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CHEMICAL CHARACTERIZATION OF *CICHORIUM INTYBUS AND PORTULACA OLERACEA* VARIETY GROWN IN EGYPT

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ABSTRACT: The present study was designed to determine the phytochemical constituents of *Cichorium intybus and Portulaca oleracea* and evaluate the antioxidant activity of their methanolic extract. It was found that *Cichorium intybus and Portulaca oleracea* contained total ash 6.08 and 7.12%, crude lipid 0.63 and 0.54%, crude protein 3.94 and 4.09%, crude fiber 9.0 and 8.34%, and total carbohydrates 80.33 and 79.91%, respectively. Total phenolics in the *Cichorium intybus* were 172.9 mg/g while total flavonoids were 139.4 mg/g. On the other hand, total phenolics and total flavonoids were 163.12 and 125.71 mg/g for *Portulaca oleracea.* HPLC analysis of *Cichorium intybus* and *Portulaca oleracea* showed the presence of 17 and 20 phenolic compounds, respectively. catechin, caffeic acid, and rutin acid were found in high levels in the *Cichorium intybus* and their amounts were 2926.598, 1268.448, and 509.948 mg/100g dw respectively. On the other hand, ferulic acid, caffeic acid, *p*-hydroxybenzoic acid and catechin were found to be in high levels in *Portulaca oleracea* and their amounts, which were 300.628, 203.678, 55.685 and 38.424 mg/100g dry weight, respectively. The obtained results also showed that the methanolic extract of Egyptian *Cichorium intybus* has the highest antioxidant activity, this may be due to their high amount of total phenolics and flavonoids.

***Key words*: *Cichorium intybus* – *Portulaca oleracea* – Phenolics – Flavonoids – antioxidant**

INTRODUCTION

Human has a long history of consuming edible plants for food and survival and now still consumes a wide variety of wild and semi-domesticated food plants, domesticated crops, vegetables, fruits, and plant food supplements, as well as plants for medicinal uses (Ju, et al., 2013 and Lau et al., 2009). There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future cases (Singh, 2015)

Cichorium intybus* commonly known as chicory belongs to the family *Asteraceae* and is widely distributed in North Africa to south Asia for several 100 years (Jamshidzadeha et al., 2006). All parts of this plant possess great medicinal importance due to the presence of several medicinally important compounds such as alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, and flavonoids. (Meehye and Shin, 1996). Leaves of chicory are good sources of phenols, vitamins (A and C) as well as potassium, calcium, and phosphorus (Mulabagal et al., 2009). Furthermore, chicory is rich cichoric acid and may stimulate the immune system as well as prevent inflammation and bacterial infections to a limited extent (Nayeemunnisa, 2009). *Cichorium intybus

has been traditionally used for the treatment of fever, diarrhea, jaundice, and gallstones. The studies on rats have shown that *Cichorium intybus* possesses anti-hepatotoxic and anti-diabetic activities (Katiyar, *et al*., 2015).

Portulaca oleracea (*Portulacaceae* family) is a well-known edible plant, which is widespread in temperate and tropical regions of the world. Many types of chemical compound are present in this plant, including unsaturated fatty acids, monoterpenoids, alkaloids, coumarins and flavonoids (Xiang *et al.,* 2006).

Recently, the development of flavonoids in medical and food fields is becoming a hot research. Flavonoids and polysaccharides are the most abundant effective components in *Portulaca oleracea*. Seven kinds of flavonoids were found in *Portulaca oleracea* including quercetin, kaempferol, myricetin, apigenin, luteolin, genistein, and genistein. The extraction and separation of flavonoids with high biological activity and high content is very important for the medical and food industry. Therefore, it is of great significance to study the extraction and the determination technologies of flavonoids from *Portulaca oleracea* (Mahmood, et al., 2020).

MATERIALS AND METHODS

1. Plant material

Egyptian *Cichorium intybus* and *Portulaca oleracea* were collected from local field in Menoufia and the leaves of plants were identified by botanical members of the Department of Botany, Faculty of Agriculture, Menoufia University. The leaves were washed and air-dried for 24 hours, then dried at 50 °C to a constant weight. The dried samples were grinded into fine powder and kept in refrigerator for analysis.

