Antioxidant Properties of the Aerial Part of Celery and Flaxseeds

Naouel OUIS[1] (✉)
Ahmed HARIRI[2]

Summary

The aim of the present study was to evaluate the antioxidant activity of essential oil and extracts obtained from the aerial parts of celery (*Apium graveolens* L.) and from flaxseeds (*Linum usitatissimum* (Linn.)). *In vitro* antioxidant activity was determined by 2,2-diphenyl-2-picryl-hydrazyl (DPPH) and ferric reducing power assay. Results showed that the essential oil of celery and methanol extracts of celery and flaxseeds exhibited a good scavenging activity of DPPH radical respectively 84 ± 0.4%, 79 ± 0.5%, and 78 ± 0.3% at concentration of 1000 µg mL^{-1} comapred to the queous extracts. These results were lower than those found with ascorbic acid 96.23 ± 1.2% and catechin 94.50 ± 1.4% at the same concentration. IC_{50} is defined as concentration of substrate that inhibits 50% of the DPPH radicals present in the reaction medium. The positive control catechin and ascorbic acid displayed lower values of IC_{50} (7.81 ± 0.1, 31.5 ± 0.3 µg mL^{-1}), followed by methanol extract of celery and flaxseeds (130 ± 0.2, 150 ± 0.4 µg mL^{-1}), essential oil of celery (180 ± 0.2 µg mL^{-1}), then aqueous extracts of flaxseeds and celery (950 ± 0.5, 980 ± 0.4 µg mL^{-1}). For aerial part of celery, significant activities for reducing iron were obtained, values observed by optical density (OD) of 1.8 ± 0.2 for essential oil and 1.7 ± 0.1 for methanol extract, while ascorbic acid and catechin provided an OD of 2.069 ± 0.03 and 2.66 ± 0.016 in the same concentration 1000 µg mL^{-1}. The results of the current study showed that flaxseeds and celery exhibited a higher antioxidant activities that could be exploited in food and pharmaceutical industries.

Key words

antioxidant activity, celery, DPPH, essential oil, extracts, ferric reducing power, flaxseeds

[1] Laboratory of Physical Chemistry of Macromolecules and Biological Interfaces, University Mustapha Stambouli of Mascara (UN 2901), BP. 763, Sidi Said, Mascara, 29000, Algeria

[2] Bioconversion Laboratory, Microbiology Engineering and Health Safety, University Mustapha Stambouli of Mascara (UN 2901), BP. 763, Sidi Said, Mascara, 29000, Algeria

✉ Corresponding author: naouel.ouis@univ-mascara.dz

Received: June 2, 2021 | Accepted: May 15, 2023 | Online first version published: July 20, 2023

Introduction

Plants are real source of natural and medicinal bioactive products once serving as the source of all drugs (Balandrin et al., 1993). Actually, the use of aromatic and medicinal plants in herbal medicine has been developed intensively by exploiting different herbs, fruits and legumes. Oxidative stress has been hypothesized to play a role in the pathophysiology of depression and anxiety disorders and is closely linked to the immune-inflammatory system. Oxidative stress occurs when redox homoeostasis within the cell is altered. Many research projects were focused on naturally bioactive products that could preserve human health from oxidative stress damage caused by reactive oxygen species (Rosenfeldt et al., 2013). The imbalance between ROS and reducing agents may lead to chemical modification of biologically relevant macromolecules (DNA, carbohydrates, proteins or lipids). These patho-biochemical mechanisms cause the development of different degenerative diseases including cardiovascular illness, diabetes, cancer, atherosclerosis, neurodegenerative disorders and arthritis (Adebayo et al., 2014).

Antioxidants are vital agents that possess the ability to protect the body from damage caused by free radical induced oxidative stress and retard the progress of several chronic diseases (Yamuna and Padma, 2013). Actually, the possible toxicity of synthetic antioxidant has been criticized. The commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are restricted because they are suspected to have some toxic effects and as possible carcinogens (Imaida et al., 1983). Hence, the studies on natural antioxidants have gained increasingly greater importance. Many antioxidant substances, naturally occurring in plant source have been identified as potential free radical or active oxygen scavengers (Duh, 1998). Due to its richness in various bioactive compounds, plants have been used for food preservation, pharmaceutical, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997). It has long been acknowledged that some plant essential oils exhibit antioxidant activity and it is necessary to investigate those plants scientifically (Al-Bayati, 2008).

Linum usitatissimum (Linn.), commonly known as flaxseed or linseed belongs to the family Linaceae. It has been consumed as a food ingredient (Ghule et al., 2012) and plays an important role in the field of diet and disease research due to its potential health benefits associated with linolenic acid 57% and a major lignin, namely ecoisolariciresinol diglucoside SDG (Milder et al., 2005). Several studies indicate the potential of flaxseed as antidiabetic (Prasad et al., 2000) and cardioprotective effect (Zanwar et al., 2011), but very few studies evaluate antioxidant activity of flaxseed and its association with ROS production.

