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Comparison of antioxidant properties of leaves of plants grown in Turkey

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ABSTRACT

In this study, DPPH radical scavenging activity, ABTS radical cation decolorization assay, FRAP reducing power, total phenolic content, total flavonoid content and phenolic compounds of leaves of plants, grown in Afyon/Turkey were investigated. These plants were poppy, daisy, dandelion, manger, chickweed, black chicory and white chicory. Phenolic acids including gallic acid, ferulic acid, chlorogenic acid, coumaric acid, ellagic acid, vanilic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid and flavonoids including catechin, apigenin, naringin, rutin and quercetin amounts in leaves were determined. Among plants leaves, leaves of daisy and manger had statistically highest DPPH value and leaves of poppy and dandelion had statistically highest ABTS value, while leaves of White chicory had statistically highest FRAP value, total phenolic content and total flavonoid content. Leaves of black chicory and white chicory had higher phenolic compounds compared to that of other plants. These results suggest the using of these leaves as sources of natural antioxidants.

***Keywords*:** Phenolic, Antioxidant, DPPH, ABTS, FRAP

Abbreviations:

DPPH: 2,2-diphenyl-1-picrylhydrazyl
ABTS: 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
FRAP: Ferric reducing/antioxidant power
TPC: Total phenolic content

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TFC: Total flavonoid content
TPTZ: 2,4,6-tri-(2-pyridyl)-s-triazine
GAE: Gallic acid equivalent
HPLC: High performance liquid chromatography

1. INTRODUCTION

Medicinal plants contain pharmaceuticals and health care components. Plant derived products, phytochemicals and pro-vitamins, which can prevent diseases, are described as functional foods (Ivanova et al., 2005). Phenolic compounds are found in several plants and have antioxidant activity. The antioxidant property of phenolics is sustained from their redox properties. So phenolic compounds can take a role as reducing agents, oxygen quenchers and metal chelators (Rice-Evans et al., 1997). Plants need phenolic compounds for growth, pigmentation and resistance to pathogens. Plants are exposed to UV-B (280-320 nm) radiation which affects DNA, negatively. Plants protect of themselves from this radiation by producing phenolic compounds (Winkel-Shirly, 2002). Natural antioxidants like phenolic compounds can be used as materials for synthetic antioxidants against oxidative degradation caused by free radicals (Moure et al., 2001).

Flavonoids are given as example for phenolic compounds. Flavonoids, which have high absorption at 250-270 nm and 335-360 nm, act as good UV screens (Carletti et al., 2003). The important flavonoid component is quercetin which is one of the most active antioxidant of medicinal plant. These plants provide to prevent cancer, cardiovascular diseases and asthma (Mohammedi and Atik, 2012). Mohammedi and Atik (2012) investigated the phenolic compound and biological activity of an Endemic Saharan species (*Tomarix paucovulata*) and reported that this plant had strong DPPH radical scavenging activity and phenolics (syringic acid, quercetin, kaempferol, iso-hammetin, iso-quercetin, catechin, epicatechin). Ozcan and Arslan (2011) investigated antioxidant effects of essential oils from rosemary, clove and cinnamon on hazelnut and poppy oils and reported that the essential oils had strong antioxidant effect on crude oils.

Jun et al. (2014) reported that phenolic acids of Canola seed were gallic acid, proto-catechuic acid, caffeic acid, trans-sinapic acid and chlorogenic acid, while p-benzoic acid, ferulic acid and trans-cinnamic acid were not determined. Wang et al. (2009) investigated the effect of UV-C on antioxidant capacity of blueberries and reported that 2.15, 4.30 and 6.45 kj/m^2 dosages caused the increase in antioxidant capacity. Zhau et al. (2012) observed that buckwheat had important effect on DPPH radical scavenging activity due to including rutin and kaempferol. Kim et al. (2008) demonstrated that buckwheat sprouts have higher amount of flavonoids (orientin, iso-orientin, vitexin, isovitexin, rutin, quercetin) compared to buckwheat seeds. Effects of buckwheat on diseases were attributed to its high levels of antioxidant activity and phenolic compounds (Wijngaard and Arent, 2006). Infrared treatment caused the increase in phenolic content of soy (Yalcin and Basman 2016). Yalcin and Schreiner (2018) reported that main phenolics of olive oil was tyrosol and hydroxytyrosol.

