Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(1):251-4

Antioxidant and anti-lipoxygenase activities of *Cydonia oblonga*

Mehmet Berkoz

Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Biochemistry, Van, Turkey

Received 18 September 2019; Accepted 12 December 2019
Available online 07.03.2020 with doi: 10.5455/medscience.2019.08.9174

Abstract

The present studies are focused to explore the antioxidant and anti-lipoxygnease activities in crude extract, aqueous and organic fractions (ethyl acetate, chloroform, butanol) of dry fruit of Cydonia oblonga. For determining the antioxidant activity; total phenolic content and 1,1-diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay were performed in the aqueous and organic fractions of *C. oblonga.* In our study, phenolic content revealed that crude extract has highest phenolic content followed by ethyl acetate fraction. The results of DPPH radical scavenging activity showed IC_{50} values of crude extract as 61.4 µg/mL, chloroform and aqueous 67.4 and 55.5 µg/mL, respectively, which are well comparable with butylatedhydroxyanisol (BHA) standard, which showed IC_{50} as 44.2 µg/mL. In addition, crude extract appeared to be the prominent inhibitor of lipoxygenase. The results suggests that the extract and different fractions of dry fruit of C. oblonga exhibits significant antioxidant and free radical scavenging activities/capacity which might be associated with the presence of main phytochemicals like flavonoids, phenols and tannins. Besides that, all these extract and fractions have well enough lipoxygenase inhibitory potential. As a result, we can claim that the presence of the scavenger molecules in the dry fruit of C. oblonga may attribute health benefits by providing the protective effects during the progressive stages of different acute and chronic disorders.

Keywords: *Cydonia oblonga*, crude extract, aqueous fraction, organic fractions, antioxidant activity, anti-lipoxygnease activity

Introduction

Antioxidant compounds are an important factor in health protection. Moreover, they reduce the risk of chronic diseases such as heart diseases and cancer. Free radicals are molecular species, containing one or more unpaired electrons and are highly reactive. They are formed by regular metabolism during oxygen utilization, to burn food for energy. Their development occurs endlessly within the body as a result of both enzymatic and non-enzymatic reactions. These reactive oxygen species (ROS) are dangerous for vital cell components like fatty acids, proteins, nucleic acids and carbohydrates [1]. Non-enzymatic antioxidant defense system is composed by a range of compounds such as a tocopherol (Vitamin E), β-carotene, sodium ascorbate (Vitamin C) and phenolic compounds which are widely found in medicinal plants. The main function of the antioxidant defense system is to inhibit or reduce damages caused by free radicals and reactive oxygen species.

***Coresponding Author:** Mehmet Berkoz, Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Biochemistry, Zeve Campus, Van, Turkey, E-mail: mehmet berkoz@yahoo.com

These damages are related to a number of chronic diseases, including cancer and some cardiovascular and neurodegenerative diseases. Phenolic compounds are derived from secondary plant metabolism and are essential for plant growth and reproduction. Flavonoids, xanthones, phenolic acids, tannins and tocopherols are the most common natural source of phenolic antioxidants [1].

Phytomedicine explored so far for phenolics found to have antioxidants activities and have been exploited to decrease the risk of cancer and heart ailments. The traditional medicinal plants have also been proven to cause inhibition of the biological enzymes for example lipoxygenase, cholinesterase and tyrosinase. The plants polyunsaturated fatty acids are converted by enzymes lipoxygenase into bioactive metabolites, which are eventually involved in inflammatory and immune responses. This occurs due to the formation of the leukotrienes (LTs). The approaches which cause inhibition of lipoxygenase isoforms and/or their bioactive metabolites can be valuable in the dealing with asthma, allergic rhinitis, rheumatoid arthritis, psoriasis, and cancer [1].

Antioxidant and radical scavenging properties of plants are subject to intensive research. The aim of the present study was to screen the antioxidant activities of some commonly available plants and fruits. Several medicinal plants in Turkey flora, which

possess pharmacological properties are partly identified. One such medicinal plant used since ancient time is Cydonia oblonga commonly known as quince. Its vast medicinal, pharmacological and antimicrobial properties were reported and well explained in folkloric medicine and greatest medicinal value, however, has been attributed to its fruit [2,3].