Celery (*Apium graveolens* L.) belonging to the Apiaceae family is a hepaxanthic herb valuable in the diets, where it provides low-calories fiber bulk (Njoku Ugochi et al., 2011). Celery, an ancient vegetable spice cultivated for the last 3000 years, has been found to have both nutritional and pharmacological activities (Chevallier, 1998). This plant contains a lot of important pharmacological substances (Momin et al., 2000). The oil and large doses of the seeds should be avoided during pregnancy, as they can act as a uterine stimulant. The characteristic odor of celery essential oil is due to a series of phthalide derivatives (Jung et al., 2011). Sedanolide, sedanonic anhydride, 3-n-butyl phthalide, and other minor phthalides are reported to be the major components of celery seed oil (Lund, 1978). Celery seeds possess a characteristic aroma and pungent taste can be used as seasoning in cocktails (Njoku Ugochi et al., 2011), and to treat bronchitis, asthma, liver, and spleen diseases (Satyavati and Raina, 1976). Several substances from celery seeds were reported for their anticarcinogenic property (Zheng et al., 1993). The root is an effective diuretic and has been taken for urinary stones and gravel. All parts are used ethnomedically to treat mild anxiety and agitation, loss of appetite, fatigue and cough and as an anthelminthic (Njoku Ugochi et al., 2011). The major bioactive substances in the celery include a class of phenolic compounds called furanocoumarins. The main linear furanocoumarins in celery include the three phototoxic furanocoumarins, psoralen, xanthotoxin and bergapten (Berenbaum, 1987). Hence, the present study is carried out to determine the *in vitro* antioxidant activity of the essential oil of celery and the extracts of celery and flaxseeds.

Material and Methods

Chemicals Substances

All chemicals and solvents (analytical grade) were purchased from Sigma Aldrich (Munich, Germany), unless otherwise specified.

Plant Material

The aerial parts of celery (*A. graveolens*) used in this study were collected during April 2018 from the region of Mascara (North-West of Algeria) and the flaxseeds (*L. usitatissimum*) were purchased from herbalist in the region of Mascara. The studied parts of each plant were the most used in traditional medicine in the region. The species were identified taxonomically and authenticated by the botanist at SNV faculty, University Mustapha STAMBOULI of Mascara. The studied plants were washed with tap water to remove all impurities and then with distilled water. The samples were dried in darkness at room temperature and chopped into small particles to increase the surface of diffusion.

Extracts Preparation

The dried seeds or plant material were made into fine powder of 40 mesh size using the pulverizer. 10 grams of each plant were macerated in hydro methanolic solution (methanol/water 70:30 v/v) at ratio of 1/10 (w/v) with magnetic stirring overnight at room temperature (Tiwari et al., 2011). This soaking was repeated threefold by renewing the solvent every 24 hours and then maceration of each solvent was combined. The hydro methanol extract was recovered after filtration through Whatman No.41 filter paper and concentration in a rotavapor (Buchi Labortechnink AG, Postfach, Switzerland). For preparation of aqueous extract, 10 grams of plant powder were macerated in 100 mL of distilled water at ratio of 1/10 (w/v) with magnetic stirring overnight at room temperature. This operation was repeated three times and then combined. The aqueous extract was recovered after filtration through Whatman No.41 filter paper. The two concentrated extracts for each plant were used for determination of *in vitro* antioxidant activity.

Isolation of Essential Oil

100 g of powdered plant (celery) in 500 mL of distilled water were submitted to hydro-distillation for 3 hours, using a Clevenger-type apparatus (ST15 OSA, Staffordshire, UK) until total recovery of oil. The extracted essential oil was dried over anhydrous sodium sulfate. In order to preserve its original quality, the oil was stored at 4 °C until tested in an opaque glass bottle sealed to protect it from air and light (main agents of degradation). The essential oil yield was evaluated by gravimetric method and expressed in terms of % w/w (ratio between the weight of the obtained oil and the weight of the sample to be treated). The purity of essential oil was determined by measurement of physicochemical indices according to the European Pharmacopoeia (2000). The pH was measured by using a digital pH meter apparatus and density by density meter. The relative density (d_{20}^{20}) was determined, which refers to the ratio of mass of the liquid sample and the mass of water, both at 20 °C. Pycnometers were used according to the amount of the essential oil available. The refraction index was evaluated in a refractometer using sodium light of wavelength of 589.3 mm (D ray), which was adjusted with distilled water (refraction index of 1.3330), the samples being kept at 20 °C. The rotatory power was measured by using a polarimeter. The miscibility with ethanol is the volume of ethanol needed to solubilize one volume of the essential oil (v/v). For evaluation of the acid index, a solution of m in (mg) of essential oil was mixed with 5mL of 95% ethanol and 5 drops of phenolphthalein 0.2%. This solution was neutralized with an ethanolic solution 0.1 M of KOH. The titration is over when the pink color begins to appear and persists for at least 15 seconds; we noted the volume V in (mL) of the KOH solution causing this color change and it was calculated by using the following formula:

$$I_a = (5.61 \times V) / m \quad (1)$$

where: I_a (acid index), V (volume in mL of the used KOH solution), m (mass of the sample test in gram).