In this study, antioxidant activities and phenolic components of leaves of plants, collected from Afyon in 2017, were investigated. These plants were poppy, daisy, dandelion, manger, chickweed, black chicory and white chicory. Antioxidant properties of the leaves of these plants

except poppy, grown in Afyon, have not been reported in literature. So this paper will be first with giving information about the benefits of these leaves.

2. MATERIALS AND METHODS

2. 1. Chemicals

HPLC grade methanol, formic acid, sodium carbonate (purity≥99%), 2,4,6-tri(2-pyridyl)-s-triazine ferric chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (98% purity), 2,2´-azine-bis(3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS) (98% purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (95% purity), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) (98% purity), potassium persulfate, $AlCl_3$, $NaNO_2$, H_2O_2, $CuCl_2$ and $FeSO_4$ were purchased from Merck (Darmstadt, Germany). Folin-Chiocalteu reagent and phenolic standards (p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, ellagic acid, cinnamic acid, vanilic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. 2. Materials

Poppy (*Papaver circulum*), Daisy (*Chamomilce romance*), Dandelion (*Codrilla cuncea*), Manger (*Pabuli herbam*), Chickweed (*Stellaria medra*), Black chicory (*Taraxacum officinalis*) and White chicory (*Chicorium intybus*) were collected from their natural habitats in Afyon in 2017. Leaves of plants were separated from plants and used for analysis. Photos of plants are given in Figure 1.

For sample extraction, 1 g of leaves was extracted by grinding for 1 min at 20000 rpm in a homogenizer ("DAIHAN" WiseTis HG-15D Digital Homogenizer, Korea) with 10 mL of methanol. The homogenate was centrifuged at 3500 rpm for 10 min (DAIHAN Scientific Co., Ltd., WiseSpin® CF-10 Microcentrifuge, Korea). The extract was separated and dried by vacum rotary evaporator (SCILOGEX RE 100-Pro, USA) at 40° C. The dry residues were redissolved before analysis.

Figure 1. Photos of Poppy (a), Daisy (b), Dandelion (c), Manger (d), Chickweed (e), Black chicory (f), White chicory (g).

2. 3. DPPH radical scavenging activity assay

Antioxidant activity of the samples was determined by using the DPPH radical scavenging method (Brand-Williams, et al., 1995). DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in 100% methanol in order to obtain a solution with a concentration of 4.1075 mol/L. The sample extract (400 µL) was added to the DPPH solution (1.6 mL). After incubation in the dark place at room temperature for 30 min, the decrease in absorbance was measured at 517 nm. The DPPH solution (4.1075 mol/L) was used as control for all samples. The DPPH radical scavenging activity was calculated according to the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}}\right) \times 100$$

The determination of antioxidant activity by the DPPH assay is based on the ability of reaction of the DPPH free radical with hydrogen donors. DPPH radical solution is decolorized after reduction with an antioxidant. So difference in color was calculated for determining antioxidant activity (Perez-Jimenez and Saura-Calixto, 2006).

2. 4. ABTS radical cation decolorization assay

ABTS radical cation decolorization of samples was determined according to the study reported by Re et al. (1999) with some modifications. ABTS (1.8 mM) and potassium persulfate (0.63 mM) were mixed and stored in the dark for 24h at room temperature for reaction. This solution was mixed with methanol until absorbance of 0.70 at 732 nm was obtained. Then the mixture (1.98mL) was added to sample extract (20 µL). After 30 min, the absorbance was measured by using of spectrophotometer (Optizen pop, Korea) at 732 nm. A standard curve was prepared by plotting the percentage of free radical scavenging activity of trolox (standard antioxidant) versus its concentration. The ABTS scavenging activity was expressed as µg trolox equivalent per g sample.

