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EFFECT OF DRYING PROCESS ON PHENOLIC COMPOUNDS AND TOTAL ANTIOXIDANT ACTIVITY OF SOME MEDICINAL AND AROMATIC PLANTS

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ABSTRACT

The effect of antioxidant properties as free radical scavenging activity and phytochemical compounds of fresh and dried samples of dill, parsley, coriander, peppermint and celery leaves were studied. Moreover, the chemical constituents and the content of minerals in these medicinal and aromatic plants were also investigated. Results explained that the contents of total phenolics ranged from 1446.88 for coriander to 3128.57mg/100gm for peppermint and after the drying process decreased to 1280.10 and 2356.45mg/100gm, respectively. Also, peppermint had the highest content of flavonoids, while the lowest content was observed in coriander leaves. Otherwise, fresh coriander leaves had the highest content of carotenoids, while the lowest content was in the fresh peppermint leaves. Results explained also that the main component of phenolic compounds was protocatchuic in all fresh and dried samples under investigation. Celery leaves had the highest protocatchuic content. Concerning minerals content reveal that the parsley leaves had the highest content of calcium, potassium and magnesium, while peppermint had the highest phosphorus and iron contents. Results also show that celery leaves had the highest sodium content. Fresh dill and peppermint recorded the highest percentages of total antioxidant activity. Also, fresh celery had gradual effect on DPPH compounds being used as synthetic free radical. Coriander and parsley showed the lowest antioxidant activity. After the drying process total antioxidant activity decreased. Slight decrease was observed with dill and peppermint leaves, while celery and parsley leaves were more effective by using the air oven drier process.

Keywords: celery, dill, peppermint, parsley, coriander, leaves, drying, phytochemical compounds, total antioxidant activity

INTRODUCTION

Dried herbs that are often used as cooking ingredients (seasonings) possess potential health benefits due to their natural phenolics, which are not fully investigated (Yanishlieva *et al.*., 2006; Clifford, 1999), though they are universally recognized for their flavor contributions. According to our recent report (Lee and Scagel, 2010) it was ascertained that dried basil being deficient in phenolics compared to fresh basil. We proceeded to determine if this was the case for other dried Lamiaceae products that are easily obtained from the market place.

Moreover, herbs, vegetables and fruits are daily dietary sources for a wide variety of phytochemicals. Flavonoids and other phenolic compounds adhering in these dietary sources possess bioactive properties, protecting cellular systems against oxidative damage (Jones *et al.* 1992).

Phytochemical compounds were reported to be protective against chronic diseases such as cancer and heart diseases. These protective effects are generally attributed to the presence of various functional components, such as phenolic compounds, ascorbic acid, vitamin E, provitamins, minerals,

and fibers (Molay, *et al.* 2007). Many of these chemicals have been claimed to contribute to the antioxidant activities of the plants and the antioxidant activity is mainly due to their redox properties derived from various possible mechanisms: free-radical-scavenging activity, transition-metal-chelating activity, and/or singlet oxygen- quenching capacity (Chen and Ahn, 1998; Luiz, *et al.* 2002). Phenolic compounds are also known to play an important role in stabilizing lipid peroxidation and to inhibit various types of oxidizing enzymes (Cos *et al.*., 1998). Since a large part of vegetables have undergone thermal processing to make them suitable for consumption, it is important to investigate in details how thermal processing affects the contents of phytochemicals and antioxidant activities (Rosa and Heaney, 1993).

Also, medicinal plants have an important value in the socio-cultural, spiritual and medicinal use in rural and tribal lives of the developing countries (Shinwari, 2005). The demand of medicinal plants is increasing both in developed and developing countries.

Aromatic and medicinal plants can be defined as plants used in official and traditional medicine, aroma and flavor (Baricevic *et al.*., 2004).

The screening studies for antioxidant properties of medicinal and food plants have been performed increasingly for the last few decades in hope of finding an efficient remedy for several present-day diseases and means to delay aging symptoms (Halliwell, 2008). The disorders related to excessive oxidation of cellular substrates (oxidative stress) include type II diabetes, neuro-degenerative diseases, or even some types of cancer. There is also a huge demand for natural antioxidants in food industry, for replacing the synthetic preservatives used to prevent fat rancidity or color loss. Oxidizing agents may damage a number of biological molecules such as nucleic acids, membrane lipids, enzymes, or synovial fluid polysaccharides.