Through in vitro and in vivo studies it is pointed out that *Cydonia oblonga* is a major source of accepted antioxidants, which might be supportive in controlling the progress of various oxidative stresses in the body thus have a good potential to use in various progressive inflammatory diseases, to use as a protective agent in hepatic and heart disorders [4]. Phytochemical screening of *C. oblonga* discovered the occurrence of alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids, and steroids [5]. Scientific literature reflected that almost all parts of this plant except dry fruit were used to isolate several active principles like sterols, triterpenes, and tannins [6]. The present studies are focused to explore the total phenolic content, DPPH- radical scavenging and lipoxygnease inhibition activity in crude extract, aqueous and organic fractions (ethyl acetate, chloroform, butanol) of dry fruit of C. oblonga.

Materials and Methods

Chemicals

1,1-diphenyl-2-picryl-hydrazil (DPPH) & all solvents were procured from Roche Diagnostics, Mannheim, Germany. DMSO, potassium hexacyanoferrate [$K_3Fe(CN)_6$], F-C reagent, sodium carbonate (Na_2CO_3), butylatedhydroxyanisol (BHA), Ferric chloride, trichloroacetic acid (TCA) were purchased from MP Biomedicals (France). Lipoxygenase sodium dihydrogen phosphate, linoleic acid, tween 20, baiclein were purchased from Sigma.

Plant material

The dry fruit of *C. oblonga* was procured from Van Province, Turkey and recognized by Prof. Dr. Ali Aslan from Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology.

Method Subsections

Preparation of plant extract

The dry fruit of *C. oblonga* (approximately 3 kg) was cleaned of dust particle and other adulterants by manual examination and picking and then thickly grounded. The coarse milled stuff was covered with 7 L methanol:water (70:30) mixture at room temperature (23-25ºC) for 72 hrs with intermittent shaking. It was first filtered through muslin cloth and then through Whatman No.1 filter paper, thrice; filtrate was pooled and then concentrated under reduced pressure at 40ºC on rotary evaporator. The resulting dark brown colored semi-solid mass (13.26%) was named as crude extract and stored at 4ºC until use. Half of the crude extract was used for further fractionation. For preparation of different fractions crude extract was mixed with 100 mL of water and 200 mL of ethyl acetate in a separating funnel and left for 24 hours. Next day aqueous and ethyl acetate layers were collected separately. Ethyl acetate layer was evaporated to obtain ethyl acetate fraction while aqueous layer was again treated with 200 mL of chloroform and then 200 mL of butanol in the similar manner as mentioned above to obtain chloroform, butanol and aqueous soluble fractions. The crude extract and fractions were stored at 4ºC in air tight bottles for further studies [7].

Estimation of total phenolics

Total phenolic content of extracts and various fractions of *C. oblonga* was measured by Folin-Chiocalteu reagent according to the method described by Hazra et al. [8] with some amendment. The extracts were diluted with distilled water to obtain the readings inside the standard curve range of 0.0 to 600 µg of gallic acid/mL. A mixture was obtained my adding 250 µL of gallic acid, 1 mL of distilled water, 250 µL of Folin-Chiocalteu reagent in a test tube. After mixing, it was kept standing for 5 min at room temperature. Then, 7% sodium carbonate aqueous solution (2.5 mL) and distilled water were added to make up the volume to 6 mL. The mixture was kept in an incubator for 90 minutes. The absorption of the subsequent blue color solution produced, was measured at 725 nm by spectrophotometer. The results were calculated as mg of gallic acid (Standard) equivalents (GAE)/g of extract by means of an equation from the standard gallic acid graph. Three readings were noted for each extract.

DPPH radical scavenging assay

The free radical scavenging activity was measured by method describing by Iqbal et al., [9] using 1,1-diphenyl-2-picryl-hydrazil (DPPH) as free radical. The solution of DPPH of 0.3 µM was prepared in ethanol. Each sample (5µL) (of different percentages) was mixed with 95µL of DPPH solution in ethanol, incubated at 37ºC for 30min. The absorbance at 515 nm was measured by ELISA plate reader (VersaMax, Molecular Devices LLC, California, USA) and percent radical scavenging activity was determined. BHA is used as standard.

