, methanol, Folin reagent, sodium carbonate, gallic acid, BHT, BHA, sodium nitrate, chloride aluminum and NaOH were purchased from Merck (Germany) and DPPH was supplied by Sigma (USA).

2.3. Extraction of cinnamon

Cinnamon was purchased from Tehran market. Its extraction was done by water distillation. At first, 150 g of cinnamon was poured into a balloon and two-third volume of the balloon was made up with distilled water. Next, the oil and refrigerant separator was connected to the balloon and the Clevenger apparatus was fixed and firmed with a clamp [14,15]. The supernatant included the extract and the highest extract amount was collected at the first hour of extraction. At the end, sodium sulfate was used to absorb the remained water. The extract was kept at 4 °C until analysis [16].

2.4. Extraction of *C. aurantium*

C. aurantium flowers were purchased from local market of Lahijan, Iran. They were milled, powdered, and extracted by 80% v/v methanol. This extract was filtered and concentrated under reduced pressure of rotary evaporator. Then the extract was stored at 4 °C until analysis [17].

2.5. Extraction of green tea leaves

Green tea leaves were prepared from a tea processing plant in Lahijan, Iran. All samples were checked for integrity, absence of dust, and contamination with insects. The leaves were milled at room temperature after rinsing and drying [18]. Then, 10 g of dry tea leaves were extracted with 150 ml of hot distilled water (80 °C) three times. The condensed extract was dried and weighed under vacuum at 40 °C [19].

2.6. Analysis of the extracts by gas chromatography/mass spectrometry

Column of gas chromatograph was Hp-5 (0.25 mm × 0.2 µm × 30 m) MSD: Hp 5975. Water of extracts was removed by 2 ml anhydrous sodium sulfate. The oven temperature was programmed at 50 ° to 265 °C with the constant heating rate of 2.5 °C/min. Helium gas at flow rate of 1.1 ml/min and ionization energy of 70 eV were used [20].

2.7. Total phenol content of the extract

Total phenolic content was determined by Folin-Ciocalteu reagents with analytical grade as standard. For this purpose, 200 µl of the extracts was added to methanol at the ratio of 1:10 and added to 1 ml Folin-Ciocalteu reagents diluted with distilled water at the ratio of 1:10. After 4 min, 800 ml of 20% sodium carbonate was added to the mixture and stored in darkness for 1 h. The absorbance was read at 756 nm. Then, the standard curve of GA (700, 800, 900, 1000, and 1100 µg/ml) was plotted. Concentration of total phenol content was calculated according to Eq. 1. Blank solution was a mixture of 2 ml of methanol, 1 ml of Folin reagent, and 800 µl of saturated sodium carbonate [21].

$$C = (C_1 \times V)/m \qquad \text{Eq. 1}$$

C = Total phenol (µg/g)
C_1 = GA equivalent (µg/ml)
V = Total volume of methanol extract (ml)
m = Weight of the plant used to obtain 200 µl of extract (g)

2.8. DPPH inactivation test of the extracts

50 µl of various dilutions of the extracts (10, 20, 30, 40, and 50 µl/ml) was mixed with 5 µl of a 0.004% methanol solution of DPPH. After storing for 30 min in darkness at room temperature, the absorbance of the samples was read at 517 nm in spectrophotometer. To obtain the concentration inactivates 50% of free radical (IC_{50}), different concentrations (10, 20, 30, 40, and 50 µl/ml) of the extract was drawn against percent of free radical scavenging (percent of inhibition) calculated by Eq. 2. The blank was DPPH solution in 0.004% methanol [20]. BHT and BHA were the standard antioxidant. Free radical scavenging activity for these synthetic antioxidant were measured in concentrations of 100 and 200 µg/ml.

$$\text{Inhibition (\%)} = \frac{A_{Blank} - A_{Sample}}{A_{Blank}} \times 100 \qquad \text{Eq. 2}$$

Inhibition (%) = Free radical scavenging activity
A_{blank} = Absorbance of the control
A_{sample} = Absorbance of the sample

2.9. Statistical analysis

All tests were performed in two replications and analyzed by SPSS ver. 20. One way ANOVA was used for data analysis. Data are presented as mean ±standard deviation. Differences were significant at $p \leq 0.05$.

3. Results and discussion

E. cinnamaldehyde (65.08%) was the most abundant compound in cinnamon extract. Linalool (32.41%) and kaempferol-3-O-glucoside (84%) were the most abundant ingredients in *C. aurantium* and green tea leaves extract, respectively (Table 1-3).