For determination of the saponification index, in a 250 mL monocolumn flask equipped with a reflux condenser, the test sample m (mg) was mixed with 25mL of 0.5 M alcoholic potassium hydroxide and a few glass beads. The refrigerant was adapted and refluxed for 30 minutes and 1mL of phenolphthalein was added to the solution. The latter was titrated immediately with 0.5 M hydrochloric acid (sample test). The blank test was carried out under same conditions (blank test). The saponification index was calculated by using the formula:

$$I_s = ((V_t - V_e) \times C.HCl \times M.KOH) / m \quad (2)$$

where: (I_s) saponification index, (V_t) volume in mL of HCl 0.5M poured (blank test), (V_e) volume in mL of HCl 0.5M poured (sample test), (*C.HCl*) concentration of HCl in mol L^{-1}, (*M.KOH*) molar mass of KOH (56.10 g mol^{-1}, (m) mass of the sample test in gram.

The ester index (I_e) was calculated from the saponification index (I_s) and the acid index (I_a) according to the formula:

$$I_e = I_s - I_a \quad (3)$$

For determination of iodine value, in a 500 mL volumetric flask, one gram of oil sample was mixed with 15mL of carbon tetrachloride and 25 mL of Wijs solution. The flask was stoppered and swirled to ensure complete mixing. The sample was then placed in the dark for 30 minutes at room temperature. The flask was removed from storage and 20 mL of 10% potassium iodide (KI) solution were added, followed by 150mL of distilled water. The mixture was titrated with 0.1N thiosulphate ($Na_2S_2O_3$) solution added gradually and with constant and vigorous shaking until the yellow colour had almost disappeared. 1.5 mL of starch indicator solution was added and the titration was continued until the blue colour disappeared. A blank determination was conducted simultaneously. The peroxide present was evaluated by titration against thiosulphate in the presence of KI using starch as an indicator.

Evaluation of the Antioxidant Activity

DPPH Free Radical-Scavenging Activity

The antioxidant potential of extracts and essential oil of all plants was determined in terms of hydrogen donating or radical-scavenging capacity, using the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. The ability of the oil or extract to scavenge DPPH was assessed by using the method described by Shen et al. (2010) with some modifications. The samples to be tested for their antiradical activities were prepared in methanol to achieve the concentration of 1 mg mL^{-1}. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, and 1.95 µg mL^{-1}. A volume of 50 µL of each solution was added to 1950 µL of methanol solution of DPPH (6.10^{-5} M) as free radical source. The mixtures were stirred vigorously for 30 seconds and then incubated in the dark for 30 min at room temperature. The absorbance was measured using UV/Vis spectrophotometer model Hitachi 4-2000 at 517 nm against pure methanol (Shimada et al., 1992). Ascorbic acid and catechin were used for comparison as positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The inhibition percentage of DPPH was estimated using the equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = ((A\ control - A\ sample) / A\ control) \times 100 \quad (4)$$

where: *A control*: absorbance of the control containing all reagents except the oil or extract; *A sample*: absorbance of the sample (presence of the essential oil or extract).

IC_{50} defined as concentration of substrate that inhibits 50% of the DPPH radicals present in the reaction medium was determined from the % inhibition versus concentration plot, using a non-linear regression algorithm.

Ferric-Reducing Power

The ferric reducing power of all tested samples was evaluated by using the potassium ferricyanide-ferric chloride method described by Yildirim et al. (2001). 1 mL of each tested sample at different concentrations was mixed with 2.5 mL of 0.2 M phosphate buffer pH 6.6 and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution,. After incubation for 20 min at 50 °C, 2.5 mL of 10% trichloracetic acid were added and the reaction mixture was centrifuged for 10 min at 3000 rpm (Sigma laborzentrifugen D-37620 Osterode am Harz, Germany). An aliquot of 2.5 mL of the supernatant from each mixture was mixed in a test tube with 2.5 mL of distilled water and 0.5 mL of ferric chloride solution (0.1%) prepared freshly in distilled water. After 20 min of reaction time at 35 °C, the absorbance was recorded at 700 nm against a blank containing all reagents except the essential oil (or extract) solutions and ferric chloride. The control was achieved by different

concentrations of ascorbic acid and catechin. Higher absorbance of the reaction mixture indicated higher reducing power. Tests were carried out in triplicate. The concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting absorbance at 700 nm against the corresponding sample concentration.