2. Determination of the chemical composition

Ash content was determined by ignition of the dried sample at 550 °C until a constant weight according to the Association of Official Analytical Chemists, (AOAC, 2000). Crude fiber was determined according to AOAC, (2000). Total nitrogen was determined (dry basis) according to the modified micro-Kjeldahl method as described by AOAC., (2000). The crude protein contents were calculated using the conversion factor 6.25. Crude lipid was determined according to AOAC., (2000). The total carbohydrate determined by difference = 100 – (% protein + % fat + % ash + % fiber).

3. Preparation of plant extracts

Dried powdered leaves, (30 g) from each plant were extracted by methanol 80% at room temperature for 24h. The resulting extracts were filtered using Whatman no. 1 filter paper and the residues were re-extracted by the same process until plant materials were exhausted. The collected filtrates were pooled and evaporated to dryness under reduced pressure to give a semisolid residue, which was then lyophilized to get powder and were stored at 4 °C until used.

4. Determination of total phenolic compounds

The phenolic level was determined by the modified Folin-Ciocalteu assay, as described by McDonald *et al.*, (2001). One milliliter of extract and 5 mL of 10% Folin-Ciocalteu reagent were mixed, and then 4 mL of 2% Na_2CO_3 was added. Reagent without extract (only absolute methanol) was used as a blank. After incubating all samples at room temperature for 60 min, their absorbance was measured at

765 nm using spectrophotometer. The calibration curve for gallic acid (0–100 µg/mL) was established to calculate the phenolic content. Total phenolics (TPC) were expressed as the mg gallic acid equivalent (GAE) per gram of extract.

5. Determination of total flavonoid compounds

The total flavonoid contents were determined using the method described by Dewanto, *et al*., (2002). Briefly, an aliquot (250 µl) of each extract or a standard solution was mixed with 1.25 ml of deionized water followed by 75 µl of a 5% NaNO2 solution. After 6 min, 150 µl of a 10% AlCl3. 6H2O solution were added to each mixture. After 5 min, 0.5 ml of 1 M NaOH was added, and the total volume was adjusted to 3.0 ml with deionized water. (+)-Catechin was used as a standard and the absorbance was measured at 510 nm, using a spectrophotometer which was corrected using a blank. The results were expressed as mg of (+)-catechin equivalents (CE)/100 g dry matter.

6. Quantitative identification of phenolics by HPLC

A modified method of (Zuo, *et al.,* 2002) analysis was carried out using HPLC (A shimazdzu lC 20 AT) equipped with a Luna TM 5 µm C18, 25 cm x 4.6 mm i.d (Phenomenex, Torrance, CA, USA) column. S.PD-20 UV Visible detector was used. A gradient elution was carried out using the following solvent systems: Mobile phase A (acetonitrile / acetic acid/double distilled water- 9/2/89 v/v/v), Mobile phase B (acetonitrile/acetic acid/double distilled water - 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition was started at 100% solvent A for 10 min then over 15 minutes a linear gradient to 60% mobile phase A, 32% mobile phase B and held at this composition for 10 min. The flow rate of the mobile phase was 1 ml/min and the temperature at the column performed at 35 ± 0.5 °C. The identification of individual components was carried out by comparing the retention times and UV-absorbance of unknown peaks with peaks obtained from the mixed known standard under the same condition.

7. *In vitro* antioxidant activity reducing power assay

A spectrophotometric method (Oyaizu 1986) was used for the measurement of reducing power. An aliquot (2.5 ml) of each extract was mixed with 2.5 ml of 200 mmol/l sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After adding 2.5 ml of 10% trichloroacetic acid (w/v), the mixture was centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with5 ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was then measured at 700 nm. Higher absorbance indicates higher reducing power. Vit.C was used as standard.

RESULTS AND DISCUSSION.

1. Chemical composition of *Cichorium intybus* and *Portulaca oleracea*

Table (1) indicate that *Cichorium intybus* contained total ash 6.08 %, crude lipid 0.63 %, crude protein 3.94 %, crude fiber 9.0 %, and total carbohydrates 80.33 %. On the other hand, *Portulaca oleracea* contained total ash 7.12 %, crude lipid 0.54 %, crude protein 4.09 %, crude fiber 8.34 %, and total carbohydrates 79.91 %. The results are in accordance with those of Jancic, (2016), and Muhammad, *et al*., (2017) referring that *Cichorium intybus* chemical composition contains, total ash 6.9%, crude lipids 0.44 %, crude protein

3.91 %, crude fiber 8.88 % and total carbohydrates 80.48 %. while Ifeoma, *et al*., (2017) reported that *Cichorium intybus* had chemical composition of total ash 10.91%, crude lipids 3.68 %, crude protein 14.7 %, crude fiber 16.7 % and total carbohydrates 70.7 %.