DPPH scavenging effect (%) = Ac – As/Ac × 100 where;

Ac = Absorbance of control (DMSO treated) and As = Absorbance of sample.

Lipoxygenase inhibition assay

5-lipoxygenase activity was analyzed by the method of Tappel [10] with slight modifications. A total volume of 200 µL containing 160µl of KH_2PO_4 buffer (100mM, pH 8.0), 10µl test compound (extract and fractions), and 20 µL purified lipoxygenase solution (130 units per well) were mixed and pre-read at 234 nm by ELISA plate reader (VersaMax, Molecular Devices LLC, California, USA). The mixture was pre-incubated at 25°C for 10 minutes. The reaction was originated by the addition of 10 µL substrate solution (linoleic acid 0.5 mM, 0.12% tween 20). The absorbance was noted every 15 min at 234 nm for any change in absorbance. Baicalein was used as a positive control. The IC_{50} values were determined at 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.015 mg dilutions. All extracts assays carried out in triplicate. The radical scavenging activity was calculated by the equation:

Inhibition (%) = (Absorbance of control - Absorbance of test solution/Absorbance of control) × 100

Results

Total phenolic content revealed that crude extract has highest phenolic content followed by ethyl acetate fraction. Total phenolic compounds behave as key constituents because of their hydroxyl groups, which bestow scavenging ability (Table 1).

Table 1. Total phenolic content of extracts and various fractions of *C. oblonga*

Fraction	Total phenolic content (mgGAE/g)
Crude extract	367
Aqueous	115.3
Ethyle acetate	65
Chloroform	244.9
Butanol	123.4

The results of DPPH radical scavenging activity showed IC_{50} values of crude extract as 61.4 µg/mL, chloroform and aqueous fractions were 67.4 and 55.5 µg/mL, respectively, which are well comparable with BHA standard which showed IC_{50} as 44.2 µg/mL. While butanol and ethyl acetate posses less antioxidant potential as compared to other fractions but, however the fruit has good antioxidant activities (Table 2). Results in Table 2 showed that all fractions along with crude extract appeared to be the prominent inhibitor of lipoxygenase. The effect of various fractions and extract on lipoxygenase inhibition challenge was very profound.

Table 2. IC_{50} values of extracts and fractions of dry fruit of Aeglemarmelos L.

Fraction	DPPH Scavenging IC_{50} µg/ml± SD	Lipoxygenase Inhibition IC_{50} µg/ml± SD
Crude extract	61.4± 0.12	99.3±0.84
Aqueous extract	55.5±0.40	101.8±1.2
Ethyl Acetate	110.7±0.71	227.3±1.89
Chloroform	67.4±0.63	102.4±0.95
Butanol	93.6±0.59	207±3.5
BHA	44.6±0.05	N.D.
Baicalein	N.D.	22.6±0.02

(SD: Standart deviation, N.D.: not determined)

Discussion

The aim of the present study was to screen indigenous plants, especially which are commonly used, to identify antioxidant ability and enzyme inhibition capacity. Most bioactive food constituents are derived from plants; this study is therefore an essential research tool to further elucidate the potential health effects of phytochemical antioxidants in diet. Due to unpleasant effects of synthetic drugs, which are commonly used against oxidative damage, it is wise and advisable to use herbal antioxidants as food supplements and traditional medicines. Therefore, globally interest is developed towards green medicines because herbs are good source of many vital nutrients required to run life healthy and smoothly [11,12]. The results of this study confirmed that crude extract has highest phenolic content followed by ethyl acetate fraction and it is well reported that majority of natural antioxidants are polyphenol in nature which confer their scavenging ability [13].

The phytochemical content and antioxidant activities among the extracts can be compared if the density of the extracts are similar. Extract with high density may show higher phytochemical content and higher activity than low density extract. Therefore, all extracts in the present study should be prepared in similar density [11-13].