The determination of antioxidant activity by the ABTS assay is based on the neutralization of a radical-cation after the one electron oxidation of the synthetic choromophore 2,2´-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS). Antioxidant activity is determined by the change of absorption spectrum after reaction (Perez-Jimenez and Saura-Calixto, 2006).

2. 5. Ferric reducing/antioxidant power (FRAP) assay

The reducing capacity of samples was performed according to the method reported by Benzie and Strain (1996). FRAP value was expressed as µg Fe^{2+} per g of sample.

The determination of antioxidant activity by the FRAP assay is based on the forming of blue color after reaction of 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) with ferric chloride hexahydrate (Perez-Jimenez and Saura-Calixto, 2006).

2. 6. Total phenolic content

Total phenolic content of samples was determined by using Folin-Chiocalteu method (Singleton et al., 1999). Sample extract (300 µL) and Folin-Chiocalteu reagent (750 µL) were mixed and incubated for 5 min. Then 750 µL of Na_2CO_3 (60g/L) was added and the mixture

was incubated in the dark for 90 min at room temperature. The absorbance was measured at 725 nm. Total phenolic content was expressed as μg catechin equivalents per g of sample through the calibration curve of catechin.

2. 7. Total flavonoid content

Total flavonoid content of samples was determined according to the method of Dewanto et al. (2002). Total flavonoid content was expressed as μg catechin equivalents per g of sample.

2. 8. Analysis of phenolic compounds

The dried sample was dissolved in 1 mL of 100% methanol and filtered through 0.45 μm nylon filter. Phenolic compounds of samples were analyzed according to the method of Caponio et al. (1999) by using HPLC (Shimadzu prominence, Japan) equipped with diode array detector (SPD-M20A) and Zorbax Eclipse C18 column (250×4.6 mm, 5 μ).

2. 9. Statistical analysis

Analysis of variance and duncan test were applied to identify difference among means of results by using SPSS.

3. RESULTS AND DISCUSSION

3. 1. DPPH radical scavenging activity of leaves of plants

The DPPH assay determines the ability of antioxidants to scavenge the radical DPPH, which causes a decrease in the absorbance at 517 nm. DPPH radical scavenging activity of leaves is presented in Table 1. Significant differences were found among DPPH radical scavenging activities of leaves. DPPH radical scavenging activities of leaves ranged from 62.4% to 82.4%. Leaves of Daisy and Manger had significantly higher DPPH radical scavenging activities compared to that of other plants. The lowest DPPH radical scavenging activity was obtained for poppy leaves. DPPH radical scavenging activities of Daisy leaves and Manger leaves were statistically similar. These results are corresponding with the results of the study reported by Zhou et al. (2005). Li et al. (2009) reported that DPPH radical scavenging activities of berry fruits ranged from 29.97% to 78.86%. Bunea et al. (2011) reported that DPPH radical scavenging activity of blueberries (wild and cultivated) ranged from 29.96% to 59.79% of inhibition. Reddy et al. (2010) investigated antioxidant activity of Indian fruits and reported that DPPH scavenging activity of fruits ranged from 32 to 891 mg trolox equivalent/100 g.

3. 2. ABTS radical cation decolorization of leaves of plants

The ABTS assay determines the ability of antioxidants to scavenge the radical cation ABTS, which causes a decrease in the absorbance at 732 nm. ABTS radical scavenging activity of leaves is presented in Table 1. Significant differences were found among ABTS cation decolorization of leaves. ABTS cation decolorization of leaves ranged from 5989.6 μg trolox/g (Manger) to 9277.2 μg trolox/g (Dandelion). ABTS radical cation decolorization of poppy leaves was statistically similar to that of Dandelion leaves. The results were higher than the results of Canola seed (2500μg/g) reported by Jun et al. (2014). Garzon et al. (2010) reported that the ABTS radical cation decolorization of Colombian wild bilberry was 45.5 μmol trolox

equivalents/g. Bunea et al. (2011) reported that ABTS radical cation decolorization of Romanian blueberries (wild and cultivated) ranged from 24.33 to 56.65 μmol trolox equivalent/g. Almeida et al. (2011) reported that antioxidant activity (ABTS) of 11 Brazillian fruits ranged from 0.99 to 15.73 μM/g.