Data Analysis

All determinations were conducted in triplicates and results for each measured parameter were expressed as mean ± SD. Data were statistically determined by analysis of variance ANOVA using the level significance ($P < 0.05$) using Microsoft Excel and SPSS statistics software 8.1.

Results and Discussion

Results of Physic-Chemical Characterization of *Apium Graveolens* Essential Oil

The essential oil of the aerial parts of *Apium graveolens* L. presents a limpid liquid aspect, volatile, mobile, with light yellow color, strong and fresh smell. This oil was characterized by a yield of 1.1 ± 0.2%. According to Marzouk et al. (2008), the plant polymorphism, seasonal, geographical variation and nutritional availability of plant affect extraction oil yields, which are higher when plants are extracted during the flowering period. The physic-chemical analysis (pH 5.3 ± 0.1, density at 20 °C 0.91 ± 0.02 g mL^{-1}, relative density 0.96 ± 0.02 g g^{-1}, refractive index at 20 °C 1.345 ± 0.003, rotatory power +3.22 ± 0.15, miscibility with ethanol at 96% 1/8 (v/v), acid index 8.246 ± 0.020 mg KOH g^{-1} EO, ester index 94.283 ± 0.020 mg KOH g^{-1} EO at 20 °C, saponification index 104.10 ± 0.01, peroxide index 14 ± 0.1 meq. O2 kg^{-1} EO, carbonyl index 126.22 ± 0.01, and iodine number 1.6 ± 0.2) showed the good quality of the studied oil.

Results of Antioxidant Activity

Free radical DPPH method and ferric reducing assay were used for the evaluation of antioxidant activity. The use of more than one method is recommended to give a comprehensive prediction of antioxidant potential (Dini et al., 2009).

DPPH Free Radical-Scavenging Activity

Results showed that the antioxidant activity was directly dependent on the concentrations used. As shown in Fig. 1, the methanol extract and essential oil of celery show a higher scavenging activity of DPPH radical (79 ± 0.5% and 84 ± 0.4%) at concentration of 1000 µg mL^{-1} compared to the aqueous extract (53 ± 0.3%) at the same concentration. This result was similar to the % of inhibition of methanol extract of celery 88.31% reported by Jung et al. (2011). A similar scavenging activity of DPPH radical was obtained by methanol extract (78 ± 0.3%) and aqeous extract (54 ± 0.2%) of flaxseed. These results are lower compared to those found with ascorbic acid and catechin (96.23 ± 1.2%, 94.50 ± 1.4%). Our results of antioxidant activity of celery were in line with the results obtained by Yao et al. (2010) and Njoku Ugochi et al. (2011). The presence of the C2-C3 double bond configured with a 4-keto arrangement is known to be responsible for electron delocalization from ring B and it increases the free radical-scavenging property (Wojdylo et al., 2007). Kim and Lee (2004) reported that caffeic and *p*-coumaric acids displayed a good antioxidant property. Results of DPPH scavenging activity of flaxseeds are in agreement with the report of Alachaher et al. (2018). According to Derwich et al. (2011), the antiradical activities could be associated with their chemical composition, especially with their major compounds. The richness of celery and flaxseeds in polyphenols (Berenbaum, 1987; Shahrani et al., 2019) explains its antioxidant effect.

The IC_{50} parameter commonly used to measure the antioxidant activity is necessary for each sample to reduce 50% of DPPH radical concentration in a defined period of time and a low IC_{50} value corresponds to a higher antioxidant activity. It was used to classify antioxidant activity of tested sample in comparison with the standard. The positive control catechin and ascorbic acid displayed lower values of IC_{50} respectively 7.81 ± 0.1 and 31.5 ± 0.3 µg mL^{-1}, followed by methanol extract of celery 130 ± 0.2 µg mL^{-1} and flaxseeds 150 ± 0.4 µg mL^{-1}, essential oil of celery 180 ± 0.2 µg mL^{-1}, then aqueous extracts of flaxseeds 950 ± 0.5 µg mL^{-1} and celery 980 ± 0.4 µg mL^{-1}. Alachaher et al. (2018) found the value of 220.05 for methanol extract of flaxseed. The obtained IC_{50} values of celery are higher to the value 88.73 µg mL^{-1} for methanol extract and 95.11 µg mL^{-1} for water extract reported by Jung et al. (2011). According to Yao et al. (2010), the antiradical activity of celery is due to the presence of flavonoids compounds such as apigenin, luteolin, and kaempferol. The richness of flaxseed extracts in flavonoids, phenolic compounds, glycosides, alkaloids, and terpenoids (Alachaher et al., 2018; Hosseinian et al., 2006) explains its good antioxidant power.