The objective of this study was to investigate and compare the antioxidant properties as free radical scavenging activity and phytochemical compounds between extracts of fresh and dried dill, parsley, coriander, peppermint and celery leaves.

MATERIALS AND METHODS

Materials

Fresh leaves of dill (Anethum graveolens), parsley (Petroselinum crispum), coriander (Coriandrum sativum), celery (Apium graveolens) and peppermint (Mentha piperita) were obtained from the Horticultural Research Institute, Agricultural Research Center. Samples for preparing dried green leaves after cleaning, dried at 40 °C for approximately 6 h in an oven and ground by blender. Fresh and dried leaves (2.5 and 0.5gm) were extracted with ethanol (100 ml) for 10 h. The extracts were filtered through Whatman No. 2 filter paper and the filtrates were used to determine total phenolic compounds and antioxidant activity.

Methods

- Moisture content, crude protein (N × 6.25), crude fiber, ash, total acidity, chlorophyll (a), chlorophyll (b), carotenoids and ascorbic acid were determined according to AOAC (1990).

- Minerals content (Na,Ca and K) were estimated using emission flame photometer (Model Corning 410).The other minerals (Fe, P and Mg) were determined using Atomic absorption spectrophotometer (Perkin-Elmer Instrument Model 2380).
- The total polyphenolic content was estimated using the Folin–Ciocalteu assay, developed by Velioglu *et al.*., (1998), with some modifications. Briefly, 125 µl of Folin–Ciocalteu reagent, 125 µl diluted sample, and 250 µl of distilled water were put into a test tube. The mixture was vortexes and allowed to stand for 5 min at room temperature. Then, 1.25 ml of sodium carbonate solution (7%) was added, followed by 1 ml of distilled water. The mixture was vortexes and allowed to stand at room temperature for 90 min. Total phenolic content was determined using a spectrophotometer (Jenway 6405 UV/VIS) at 760 nm. Gallic acid was used as standard, and total phenolic content was expressed as equivalents of gallic acid (GAE)/100 g sample.
- Total flavonoids content was determined by modifying a colorimetric method described previously Dewanto *et al.*., (2002) and Eberhardt *et al.*., (2005). Briefly, 0.2 ml of the water-soluble extract, 0.8 ml of distilled water and 50 µl of a 5% $NaNO_2$ solution were mixed in a test tube. After 6 min, 100 µl of a 10% $AlCl_3 - 6H_2O$ solution was added and allowed to stand for another 5 min before 0.5 ml of 1 M NaOH was added. Then, 850 µl of distilled water was added to bring the mixture to 2.5 ml and mixed well.

The absorbance was measured immediately against the blank at 510 nm using a spectrophotometer (Jenway 6405 UV/VIS) in comparison with the standards prepared similarly with known quercetin concentrations (10–800 µg/ml).The results are expressed as mean (micromoles of quercetin equivalents,QE/100 g sample).

- Extraction, separation and quantification of phenolic compounds were determined according to the method described by Goupy *et al.*.(1999).
- Antioxidant activity was determined by the 2,2,-diphenyl- 2-picryl-hydrazyl (DPPH) method of Zhang and Hamauzu (2004). with some modifications. Broccoli content of the methanol extracts of fresh or processed broccoli were adjusted to 6 mg/ml (on dry basis), which was chosen as an appropriate concentration for assessing antioxidant activity after preliminary studies of the different concentrations. An aliquot of 1.5 ml of 0.1 mM DPPH radical in methanol was added to a test tube with 0.5 ml of broccoli extract, at 6 mg/ml. Instead of methanolic extract of broccoli, pure methanol was used as control. The reaction mixture was let to stand at room temperature in the dark for 60 min before the decrease in absorbance at 517 nm was measured. Pure methanol was used to calibrate the spectrophotometer. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

$$AA\ (\%) = Abs\ control - Abs\ sample\ /\ Abs\ control \times 100:$$

Statistical analysis: All data were recorded as means and analyzed by SPSS for Windows (ver.10.1.). One-way analysis of variance (ANOVA) and Duncan comparisons were tested for any significant differences between raw and dried medicinal and aromatic samples.