3. 3. FRAP ferric reducing power of leaves of plants

FRAP assay is different from ABTS and DPPH assays. Because FRAP assay is based on reducing ability, while ABTS and DPPH assays are based on free radical scavenging capacity. The FRAP assay determines the ability of antioxidants to reduce ferric tripyridyl triazine complex to its ferrous form (colored form), which causes an increase in the absorbance at 595 nm. Reducing power of leaves is given in Table 1. Significant differences were found among reducing powers of leaves. Ferric reducing power of leaves ranged from 4726.5 μgFe^{2+} /g (Manger) to 14558.5 Fe^{2+} (white chicory) μg /g. There are some examples to FRAP of fruits in literature. Garzon et al. (2010) reported that the ferric reducing antioxidant potential (FRAP) value of Colombian wild bilberry was 87 μmol trolox equivalent/g or 116.0 μmol ferric iron reduced/g. Bunea et al. (2011) reported that FRAP of Romanian blueberries (wild and cultivated) ranged from 33.03 to 73.71 μM Fe^{2+} /g.

Table 1. Antioxidant activities of leaves of plants.

Samples	DPPH (%)	ABTS (μg/g)	FRAP (μg Fe^{2+}/g)
Poppy	62.4 ±0.76e	9277.2 ±36.62a	8125.5 ±34.65e
Daisy	81.0 ±1.00a	8026.2 ±56.29d	5935.9 ±54.07f
Dandelion	77.2 ±0.28b	9237.9 ±59.88a	13091.1 ±58.67b
Manger	82.4 ±1.15a	5989.6 ±54.14f	4726.5 ±55.32g
Chickweed	69.8 ±0.56c	8655.8 ±62.17c	9275.3 ±59.89d
Black chicory	68.2 ±0.01d	9189.7 ±3.53b	9937.3 ±2.06c
White chicory	76.5 ±0.40b	7193.5 ±41.14e	14558.5 ±50.26a

*Values followed by the same letter in the same column are not significantly different ($p<0.05$)
± Standard deviation

3. 4. Total phenolic content (TPC) of leaves of plants

Total phenolic content of leaves is presented in Table 2. Significant differences were found among total phenolic contents of leaves. Total phenolic contents of leaves ranged from 1664.6 μg catechin equivalent/g sample (Manger) to 4214.2 μg catechin equivalent/g sample (White chicory). Total phenolic content of Poppy leaves was statistically similar to that of Daisy leaves and Chickweed leaves. The results were higher than the results of fruits reported by Almeida et al. (2011). Almeida et al. (2011) investigated antioxidant properties of 11 fruits and

reported that total phenolic content of fruits ranged from 13.5 to 159.9 mg gallic acid equivalent/100g. Li et al. (2009) reported that total phenolic content of berry fruits ranged from 22.83 to 131.88 g/kg. Garzón (2010) reported that total phenolic content of Colombian wild bilberry was 758.6 mg gallic acid equivalent/100g. Bunea et al. (2011) reported that total polyphenols of blueberries (wild and cultivated) ranged from 424.84 to 819.12 mg gallic acid equivalent /100 g.

3. 5. Total flavonoid content of leaves of plants

Total flavonoid content of leaves is presented in Table 2. Significant differences were found among total flavonoid contents of leaves. Total flavonoid content of leaves ranged from 1125.5 µg catechin equivalent/g sample (Daisy) to 3818.4 µg catechin equivalent/g sample (White Chicory). The results were higher than the results reported by Bunea et al. (2011). Bunea et al. (2011) reported that total flavonoid content of blueberry varieties was in the range of 84.33-1125 µg quercetin equivalent/g.

Table 2. Total phenolic content (TPC) and total flavonoid content (TFC) of leaves of plants.