Reducing Power

The reducing power assay is often used to estimate the ability of natural antioxidant to donate an electron or hydrogen to form more stable product "reduction of ion" (Shimada et al., 1992; Ho et al., 2012). The determination of the ferric reducing antioxidant was based on the reduction of Fe^{+3}/ferriccyanide complex to the ferrous form in the presence of antioxidants in the tested samples. The Fe^{+2} was then monitored by measuring the formation of Perl's Prussian blue at 700 nm. In fact, it is widely accepted that higher absorbance at 700 nm is correlated to power reducing (Shimada et al., 1992). This author reported that the reductive potential might be related to the presence of phenolic compounds, such as isothymol and carvacrol, due to the hydroxyl substitutions in the aromatic ring which possess potent hydrogen-bonding abilities. The reducing capacity of all extracts increased in a concentration-dependent manner (Fig. 2).

For aerial part of celery, we note that significant activity for reducing iron values was observed by optical density of 1.8 ± 0.2 for essential oil and 1.7 ± 0.1 for methanol extract, while ascorbic acid and catechin provide an OD of 2.069 ± 0.03 and 2.66 ± 0.016 in the same concentration 1000 µg mL^{-1}. Our results are in agreement with the work of Jung et al. (2011). For flaxseeds, the higher activity was obtained by methanol extract 1.65 ± 0.02 followed by aqueous extract 0.8 ± 0.01. We can classify power reduction of iron as follows: catechin, ascorbic acid, essential oil of celery, methanol extract of celery, methanol extract of flaxseeds, aqueous extract of celery 0.9 ± 0.02 and then aqueous extract of flaxseeds. Several authors (Gao et al., 2000; Amarowicza et al., 2004) have observed a direct correlation between antioxidant capacities and

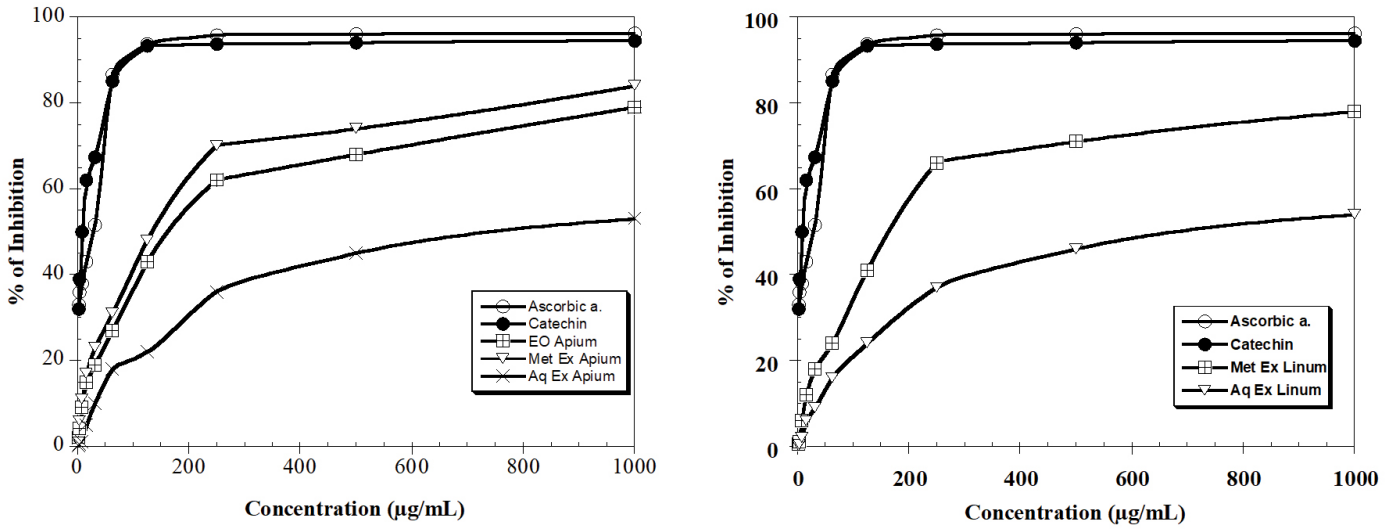

Figure 1. Free radical-scavenging activities of positive controls (ascorbic acid, catechin), essential oil of celery, methanol and aqueous extracts of celery and flaxseeds. Values represent Mean ± SD; n=3; Confidence level $P \leq 0.05$