3.1. Total phenol content

As shown in Tables 4 and 5, total of 15 combinations were analyzed in our study. Results of

total phenol content (Table 4) revealed that out of the samples containing one extract, green tea leaves had the highest phenolic content ($p < 0.05$). In the treatments containing two extracts, those with considerable level of *C. aurantium* (treatment 5) had the lowest amount of total phenol content. However, the mean phenolic content of treatment 5 was higher than the mean phenolic content of *C. aurantium* extract alone ($p < 0.05$). Except for treatments 5 and 12 whose the mean difference was significant, the mean differences among the other samples were not significant ($p > 0.05$). As a main finding, the mixture of green tea leaves:cinnamon extracts (75:25) contained the highest phenolic content among the all combinations. In our study, phenolic content of *C. aurantium* was 15.56 ±0.06 µg/g GA or 0.01556 mg/g GA, differing from some other studies. For instance, Hashemi et al. studied the antioxidant potency of *C. aurantium* in comparison to TBHQ and observed 7.1 mg/g GA equivalent of phenolic content [22]. Moreover, Karimi et al. examined various extracts of this plant's bloom to evaluate their phenolic compounds and observed that one gram of *C. aurantium* extract contained 4.43 mg of phenolic content based on GA content [23]. In addition, Moulehi et al. analyzed this plant's fruit peel and pit and obtained 5.1 and 1.53 mg of phenolic content based on GA, respectively [24]. Zeghad et al. calculated total polyphenol content of *C. aurantium* using classical spectrophotometric method as 14.34 ±0.30 mg/g GA that is far from our result [25].

Table 1- Chemical analysis of cinnamon extracts by gas chromatography/mass spectrometry

Number	Component	Amount (%)	RT (min)	Number	Component	Amount (%)	RT(min)
1	α-thujene	0.11	12.68	15	Z-cinnamaldehyde	2.93	29.60
2	α-pinene	1.15	13.14	16	E-cinnamaldehyde	65.08	32.48
3	Camphene	0.53	14.34	17	Eugenol	3.63	36.15
4	Benzaldehyde	0.22	15.01	18	Phenylperopyl iso-butyrate	0.15	36.98
5	β-pinene	0.30	15.89	19	α-copaene	0.35	37.90
6	α-phellandrene	0.67	17.73	20	β-caryophyllene	4.38	39.12
7	α-terpinene	0.63	18.00	21	Cinnamyl acetate	7.02	40.24
8	p-cymol	1.51	18.28	22	o-methoxy cinnamaldehyde	0.25	40.28
9	β-phellandrene	3.76	18.75	23	α-humulene	0.65	40.40
10	Linalool	2.48	22.93	24	Cinnamaldehyde isomer	0.21	43.75
11	Camphor	0.48	25.44	25	Caryophyllene alcohol	0.14	45.77
12	Cinnamic alcohol	0.34	26.25	26	Unknown Sesquiterpene	0.37	45.92
13	4-terpineol	0.37	27.54	27	Hexadecanal	0.13	47.03
14	α-terpineol	0.75	28.30	28	Benzyl benzoate	1.39	52.63

Table 2- Chemical analysis of *Citrus aurantium* extracts by gas chromatography/mass spectrometry

Number	Component	Amount (%)	RT (min)	Number	Component	Amount (%)	RT (min)
1	α-pinene	0.30	9.46	17	Nerol	16.49	12.35
2	Camphene	0.02	9.67	18	Geraniol	2.74	12.71
3	β-pinene	0.55	9.86	19	Linalyl acetate	3.05	12.78
4	Myrcene	5.32	9.96	20	Neryl acetate	2.74	13.66
5	δ-3-carene	0.14	10.15	21	Geranyl acetate	8.31	13.90
6	α-terpinene	0.03	10.26	22	β-elmene	0.02	13.99
7	Limonene	1.05	10.42	23	Trans-caryophyllene	0.88	14.46
8	Trans-β-ocimene	0.64	10.47	24	β-farnesene	0.05	14.58

9	Cis-β-ocimene	3.27	10.55	25	α-hamulene	0.13	14.71
10	ϒ-terpinene	0.07	10.70	26	α-farnesene	0.05	15.05
11	Linalool oxide	0.09	10.87	27	Bicyclogermacarene	0.29	15.07
12	Terpinolene	1.03	10.99	28	δ-cadinene	0.08	15.33
13	Linalool	32.41	11.11	29	Nerolidol	0.51	15.66
14	Citronellal	0.04	11.61	30	Caryophyllene oxide	0.17	16.05
15	Terpineol-4	0.40	12.01	31	Viridiflorol	0.06	16.08
16	α-terpineol	16.51	12.02				

Table 3- Chemical analysis of green leave extracts by gas chromatography/mass spectrometry