Samples	TPC (µg/g)	TFC (µg/g)
Poppy	2612.5±29.99de	1349.0±27.80f
Daisy	2425.2±56.10e	1125.5±54.18g
Dandelion	3767.4±47.81b	3566.1±30.28b
Manger	1654.6±42.62f	872.7±23.60h
Chickweed	2714.6±52.50d	1613.8±49.79e
Black chicory	3134.1±1.40c	2377.3±16.57d
White chicory	4214.2±42.80a	3818.4±53.22a

*Values followed by the same letter in the same column are not significantly different ($p<0.05$) ± Standard deviation

3. 6. Phenolic compounds of leaves of plants

Phenolics compounds of leaves are given in Table 3 and Table 4. LOD, wavelength and retention times of phenolic compounds are given in Table 5. Gallic acid, ferulic acid, chlorogenic acid, cumarric acid, ellagic acid, vanilic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid of leaves ranged between 0.02 (Dandelion) – 0.90 (Daisy) µg/g, 0.01 (Poppy, White chicory) - 0.59 (Chickweed) µg/g, 0.02 (Poppy) - 14.59 (Manger) µg/g, 0.33 (Chickweed) - 11.19 (Dandelion) µg/g, 0.48 (Daisy) -4.73 (Manger) µg/g, 0.22 (Dandelion) - 25.43 (White chicory) µg/g, 0.74 (Poppy) - 94.47 (Black chicory) µg/g, 0.13 (Dandelion, Manger) - 1.35 (Daisy) µg/g, 0.07 (Poppy) - 17.01 (Black chicory) µg/g and 0.24 (Manger) - 10.48 (Poppy) µg/g, respectively. Gallic acid was not

determined in Manger, Black chicory and White chicory, while ferulic acid was not determined in Dandelion. Catechin, apigenin, naringin, rutin and quercetin of leaves ranged from 0.19 (Daisy) to 3.42 (Chickweed) µg/g, from 0.09 (Manger) to 6.31 (Daisy) µg/g, from 0.04 (Poppy) to 0.30 (Black chicory) µg/g, from 0.07 (Poppy, White chicory) to 7.47 (Daisy) µg/g and from 0.08 (Chickweed, Black chicory, White chicory) to 3.26 (Daisy) µg/g, respectively. Naringin was not determined in Daisy, Dandelion, Manger, Chickweed and White chicory. Jun (2014) reported that phenolic acids found in Canola seed were gallic acid (10.4 mg/g), protocatechic acid (4.8 mg/g), caffeic acid (0.1 mg/g), trans-sinapic acid (41.5 mg/g) and chlorogenic acid (2.5 mg/g), while p-hydroxybenzoic acid, ferulic acid and trans-cinnamic acid were not determined. According to Mohammedi and Atik (2012), an Endemic Saharan species (*Tamorix pauciovulatal*) had syringic acid (1.07 mg/100 g), quercetin (34.1 mg/100 g), kaempherol (5.77 mg/100 g) and isohammetin (5 mg/100 g). Zhou et al. (2005) observed that p-hydroxybenzoic acid, vanillic acid, syringic acid, coumaric acid and ferulic acid contents of wheat brans ranged between 8.89-19.98 µg/g, 14.45-33.11 µg/g, 36.45-55.70 µg/g, 5.81-8.60 µg/g and 130.06-146.38 µg/g, respectively. Li et al. (2009) reported that the highest level of caffeic acid, gallic acid and trans-cinnamic acid were found in chokecherry (6455 mg/kg), raspberry (1129mg/kg) and strawberry (566mg/kg).

Table 3. Phenolic acids of plant leaves (µg/g).