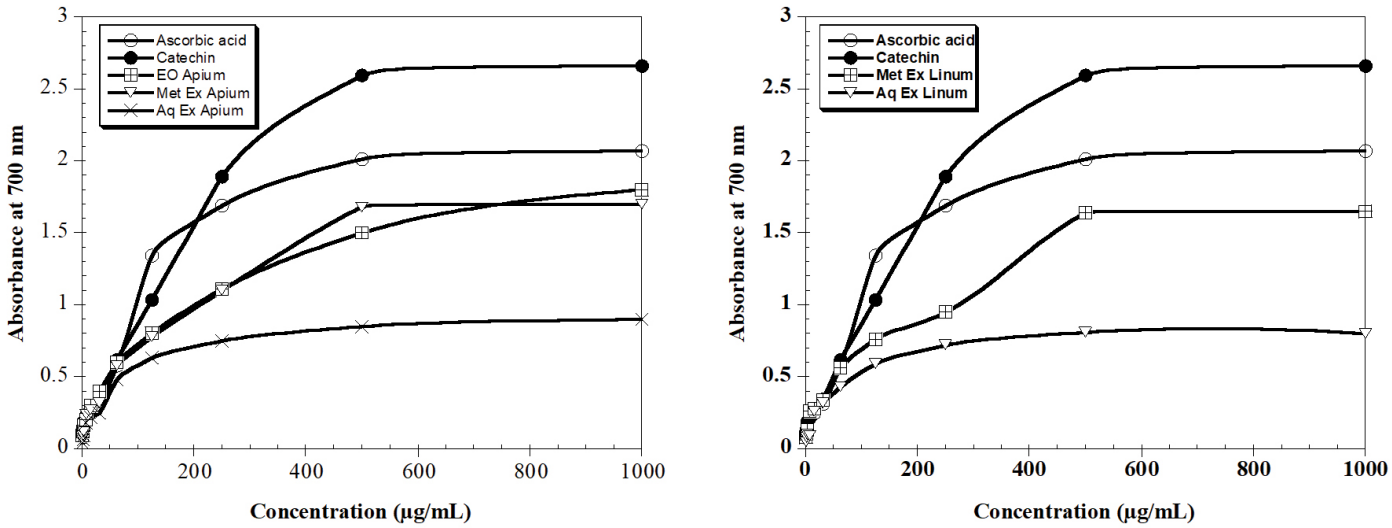

Figure 2. Antioxidant capacities of positive control (ascorbic acid, catechin), essential oil of celery, methanol and aqueous extracts of celery and flaxseeds. Values represent Mean ± SD; n=3; Confidence level $P \leq 0.05$

reducing power of certain plant extracts. The reducing capacity (FRAP) is generally associated with the presence of reductones (Krishnamoorthy et al., 2011). Reductones are reported to react with certain precursors of peroxide, thus preventing peroxide formation. According to Gordon (1990), antioxidant activity of reductones is based on the breaking of the free radical chain by donating a hydrogen atom. The marked antioxidant potential of celery and flaxseeds may be attributed to the abundance of polyphenols which may act as reductones to convert free radical into more stable products and terminate free radical chain reaction. Our results are in agreement with those reported by Alachaher et al. (2018) and Sangeetha et al. (2010).

The positive control (catechin and ascorbic acid) displayed lower values of IC_{50} 53 ± 0.3 and 58.3 ± 0.4 µg mL^{-1}, followed by essential oil and methanol extract of celery (59 ± 0.3, 62 ± 0.2 µg mL^{-1}), methanol extract of flaxseeds 64 ± 0.2 µg mL^{-1}, then aqueous extracts of celery 82 ± 0.3 µg mL^{-1} and flaxseeds 100 ± 0.1 µg mL^{-1}.

Conclusion

The results from this study demonstrate clearly that essential oil of celery and methanol extract of celery and flaxseeds exert a strong antioxidant potential determined by two methods: DPPH free radical scavenging and ferric ion reduction. Our results denote that methanol is the most efficient extraction solvent compared to the water solvent. *Apium graveolens* L. species exhibits higher antioxidant activity compared to *Linum usitatissimum*. It may provide a valuable source of natural antioxidants for commercial exploitation.

References

Adebayo A. H., Song F. H., Liu X. T., Dai H., Huang P., Zhang L. X. (2014). *Citrillus lanatus* Extract Reverses Oxidative and Haematological Dysfunction in Carbon Tetrachloride Induced Liver Damaged Rats. Int J Pharm Sci 10 (4): 218-224. doi: 10.3923/ijp.2014.218.224

Alachaher F. Z., Dali S., Dida N., Krouf D. (2018). Comparison of Phytochemical and Antioxidant Properties of Extracts from Flaxseeds (*Linum usitatissimum*) Using Different Solvents. Int Food Res J 25 (1): 75-82.