RESULTS AND DISCUSSION

Results in Table (1) Show the chemical constituents of some medicinal and aromatic plants namely (celery, dill, peppermint, parsley and coriander). Moisture content ranged from 84.34 to 89.72% in both peppermint and dill leaves on fresh weight basis, respectively. Also protein content ranged from 10.27% in celery leaves to 23.12% in peppermint while the percentages of protein in parsley, dill and coriander were 22.89, 21.57 and 20.96%, respectively.

Results also explain the highest percentages in crude fiber shown in both peppermint and celery leaves which were 12.66 and 12.32% (on DWB), respectively, while the lowest percentage was observed in coriander leaves 7.12 %. Ash percentages ranged from 12.78 in dill to 14.92% in peppermint leaves , meanwhile, coriander had the highest content in total acidity 29.87, while peppermint had the lowest content 14.59.

Table (1): Chemical constituents of some fresh medicinal and aromatic plants (on dry weight basis)

Constituents(%)	Celery	Dill	Peppermint	Parsley	Coriander
Moisture content	88.46±1.68	89.72±0.83	84.34±0.64	85.64±1.01	88.25±0.22
Crude protein	10.27±0.33	21.57±1.11	23.12±0.71	22.89±0.07	20.96±0.96
Crude fiber	12.32±0.40	8.75±0.18	12.66±0.24	11.18±0.12	7.12±0.08
Ash	12.84±0.88	12.78±0.14	14.92±0.32	13.54±0.12	13.79±0.03
Total acidity*	23.25±0.45	20.28±0.21	14.59±0.60	28.27±0.51	29.87±0.18

*** mL 0.1N NaOH/100gm sample. Data are means of three replicate experiments ± SD.**

The phytochemical compounds in some fresh medicinal and aromatic plants compared to that in dried are shown in Table (2). Results reveal that the contents of total phenolics ranged from 1446.88 for coriander to 3128.57 mg/100gm for peppermint (on dry weight basis). After being dried these contents decreased to 1280.10 and 2356.45mg/100gm, respectively. Also, peppermint had the highest content of flavonoids while the lowest content was observed in coriander leaves. Otherwise, fresh coriander leaves had the highest content of carotenoids which was 184.23 mg/100gm, while the lowest content 112.47 mg/100 gm was shown in fresh peppermint leaves. However the highest content of ascorbic acid was observed in fresh parsley leaves which decreased from 762.94 to 122.18 mg/100gm (on DWB) after being dried by enforced hot air oven drier. Results indicate also that peppermint leaves had the lowest content of ascorbic acid which was 162.57mg/100gm. Moreover, total chlorophyll ranged from 507.91 to 2432.03 mg/100gm decreased to 252.32 and 1285.82 mg/100 gm for dill and peppermint leaves, respectively. The high content of medicinal and aromatic plants in these phytochemical compounds play an important role as beneficial effect being due to the action of antioxidant compounds, which are capable of neutralizing free radicals and reduce oxidative damage in the body (Clifford, 1995).

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Phenolic compounds in some fresh and dried medicinal and aromatic plants are shown in Table (3). Thirteen compounds of some fresh and dried medicinal and aromatic plants polyphenols were identified by HPLC analysis. The detected polyphenolic compounds were protocatchuic, P. hydroxy penzoic, catechein, chlorogenic, catechol, syringic, caffene, vanillic, P.coumaric, salycillic, cinnamic, chrisin and caffeic as shown in Table (3). The main component of phenolic compounds was protocatchuic in all fresh and dried samples under investigation. Celery leaves had the highest protocatchuic content which was 617.96 mg/kg. This content decreased to 104.17 mg/kg after drying process. Fresh dill and peppermint had 433.14 and 411.69. These contents decreased to 305.38 and 80.61mg/kg, respectively. Also, dill had the highest content of chlorogenic compound 347.54mg/kg followed by celery which was 256.74mg/kg while the lowest chlorogenic content shown in coriander (15.40 mg/kg). Results also explained that coriander had 400.88 and 308.34 mg/kg in both P.coumaric and salycillic, respectively.