Mastud, *et al*., (2018) showed that the *Portulaca oleracea* powder was a good source of crude fiber (4.5±0.01%) and protein (16.38±0.05%) and added that *Portulaca oleracea* contained 18.20±0.50% ash, 2.33±0.70% fat, and 58.89% carbohydrate, while El-Hadidy, (2014) and Obied *et al*., (2016) showed that *Portulaca oleracea* contained 17.50-29.04% protein, 5.00- 12.00% crude fibers, 17.80-23.01% ash.

2. Total phenolic compounds and total flavonoids of *Cichorium intybus* and *Portulaca oleracea* extract.

Plant polyphenols are a major group of compounds acting as primary antioxidants or free radical scavengers. Therefore, it was reasonable to determine the total phenolic and flavonoid contents in the plant extracts. The results obtained shown in Table (2) indicates that the total phenolics in the *Cichorium intybus* were 172.9 mg/g GAE equivalent, while total flavonoids were 139.4 mg/g catechin equivalent. On the other hand, total phenolics and total flavonoids were 163.12 mg/g and 125.71 mg/g for *Portulaca oleracea*.

These data agree with Nasir *et al*., (2012) who reported that total phenolics in the *Cichorium intybus* extract were 285 mg/g while total flavonoids were 150 mg/g. On the other hand, Mahmood Habibian *et al*., (2020) reported that total phenolics in the *Portulaca oleracea* extract was 142 mg/g while total flavonoids were 81.2 mg/g.

3. Quantitative analysis of phenolic compounds in *Cichorium intybus* and *Portulaca oleracea* by HPLC

Polyphenolic compounds in *Cichorium intybus* and *Portulaca oleracea* were analyzed by High Performance Liquid Chromatography (HPLC), and the concentrations of all tested phenolic compounds were given in Table (3).

Table (1): The chemical composition of *Cichorium intybus* and *Portulaca oleracea* (W/W %) on dry weight basis.

Plants name / Components	*Cichorium intybus*	*Portulaca oleracea*
Ash	6.08	7.12
crude lipid	0.63	0.54
Total Protein	3.94	4.09
Crude Fiber	9.0	8.34
Total carbohydrate	80.33	79.91

Table (2): Total phenolic and total flavonoids contents of *Cichorium intybus* and *Portulaca oleracea.*

Plants	Total phenolics (mg/g) dry weight	Total flavonoids (mg/g) dry weight
Cichorium intybus	172.9	139.4
Portulaca oleracea	163.12	125.71

Table (3): HPLC analysis of phenolic compounds in *Cichorium intybus* and *Portulaca oleracea.*

No	Phenolic compounds	*Cichorium intybus* (mg / 100g dry weight)	*Portulaca oleracea* (mg / 100g dry weight)
1	Gallic acid	21.407	27.633
2	Protocatechuic	47.778	15.895
3	*p*-hydroxybenzoic acid	7.462	55.685
4	Gentisic acid	18.890	2.610
5	Catechin	2926.598	38.424
6	Chlorogenic acid	1.403	1.578
7	Caffeic acid	1268.448	203.672
8	Syringic acid	2.062	14.891
9	Vanillic acid	17.228	20.424
10	Scopoletin	0.000	0.000
11	Ferulic acid	90.918	300.628
12	Sinapic acid	9.857	27.839
13	*p*-counmaric acid	0.000	16.887
14	Rutin	509.948	24.205
15	Naringenin	0.000	6.732
16	Apigenin-7-glucoside	27.697	13.576
17	Rosmarinic acid	3.469	0.000
18	Cinnamic acid	2.205	0.501
19	Luteolin	0.605	5.296
20	Apigenin	0.000	6.095
21	Kaempferol	0.000	0.906
22	Chrysin	10.559	1.882

HPLC analysis for the phenolic compounds showed the presence of 17 compounds which were varied in their amounts. It was observed that catechin, caffeic acid, and rutin acid were found in high levels and their amounts were 2926.598, 1268.448, and 509.948 (mg/100g dw) respectively. While ferulic, (90.918) protocatechuic (47.778), apigenin-7-glucoside (27.697), gallic acid (21.407), gentisic acid (18.890) and vanillic acid (17.228), were found to be in moderate amounts.