Al-Bayati F. A. (2008). Synergistic Antibacterial Activity between *Thymus vulgaris* and *Pimpinella anisum* Essential Oils and Methanol Extracts. J Ethnopharmacol 116 (3): 403-406. doi: 10.1016/j.jep.2007.12.003

Balandrin M., Kinghorn A., Farnsworth N. (1993). Plant-Derived Natural Products in Drug Discovery and Development. In: Human Medicinal Agents from Plants (Kinghorn A. D., Balandrin M. F., eds.), ACS Symposium Series 534, American Chemical Society, Washington DC, pp. 1-12

Berenbaum M. (1987). Charge of the Light Brigade: Phototoxicity as a Defense against Insects. In:, Light Activated pesticides (Heitz J. K., Downum K. R., eds), ACS Symposium Series 339, American Chemical Society, Washington, D.C., pp. 206–216

Chevallier A. (1998). The Encyclopedia of Medicinal Plants. DK Publishing Inc., New York. pp. 61.

Derwich E., Benziane Z., Boukir A. (2011). Antibacterial Activity and Chemical Composition of the Leaf Essential Oil *Mentha rotundifolia* from Morocco. Elect J Env Agricult Food Chem 9 (1): 19-28

Dini I., Tenore G. C., Dini A. (2009). Saponins in *Ipomoea batatas* Tubers: Isolation, Characterization, Quantification and Antioxidant Properties. Food Chem 113 (2): 411-419. doi: 10.1016/j.foodchem.2008.07.053

Duh P. D. (1998). Antioxidant Activity of Burdock (*Arctium lappa* Linne): Its Scavenging Effect on Free Radical and Active Oxygen. J Am Oil Chem Soc 75 (4): 455-461. doi: 10.1007/s11746-998-0248-8

European Pharmacopoeia, 2000. Dritter Nachtrag, 3rd ed. Council of Europe, Strasbourg, pp. 499-500

Gao X., Bjork L., Trajkovski V., Uggla M. (2000). Evaluation of Antioxidant Activities of Rosehip Ethanol Extracts in Different Test Systems. J Sci Food Agric 80 (14): 2021-2027. doi: 10.1002/1097-0010(200011)80:14<2021::AID-JSFA745>3.0.CO;2-2

Ghule A. E., Jadhav S. S., Bodhankar S. L. (2012). Effect of ethanolic extract of seeds of *Linum usitatissimum* (Linn.) in hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. Asian Pacific Journal of Tropical Disease 2: 405-410. doi: 10.1016/S2222-1808(12)60088-7

Gordon, M.H. (1990) The Mechanism of Antioxidant Action *in Vitro*. In: Food Antioxidants (Hudson B. J. F., ed), Springer, Berlin, pp. 1-18. doi: 10.1007/978-94-009-0753-9_1

Ho Y. L., Huang S. S., Deng J. S., Lin Y. H., Chang Y. S., Huang G. H. (2012). *In vitro* Antioxidant Properties and Total Phenolic Contents of Wetland Medicinal Plants in Taiwan. Bot Stud 53: 55-56.

Hosseinian F. S., Muir A. D., Westcott N. D., Krol E. S. (2006). Antioxidant Capacity of Flaxseed Lignans in Two Model Systems. J Am Oil Chem Soc 83: 835-840. doi: 10.1007/s11746-006-5034-x

Imaida K., Fukushima S., Shirui T., Ohtani M., Nakanishi K., Ito N. (1983). Promoting Actions of Butylated Hydroxyl Anisole and Butylated Hydroxyl Toluene on 2-Stage Urinary Bladder Carcinogenesis and Inhibition of c-Glutamyl Transpeptidase-Positive Foci Development in the Liver of Rats. Carcinogenesis 4: 895-899

Jung W. S., Chung I. M., Kim S. H., Kim M. T., Ahmad A., Praveen N. (2011). *In vitro* Antioxidant Activity, Total Phenolics and Flavonoids from Celery (*Apium graveolens*) Leaves. J Med Plant Res 5 (32): 7022-7030. doi:10.5897/JMPR11.1129

Kim D. O., Lee C. Y. (2004). Comprehensive Study on Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Various Polyphenolics in Scavenging a Free Radical and Its Structural Relationship. Crit Rev Food Sci Nutr 44 (4): 253-273. doi: 10.1080/10408690490464960.

Krishnamoorthy M., Sasikumar J. M., Shamna R., Pandiarajan C., Sofia P., Nagarajan B. (2011). Antioxidant Activity of Bark Extract from Mangroves, *Bruguiera cylindrical* (L.) Blime and *Ceriops decandra* Per. Indian J Pharmacol 43 (5): 557-562. doi: 10.4103/0253-7613.84972.

Lis-Balchin L., Deans S. G. (1997). Bioactivity of Selected Plant Essential Oils against *Listeria monocytogenes*. J Appl Microbiol 82 (6): 759-762. doi: 10.1046/j.1365-2672.1997.00153.x

Lund E. D. (1978). Flavors and Nonalcoholic Beverages: Thin Layer and High Pressure Liquid Cheomatographic Analysis of Celery Seed Oil. J Assoc Anal Chem 61: 1083-1088.