Samples	Gallic acid	Perulic acid	Chlorogenic acid	Coumaric acid	Ellagic acid
Poppy	0.63±0.09b	0.01±0.00 d	0.020.00 g	4.43±0.13 c	2.22±0.07 d
Daisy	0.90±0.03a	0.19±0.02 b	4.72±0.17 b	1.88±0.09 d	0.48±0.03 g
Dandelion	0.02±0.00d	n.d.	0.14±0.03e	11.19±0.37 a	3.54±0.09 b
Manger	n.d.	0.09±0.01c	14.59±0.35 a	6.15±0.25 b	4.73±0.13 a
Chickweed	0.27±0.07c	0.59±0.09a	1.83±0.13 d	0.33±0.07 e	1.91±0.15 e
Black chicory	n.d.	0.06±0.01c	1.31±0.05d	6.92±0.13 b	2.79 ±0.17c
White chicory	n.d.	0.01±0.00 d	2.28±0.15 c	6.75±0.15 b	1.26±0.15 f

Table 3(continue). Phenolic acids of plant leaves (µg/g).

Samples	Vanilic acid	Caffeic acid	Cinnamic acid	4-hydroxy benzoic acid	2,5-dihydroxy benzoic acid
Poppy	8.94±0.15b	0.74±0.09f	0.24±0.07c	0.07±0.01f	10.48±0.43a
Daisy	1.35±0.05d	11.99±0.30c	1.35±0.09a	4.49±0.15c	0.48±0.09de

Dandelion	0.22±0.03e	3.87±0.13e	0.13±0.03d	9.60±0.37b	0.74±0.13d
Manger	3.51±0.10c	13.82±0.39b	0.13±0.02d	1.85±0.13d	0.24±0.07e
Chickweed	1.44±0.09d	5.17±0.20d	0.32±0.03b	1.16±0.09e	3.2±0.17c
Black chicory	9.44±0.13b	94.47±0.50a	1.25±0.13a	17.01±0.40a	5.83±0.39b
White chicory	25.43±0.39a	3.61±0.13e	0.15±0.01d	8.97±0.31b	0.91±0.07d

*Values followed by the same letter in the same column are not significantly different ($p<0.05$) ± Standard deviation

Table 4. Flavonoids of leaves of plants (μg/g).

Samples	Catechin	Apigenin	Naringin	Rutin	Quercetin
Poppy	2.40±0.10b	3.27±0.15b	0.04±0.01b	0.07±0.01e	0.11±0.03b
Daisy	0.19±0.03e	6.31±0.20a	n.d.	7.47±0.31a	3.26±0.17a
Dandelion	3.29±0.11a	0.67±0.09d	n.d.	0.26±0.03d	0.10±0.03b
Manger	0.96±0.09d	0.09±0.01e	n.d.	2.70±0.13b	0.09±0.01b
Chickweed	3.42±0.13a	0.48±0.07d	n.d.	0.12±0.01e	0.08±0.01b
Black chicory	1.69±0.07c	1.29±0.09c	0.30±0.09a	1.10±0.09c	0.08±0.01b
White chicory	1.25±0.05d	1.52±0.13c	n.d.	0.07±0.01e	0.08±0.02b

*Values followed by the same letter in the same column are not significantly different ($p<0.05$) ± Standard deviation

3. 7. Correlation

Correlation (R) between the total phenolic content and FRAP values of leaves were high (0.96). Reddy et al. (2010) observed correlation between the DPPH and ABTS (R=0.94). Almeida et al. (2011) observed that the correlation between ABTS and DPPH assays of the 11 Brazillian fruits was positively high (R=0.92), indicating that samples had comparable activities in two assays. Correlation between ABTS and TPC assays of the 11 Brazillian fruits was 0.94 (R), while relation between DPPH and TPC assays was 0.88.

4. CONCLUSIONS

In this study, healthy components of plants, grown in Afyon, were determined to research of the using of them as medicinal. These plants were Poppy, Daisy, Dandelion, Manger,

Chickweed, Black chicory and White chicory. Antioxidant activities (DPPH, ABTS, FRAP), total phenolic content, total flavonoid content and phenolic compounds of leaves of eight plants were analyzed. All leaves had high antioxidant activities, high phenolics and high flavonoids. Daisy and Manger leaves had the highest DPPH value, while poppy and dandelion had highest ABTS value. Leaves of White chicory had the highest FRAP value, total phenolic content and total flavonoid content among leaves of plants. All plants leaves can be used as sources of natural antioxidants especially leaves of White chicory.

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