Chrysin (in mg/100g dw) (10.559), sinapic acid (9.857), phydroxybenzoic acid (7.462), rosmarinic acid (3.469), cinnamic acid (2.205), syringic acid (2.062), chlorogenic acid (1.403) and luteolin (0.605) were found to be in low amounts in the methanolic *Cichorium intybus* extract. These data agree with those of Ifeoma, *et al.,* (2017) and Rezvan, *et al.,* (2018).

While the HPLC analysis of *Portulaca oleracea* for the phenolic compounds showed the presence of 20 compounds which were varied in their amounts. It was observed that ferulic acid, caffeic acid, *p*hydroxybenzoic acid and catechin were found to be in high levels and their amounts which were 300.628, 203.678,

55.685 and 38.424 mg/100g dry weight respectively. While (in mg/100g dw) sinapic acid (27.939), gallic acid (27.633), rutin (24.205), vanillic acid (20.424), *p*-coumaric acid (16.887), protocatechuic (15.895), syringic acid (14.891) and apigenin-7-glucoside (13.576), were found in moderate amounts.

Naringenin (6.732), apigenin (6.095), luteolin (5.296), gentisic acid (2.610), chrysin (1.882), chlorogenic acid (1.578), kaempferol (0.906) and cinnamic acid (0.501) were found to be in low amounts (in mg/100g dw) in the methanolic *Portulaca oleracea* extract. These data agree with those of Erkan, *et al*., (2012) who reported that *Portulaca oleracea* is a rich source in phenolic compounds.

4. Reducing power activity for methanolic extract of *Cichorium intybus* and *Portulaca oleracea*

The methanolic leaf extract of *Cichorium intybus* and *Portulaca oleracea* have been reported to exhibit antioxidant activity both *in vitro* and *in vivo* in numerous studies due to their high amount of polyphenols such as, phenolics and flavonoids contents (Erkan 2012; Oliveira *et al.,* 2009).

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism in phenolic antioxidant action (Nabavi *et al.,* 2009). In this work, the presence of reductants (antioxidants) in the samples would result in the reduction of Fe^{+3} to Fe^{+2} by donating an electron.

Data in Fig (1) showed that the reducing power of *Cichorium intybus* extracts (2.5% and 5% concentration), which were (94.3 and 219.3 mMol Ascorbic Eq), while such parameter for 2.5% and 5% *Portulaca oleracea* extracts were (81.2 and 198.3 mMol Ascorbic Eq). These results are in accordance with those of Shad et al., 2013; Oliveira et al., 2009 who found that reducing power was (72.37 ± 7.26 mMol Ascorbic Eq) for *Cichorium intybus* extract and it was (70.3 ± 5.7 mMol Ascorbic Eq) for *Portulaca oleracea* extract.