Marzouk B., Fredj M. B. H., Chraif I., Mastouri M., Boukef K., Marzoul Z. (2008). Chemical Composition and Antimicrobial Activity of Essential Oils from Tunisian *Mentha pulegium* L. J Food Agric Environm 6 (1): 78-82.

Milder I. E. J., Arts L. C. W., Van de Putte B., Venema D. P., Hollman P. C. H. (2005). Lignan Contents of Dutch Plant Foods: A Database Including Lariciresinol, Pinoresinol, Secoisolariciresinol and Matairesinol. Brit J Nutr 93 (3): 393-402. doi: 10.1079/bjn20051371

Momin R. A., Ramsewak R. S., Nair M. G. (2000). Bioactive Compounds and 1,3-Di[(cis)-9-octadecanoryl]-2-[(cis, cis)-9,12-octadecadienoyl] Glycerol from *Apium graveolens* L. Seeds. J Agric Food Chem 48 (9): 3785-5788. doi: 10.1021/jf991383r

Njoku Ugochi U., Joshua Elijah E., Offor Leonard I., Okoli Charles O. (2011). Antioxidant Properties of *Apium graveolens*. Res J Pharmacogn Phytochem 3 (5): 103-108.

Prasad K., Mantha S. V., Muir A. D., Westcott N. D. (2000). Protective Effect of Secoisolariciresinol Diglucoside against Streptozorocin Induced Diabetes and Its Mechanism. Mol Cell Biochem 206 (1-2): 141-150. doi: 10.1023/a:1007018030524

Rosenfeldt F., Wilson M., Lee G., Kure C., Braun L., Haan J. (2013). Oxidative Stress in Surgery in an Ageing Population: Pathophysiology and Therapy. Exp Gerontol 48 (1): 45-54. doi: 10.1016/j.exger.2012.03.010

Sangeetha S., Marimuthu P., Doraisamy P., Sarada D. V. L. (2010). Evaluation of Antioxidant Activity of the Antimicrobial Fraction from *Sphaeranthus indicus*. Int J Applied Biol Pharm Technol 1 (2): 431-436

Satyavati G. V., Raina M. K. (1976). Medicinal Plants of India, Pub. Indian Council of Medical Research, New Delhi, India, 107: 80.

Shahrani M., Asgharzadeh N., Amini-Khoei H., Mirzaeian Z., Ahmadi A., Lori Goeini Z. (2019). Phytochemical Study and Antidepressant Effect of Essential Oil of *Apium graveolens* L. J Pharm Res Int 27 (4): 1-10. doi: 10.9734/jpri/2019/v27i430179

Shen Q., Zhang B., Xu R., Wang X., Li P. (2010). Antioxidant Activity *in vitro* of Selenium-Contained Protein from the Se-Enriched *Bifidobacterium animalis* 01. Anaerobe 16 (4): 380-386. doi: 10.1016/j.anaerobe.2010.06.00

Shimada K., Fujikawa K., Yahara K., Nakamura T. (1992). Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. J Agric Food Chem 40 (6): 945-948. doi: 10.1021/jf00018a005

Tiwari P., Kumar B., Kaur G., Kaur H. (2011). Phytochemical Screening and Extraction. Int J Pharm Sci 1 (1): 98-106

Wojdylo A., Oszmianski J., Czemerys R. (2007). Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs. Food Chem 105 (3): 940-949. doi: 10.1016/j.foodchem.2007.04.038

Yamuna S. T., Padma P. R. (2013). Antioxidant Potential of the Flowers of *Caesalpinia pulcherrima*, Swartz in an *in vitro* System Subjected to Oxidative Stress. J Pharm Res 7 (7): 661-665. doi: 10.1016/j.jopr.2013.07.026

Yao Y., Sang W., Zhou M., Ren G. (2010). Phenolic Composition and Antioxidant Activities of 11 Celery Cultivars. J Food Sci 75 (1): 9-13. doi: 10.1111/j.1750-3841.2009.01392.x

Yildirim A., Mavi A., Kara A. A. (2001). Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts. J Agric Food Chem 49 (8): 4083–4089. doi: 10.1021/jf0103572

Zanwar A. A., Hegde M. H., Bodhankar S. L. (2011). Cardioprotective Activity of Flax Lignin Concentrate Extracted from Seeds of *L. usitatissimum* in Isoprenalin Induced Myocardial Necrosis in Rats. Interdiscip Toxicol 4 (2): 90-97. doi: 10.2478/v10102-011-0016-8

Zheng G. Q., Zhang J., Kenney P. M., Lam L. K. T. (1993). Chemoprevention of Benzo [a] Pyrene Induced for Stomach Cancer in Mice by Natural Phthlides from Celery Seed Oil. Nutr Cancer 19 (1): 77-86. doi: 10.1080/01635589309514238

aCS88_25