Table (3): Phenolic compounds in some fresh and dried medicinal and aromatic plants (mg/kg on dry weight basis)

Phenolic compounds	Celery		Dill		Peppermint		Parsley		Coriander	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Protocatchuic	617.96	104.17	433.14	305.38	411.69	80.61	349.02	86.24	338.60	126.81
P.hydroxy penzoic	11.27	1.19	15.09	1.25	11.77	1.67	8.41	1.22	30.74	24.05
Catechein	35.23	2.24	53.39	99.74	57.54	3.92	51.68	3.36	46.56	40.52
Chlorogenic	256.74	81.16	347.54	83.31	97.28	2.14	81.38	2.51	15.40	8.08
Catechol	53.62	18.41	31.70	2.21	18.70	2.10	23.07	7.51	51.83	17.28
Syringic	46.49	6.18	50.68	3.19	11.76	1.71	44.57	2.89	19.52	3.71
Caffene	99.19	37.55	271.91	22.04	_	_	97.36	8.69	39.87	26.57
Vanillic	28.75	7.73	14.05	1.83	_	_	4.31	1.72	33.98	5.22
P.Coumaric	185.63	86.44	132.48	48.01	117.30	18.42	5.65	438.70	400.88	140.35
Salycillic	141.54	55.33	17.62	1.75	_	_	2.44	1.73	308.34	24.35
Cinnamic	5.26	1.94	3.78	1.10	5.74	1.89	6.69	2.60	32.18	1.72
Chrisin	_	_	32.86	1.98	_	_	10.71	3.17	6.96	1.06
Caffeic	_	_	67.40	28.10	27.16	5.69	13.11	2.11	10.14	6.90

Mineral contents in some fresh medicinal and aromatic plants were determined and the obtained results are presented in Table (4). These results reveal that parsley leaves had the highest content of calcium, potassium and magnesium which were 2041.37, 3868.97 and 475.86 mg/100gm, respectively. Peppermint had the highest phosphorus and iron content 770.82 and 59.34 mg/100gm (on DWB), respectively. Results also show that celery leaves had the highest sodium content. On the other side, results explain that celery had the lowest content of calcium and iron, 518.86 and 18.24 mg/100gm (on DWB), respectively, while dill had the lowest content of phosphorus. Also, peppermint recorded the lowest content in both potassium and magnesium while parsley showed the lowest sodium content.

Table (4): Mineral contents of some fresh medicinal and aromatic plants (mg/100 gm on dry weight basis)

Mineral contents	Celery	Dill	Peppermint	Parsley	Coriander
Calcium	518.86±3.04	1520.82±2.33	1617.40±4.47	2041.37±7.02	794.33±2.96
Phosphorus	349.56±2.66	197.94±1.05	770.82±3.90	344.80±1.18	531.90±1.82
Iron	18.24±0.57	46.87±1.17	59.34±1.01	30.72±0.08	36.59±0.16
Sodium	829.67±4.03	354.17±3.11	195.63±0.96	172.83±2.00	515.64±4.01
Potassium	3546.75±6.68	3354.17±7.78	2261.74±3.47	3868.97±5.59	3056.73±3.55
Magnesium	377.36±2.39	228.27±1.81	121.81±0.15	475.86±1.49	172.21±0.94

Data are means of three replicate experiments ± SD.

Results in Figures (1 and 2) show the total antioxidant activity (TAA) in different fresh and dried medicinal and aromatic plants using 5% extract prepared by 5g fresh samples in 100ml 80% methanol.