Number	Combination	Amount (%)	RT (min)	Number	Combination	Amount (%)	RT (min)
1	Catechin	4.48	9.05	11	Kaempferol glycoside	0.82	13.72
2	Callocatechin	2.44	9.41	12	Quercetin-3-0-rhamnoside	0.31	14.05
3	Catechin gallate	0.31	9.85	13	Quercetin glycoside	1.06	14.33
4	Callocatechin gallate	1.67	10.20	14	Myricetin-3-0- rutinoside	0.57	14.62
5	Epicatechin	9.71	10.59	15	Gallic acid	0.76	14.96
6	Epigallocatechin	31.78	10.83	16	Theaflavin	0.09	15.38
7	Epicatechin gallate	6.60	11.24	17	Theaflavin-3-3- digallate	0.06	15.69
8	Epigallocatechin gallate	35.97	12.62	18	Theaflavin-3- gallate	0.01	15.98
9	Kaempferol-3-0-glucoside	0.84	12.93	19	Thearubigins	0.48	19.28
10	Kaempferol-3-0-rutinoside	0.03	13.44	20	Trace element	2.14	16.45

Table 4- Total phenolic content of the extracts by Folin-Ciocalteu method

Number	Cinnamon (%)	*Citrus aurantium* (%)	Green leaves (%)	Total phenol (µg/g GA)
1	100	0	0	22.97 ±0.03
2	0	100	0	15.56 ±0.06
3	0	0	100	27.95 ±0.07
4	50	50	0	22.52 ±0.02
5	25	75	0	19.49 ±0.01
6	75	25	0	23.69 ±0.10
7	50	0	50	25.85 ±0.09
8	25	0	75	28.98 ±0.12
9	75	0	25	23.69 ±0.02
10	0	50	50	23.81 ±0.01
11	0	25	75	25.42 ±0.07
12	0	75	25	20.51 ±0.03
13	40	30	30	26.25 ±0.06
14	50	10	40	26.89 ±0.03
15	60	20	20	24.94 ±0.07

For cinnamon, we obtained total phenol content of 22.97 ±0.03 µg/g or 0.02297 mg/g GA equivalent which was significantly different from other studies. Study of Kamali et al. showed that the highest amount of phenolic compounds in cinnamon was achieved by acetone extraction elicited by cold solvent method as 1.678 mg of phenolic content based on GA equivalent [26]. Moreover, Wijewardhana et al. found that cinnamon had a high total phenolic content yielding

18.94 ±0.46 mg GA/100 g of dry weight [27] which is different from our finding.

In our study, phenolic content of green tea leaves extracts was 27.95 ±0.07 µg/g of GA equivalent (0.02795 mg/g GA equivalent). Mirahhmadi et al. found that the aqueous extract of Iranian green tea leaves had significantly higher antioxidant activity than BHT, BHA, and alpha-tocopherol in sunflower oil [28]. Ramirez-Aristizabal et al. compared the antioxidant characteristics of four brands of green tea sold in Colombia prepared by different thermal conditions. They found that total phenol content varied between 2.53–14.63 mg GA/g for cold extraction and 29.34–55.06 mg GA/g for hot extraction [29]. Kopjar et al. studied phenol content of green, yellow and black tea leaves and reported total phenol of 3.823 ±0.142 g for pulverized and 3.662 ±0.199 g for intact products [30]. Pereira et al. determined phenolic compounds of green, black and white tea of *Camellia sinensis*. They find that green tea had a high content of total phenols (55.40 mg of pyrogallol/g) [31]. In accordance, Izzreen and Fadzelly observed high total phenol for shoot, young and mature green tea leaves varied from 80.27 ±0.61, 72.70 ±0.46 and 3.87 ±1.36 g GA/g dry weight) which were inconsistence with our results [32].

3.2. DPPH scavenging

As demonstrated in Table 5, the lowest IC_{50} was observed for treatment 14 (307.62 µg/ml = 0.307 mg/ml) and the highest for treatment 2 (2100.3 µg/ml = 2.100 mg/ml). A lower IC_{50} shows the higher power of the extract in radical scavenging activity. Synthetic antioxidants, such as BHA and BHT were the standard samples, which had the inhibition percentage of 74% and 52.7%, at the concentrations of 200 µg/ml (Table 6).

The results revealed that the cinnamon extract had a superior DPPH inhibitory power among treatments containing one extract, and green tea leaves had a higher power than *C. aurantium* extract. Mean difference was not significant between cinnamon and green tea leave extract, but it was significant with *C. aurantium* extract. Among the treatments containing two extracts, those including more than 50% of *C. aurantium* had the least inhibitory power. Despite what was expected, except for treatment 14, the treatments containing all three extracts (13 and 15) did not show the highest inhibitory power.