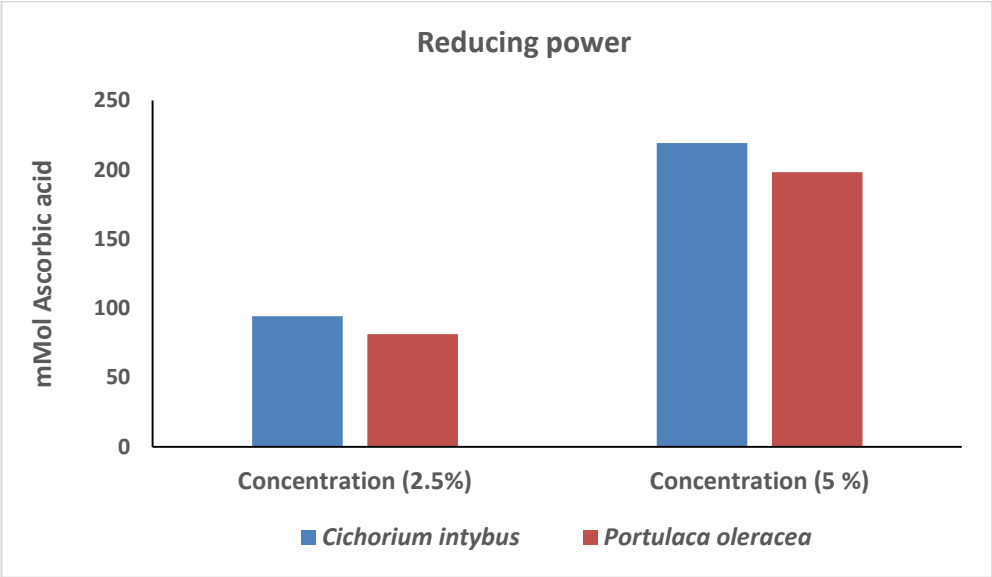

Fig (1) Reducing power activity for *Cichorium intybus* and *Portulaca oleracea* extracts

The data also revealed that the reducing power of the leaf extract of *Cichorium intybus* was better than the methanolic leaf extract of *Portulaca oleracea.* In plants, reducing power assay has been considered as one of the most important indicators of antioxidant activity (Umamaheswari and, Chatterjee, 2008). The presence of reductants in the plant extract is commonly correlated with its reducing capabilities as reductants have shown the antioxidant action by terminating the radical chain reaction as they donate hydrogen atom in the reaction mixture. It has been observed that the total reduction ability of converting Fe3+ to Fe2+ in presence of both the extracts increased in a concentration dependent manner suggesting that both the extracts donates an electron in the reaction mixture which reacted with free radicals breaking down the chain reaction and transform them into much stable non-radical products.

Conclusion

The results of our study suggest that the methanolic leaf extracts of *Cichorium intybus* and *Portulaca oleracea* cultivated in Egypt are rich in polyphenolics and flavonoids compounds. The leaf extracts of these plants can be used as natural sources of antioxidants to prevent the progression of many diseases. The total phenolics and total flavonoids contents in the *Cichorium intybus* methanolic extract were higher than that in the *Portulaca oleracea* extract, and its methanolic extract was found to have marked *in-vitro* antioxidant activity due to their high amount of total phenolics and flavonoids, that justifies its use in traditional system of medicine in Egypt.

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الخصائص الكيميائية لنباتات الشكوريا والرجله المزروعة في مصر

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الملخص العربى

صممت الدراسة الحالية لتحديد المكونات الكيميائية لنباتى الشكوريا و الرجلة وتقييم النشاط المضاد للأكسدة للمستخلص الميثانولى للنباتين . وجد أن نباتي الشكوريا و الرجلة يحتويان على إجمالي الرماد 6,08 و 7,12٪ ، والدهون الخام 0,63 و 0,54٪ ، والبروتين الخام 3,94 و 4,09٪ ، والألياف الخام 9,0 و 8,34٪ ، والكربوهيدرات الكلية 80,33 و 79,91٪ على التوالي. وقد كان إجمالي الفينولات في نبات الشكوريا 172,9مجم / جم بينما بلغ إجمالي مركبات الفلافونويد 139.4 مجم / جم ، من ناحية أخرى كان إجمالي الفينولات وإجمالي مركبات الفلافونويد 163,12 مجم / جم و 125,71 مجم / جم في نبات الرجلة . أظهر التحليل الكروماتوجرفى لنباتى الشكوريا و الرجلة عن وجود 17 و 20 مركبًا من المركبات الفينولية على التوالي. لوحظ أن الكاتشين وحمض الكافيك وحمض روتين وجدت بمستويات عالية في الشكوريا وكانت مقاديرها 2926,598 و 1268,448 و 509,948 ملغم / 100 جم وزن جاف على التوالي. من ناحية أخرى ، لوحظ في نبات الرجلة أن حمض الفيروليك وحمض الكافيين و حمض البتاهيدروكسي بنزويك و الكاتشين كانت بمستويات عالية وكانت مقاديرها 300,628 و 203,678 و 55,685 و 38,424 ملجم / 100 جم وزن جاف على التوالي . كما أوضحت النتائج أن المستخلص الميثانولي لنبات الشكوريا المصري له نشاط مضاد للأكسدة عال، وقد يرجع ذلك إلى ارتفاع كمية الفينولات والفلافونويد.

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