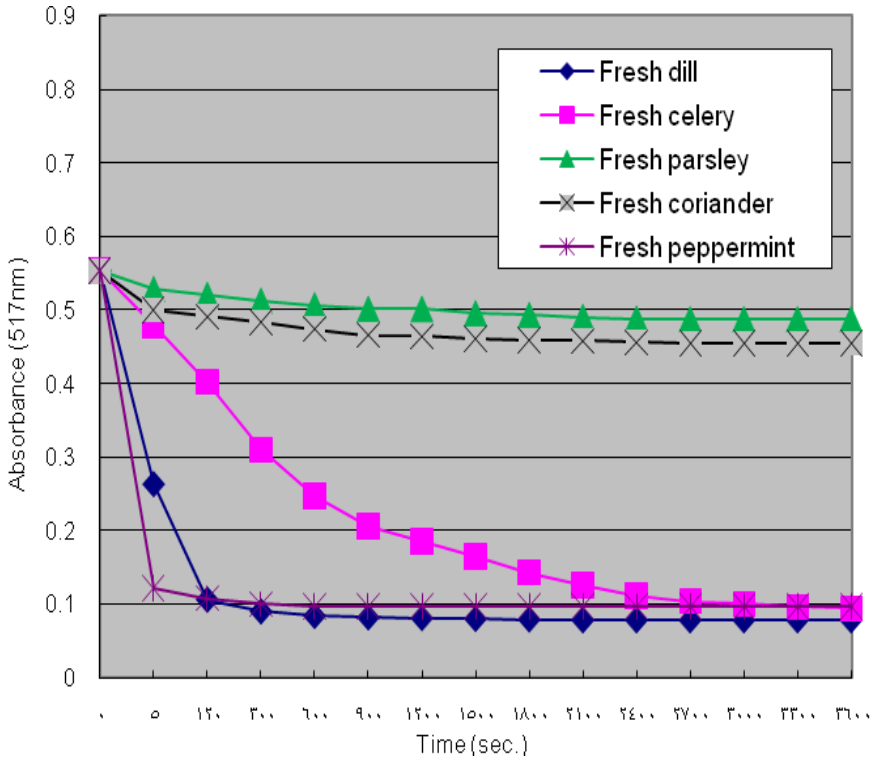

Fig. (1): Total antioxidant activities of some fresh medicinal and aromatic plants

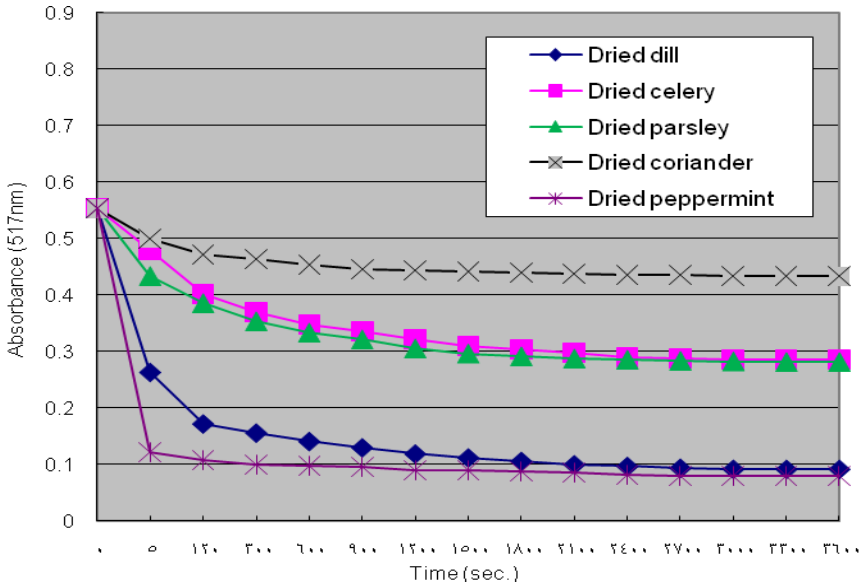

Fig.(2): Total antioxidant activities of some dried medicinal and aromatic plants.

These results reveal that antioxidant activity of fresh dill and peppermint had the highest percentages of total antioxidant activity. Also, fresh celery had gradual effect on DPPH compound which used as synthetic free radical. Coriander and parsley explained the lowest antioxidant activity obtained which recorded of TAA. After drying process total antioxidant activity decreased. Slight decrease was observed with dill and peppermint leaves while celery and parsley leaves were more effective by using air oven drier process. These results indicate the presence of antioxidant compounds such as ascorbic acid, polyphenols, flavonoids and the higher percentages which act together as an important role of antioxidant activity. Also, it contains highest antioxidant medicinal plants activity as correlated well with the contents for both chlorogenic and protocatchuic acid and the correlated results obtained by Scherer and Godoy (2009) which used a new antioxidant activity index by DPPH method and they found that gallic acid showed higher AAI value 27 followed by protocatchuic acid 20 these two compounds may play an important role as strongly antioxidant compounds in medicinal plants.

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تـأثير عمليـة التجفيـف علـي المركبـات الفينوليـة ونشـاط مضـادات الأكسـدة لـبعض النباتات الطبية والعطرية