Table 5- Antioxidant activity of the extracts by DPPH test

Number	Cinnamon (%)	*Citrus aurantium* (%)	Green leaves (%)	IC_{50} (µg/ml)
1	100	0	0	350.70 ±0.26
2	0	100	0	2100.30 ±0.50
3	0	0	100	388.28 ±2.07
4	50	50	0	407.68 ±0.20
5	25	75	0	680.62 ±0.80
6	75	25	0	337.05 ±1.80
7	50	0	50	325.11 ±2.70
8	25	0	75	330.21 ±1.40
9	75	0	25	316.02 ±2.20
10	0	50	50	335.45 ±2.50
11	0	25	75	342.46 ±4.60
12	0	75	25	692.75 ±3.30
13	40	30	30	335.40 ±3.20
14	50	10	40	307.62 ±1.50
15	60	20	20	345.18 ±2.90

Table 6- Radical scavenging index of BHA and BHA

Concentration of BHA (µg/ml)	RSI (%)	Concentration of BHT (µg/ml)	RSI (%)
100	48	100	39
200	74	200	52.7

Furthermore, all treatments had less inhibitory power than the two synthetic antioxidants BHA and BHT (200 µg/ml). Karimi et al. studied the biological activities of *C. aurantium* bloom extract in water, methanol, and ethanol fractions. They showed that the methanolic extract was stronger than two others and the concentration of 300 µg/ml had 55.3% scavenging power which is inconsistent with our study [23].

Moreover, Ben Hsouna et al. evaluated the antioxidant power of essential oil extracted from *C. aurantium* by performing beta-carotene inhibition tests and found that EC_{50} was 1.8 µg/ml [33]. Moulehi et al. reported that EC_{50} for *C. aurantium* pit extract equaled 0.188 mg/ml [24]. Hashemi et al. investigated the antioxidant power of *C. aurantium* compared to TBHQ in corn oil treated by UV and found that phenolic compounds and IC_{50} of the extract were 65 ±1.5 µg/ml and 73 ±2.1 µg/ml, respectively [34], which are lower than our results. Zeghad et al. reported antioxidant activity of *C. aurantium* by DPPH assay as 0.810 ±0.005 mg/ml [25].

Hosseini et al. explored the DPPH scavenging power in essential oil of several herbs and cinnamon. IC_{50} for cinnamon was 7795 mg/ml which were inconsistent with 350.7 ±0.26 µg/ml found in present study [35]. Gulcin et al. studied antioxidant activities of aqueous and ethanolic extracts of cinnamon (*C. verum*) bark through which IC_{50} of 21.25 and 15.71 µg/ml were reported, respectively [36]. Yang et al. studied antioxidant activity (DPPH scavenging assay) of various parts of *C. cassia* extracted with ethanol and found that IC_{50} were in the range of 0.072–0.208 mg/ml [37].

Kopjar et al. studied antioxidant activity of green, yellow and black tea leaves and showed that DPPH for pulverized and intact green tea were 20.382 ±0.046 and 19.159 ±0.162 µmol/100 g, respectively [30]. Wijewardhana et al. reported that free radical inhibition of cinnamon bark by DPPH assay was 149.15 ±1.73 mg/ml and IC_{50} was 0.009 ±0.76 mg/ml [27]. Pereira et al. determined IC_{50} of green tea infusions as 18.79 ±0.26, 35.10 ±1.34, and 13.51 ±0.98 µg/ml for three different brands [31]. Izzreen et al. explored antioxidant properties of different parts of *Camellia sinensis* leaves and reported that the IC_{50} for shoot, young and mature leaves varied between 0.03 ±0.03 and 0.04 ±0.00 mg/ml [32] which are different from our result of 388.28 ±2.07 µg/ml.

In general, the studies have shown that the content of phenol compounds depends on the source, genetics, environmental condition, harvest time, and the processes done on the plant through which a range of 24 to 40% has been reported [38]. It is assumed that the differences between our results and the others might be due to the drying process, method of extraction and plants' maturity.

4. Conclusion

Antioxidant activity of the extracts by total phenol content assay showed that treatments containing higher percentage of green tea leaves and cinnamon extracts had the highest phenol (28.98 ±0.12 µg/mg GA) and those containing the highest amount of *C. aurantium* had the lowest phenol (15.56 ±0.06 µg/mg GA). DPPH assay revealed that the lowest IC_{50} belonged to the mixture of three extracts (307.62 µg/ml) where the highest was related to the *C. aurantium* extract treatment (2100.3 µg/g GA). Although the all three extracts showed a good radical scavenging power, they were less effective compared to BHT and BHA.

5. Conflict of interest

Authors declare no conflict of interest.

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