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تم دراسة تأثير خواص مضادات الأكسدة كمثبطات للشقوق الحرة والمتمثلة في المركبات الفيتوكيميائية والمستخلصة من أوراق الشبت والبقدونس والكسبرة والنعناع والكرفس الطازجة والمجففة. علاوة علي ذلك تم دراسة التركيب الكيميائي والمحتوي من العناصر المعدنيـة لهذه النباتات الطبية والعطرية. أوضحت النتائج أن المحتوي من الفينولات الكلية تراوحت مابين ١٤٤٦,٨٨ الي ٣١٢٨,٥٧ مليجرام لكل ١٠٠ جرام لأوراق الكسبرة والنعناع علي التوالي. وبعد المعاملة بالتجفيف انخفض المحتوي الي ١٢٨٠,١٠ و ٢٣٥٦,٤٥ مليجرام لكل ١٠٠ جرام علي التوالي. أيضاً وجد أن اعلي محتوي من الفلافونات كان في اوراق النعناع بينما سجلت أوراق الكسبرة أقل محتوي بالمقارنة بأوراق النباتات الاخري. وعلي العكس سجلت عينات أوراق الكسبرة الطازجة أعلي محتوي من الكاروتينات بينما كانت أوراق النعناع أقل محتوي. كما أظهرت نتائج تحليل الفينولات أن المركب الرئيسي هو البروتوكاتشويك لكل أوراق النباتات الطازجة والمجففة تحت الدراسة وكانت اوراق الكرفس أكثر النباتات في المحتوي من البروتوكاتشويك. أما عند تقدير العناصر المعدنية فقد لوحظ ان اوراق البقدونس تحتوي علي أعلي كمية من الكالسيوم والبوتاسيوم والماغنسيوم بينما أحتوي النعناع علي أعلي معدل من الفوسفور والحديد. أظهرت ايضاً النتائج أن أوراق الكرفس تحتوي علي أعلي معدل من الصوديوم. وبالنسبة لنشاط مضادات الأكسدة الكلية سجلت كل من الشبت والنعناع أعلي معدل من النشاط وكانت أوراق الكرفس ذات تأثير متدرج علي مركب ٢-٢ داي فينيل١ بيكريل هيدرازيل والمستخدم كمركب صناعي يمثل الشقوق الحرة . بينما كان كل من أوراق الكسبرة والبقدونس أقل نشاط كمضادات للأكسدة بالمقارنة بالنباتات الاخري ايضاً لوحظ ان اجراء عملية التجفيف بواسطة الأفران الحرارية تقلل بنسبة ضئيلة نشاط مضادات الأكسدة الكلية لكل من أوراق الشبت والنعناع ولكنها تؤثر بشكل أكبر علي أوراق الكرفس والبقدونس. .

قام بتحكيم البحث

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Table (2): The content of phytochemical compounds in some fresh and dried medicinal and aromatic plants (mg/100gm DM)

Phytochemical compounds	Celery		Dill		Peppermint		Parsley		Coriander	
	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried	Fresh	Dried
Total phenolics	1884.60± 5.16	1401.90± 4.06	2018.40± 8.12	1700.89± 3.33	3128.57± 4.11	2356.45± 7.15	1978.16± 6.03	1641.10± 2.34	1446.88± 5.04	1280.10± 6.54
Flavonoids	987.11± 3.92	704.04± 6.11	746.00± 4.62	583.20± 2.57	1255.50± 5,18	723.14± 2.83	912.00± 3.80	884.31± 4.56	724.25± 2.60	608.18± 3.75
Carotenoids	165.21± 1.78	113.75± 1.89	122.13± 2.07	94.18± 0.54	112.47± 1.25	100.86± 1.35	113.95± 2.08	89.34± 0.15	184.23± 3.25	146.87± 1.96
Ascorbic acid	184.33± 2.17	46.83± 0.43	232.67± 1.65	57.95± 1.01	162.57± 1.08	45.36± 0.12	762.94± 3.91	122.18± 1.17	302.44± 1.15	76.11± 0.55
Chlorophyll(A)	425.23± 2.11	233.75± 2.31	382.24± 3.10	194.82± 2.11	1324.78± 5.88	820.88± 4.00	563.44± 4.02	344.41± 1.00	654.88± 1.99	395.44± 2.03
Chlorophyll(B)	169.12± 2.27	64.53± 1.11	125.67± 1.49	57.50± 0.83	1107.25± 3.39	464.94± 2.07	216.82± 2.00	92.68± 1.10	256.64± 0.73	122.18± 1.17
Total chlorophyll	594.35± 3.09	298.28± 1.66	507.91± 3.35	252.32± 1.44	2432.03± 5.00	1285.82± 3.21	780.26± 4.41	437.09± 1.15	911.52± 3.17	517.62± 2.58

Data are means of three replicate experiments ± SD.