DPPH free radicals dissolve in methanol and show absorption at wavelength 515 nm. Antioxidant will transfer the hydrogen to DPPH, which will be stable. Colors of DPPH would be changed from purple to yellow when the free radicals are scavenged by antioxidant. FRAP reagent is ferric (III) chloride which is combined with 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in acetate buffer of pH 3.6. Reduction potential of Fe (III)/Fe (II) is 0.77 V. Antioxidant with reduction potential lower than 0.77 V will reduce Fe (III) to Fe (II). Blue color complex of Fe (II)-TPTZ shows absorption at λ515 nm. The amount of Fe (III) is reduced to Fe (II) and then forms Fe (II)-TPTZ, related to intensity of blue color. Sample with IC_{50} or EC_{50} lower than 50 µg/mL can be classified as very strong antioxidant, 50-100 µg/mL as strong, 101-150 µg/mL as medium and greater than 150 µg/mL as weak antioxidant [11-13].

Determination of antioxidant activities by DPPH method is presented by IC_{50} DPPH, the concentration of extract (antioxidant sample) that can scavenge free radical DPPH 50% and figured by decreasing absorbance of DPPH after adding extract. IC_{50} DPPH can be determined using regression linear equation of calibration curve of each extract.

In the present research, DPPH scavenging activity of dry fruit of *C. oblonga* were evaluated using its crude and aqueous extracts, ethyl acetate, chloroform, and butanol fractions. Aqueous extract of *C. oblonga* dry fruit showed the highest antioxidant activity by DPPH assay, which had the lowest IC_{50} of DPPH scavenging activity (55.5 µg/mL). Crude extract of *C. oblonga* dry fruit also exhibits strong antioxidant activity. Also, chloroform and butanol fractions of *C. oblonga* dry fruit show strong antioxidant activity. But, ethyl acetate fraction of *C. oblonga* dry fruit shows medium grade antioxidant activity. IC_{50} of DPPH scavenging activity of ascorbic BHA was found as 44.6±0.05 µg/mL. It means that antioxidant potential of BHA is around 2.5 times antioxidant potential of ethyl acetate fraction of *C. oblonga* dry fruit by DPPH assay.

Lipoxygenase inhibition activity showed that all fractions along with crude extract appeared to be the prominent inhibitor of soybean lipoxygenase. The effect of various fractions and extract on lipoxygenase inhibition was very profound. It is evident from IC_{50} values in Table 2 that crude extract, aqueous and chloroform fractions have good lipoxygenase inhibition potential (99, 101 and 102 µg/mL), respectively.

The important role of the 5-lipoxygenase pathway in carcinogenesis is vital as inhibition of the 5-lipoxygenase pathways clearly has chemo preventive effects on various cancers [14]. We observed in our study that all fractions and crude extract of *C. oblonga* strongly inhibit lipoxyganase and have potential of scavenging of DPPH radical, this can be assumed that the components present in fractions and extract may block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase and may serve as strong scavenger of free radicals to save cellular organs.

Conclusion

The results suggest that the extract and different fractions of dry fruit of *C. oblonga* exhibits significant antioxidant and free radical

scavenging activities/capacity which might be associated with the presence of main phytochemicals like flavonoids, phenols and tannins. All these extract and fractions have good enough lipoxygenase inhibitory potential. Therefore the presence of the scavenger molecules in the dry fruit of *C. oblonga* may attribute health benefits by providing the protective effects during the progressive stages of different acute and chronic disorders.

Acknowledgment
The author would like to thank Prof. Dr. Ali Aslan for recognizing of Cydonia oblonga.

Financial Disclosure
Financial support for this study was provided by the researcher.

Ethical approval
No ethic approvell is needed to this research.

Mehmet Berkoz ORCID: 0000-0003-4219-8054

References

1. Fleschin S, Fleschin M, Nita S, et al. Free radical mediated protein oxidation in Biochemistry. Roun Biotechnol Lett. 2000;5:479-95.
2. Reddy VD, Padmavathi P, Paramahamsa M, et al. Amelioration of alcoholinduced oxidative stress by Emblicaofficinalis (Amla) in rats. Ind J Biochem Biophys. 2010;47:20-5.
3. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant Salmonella typhi. Phytother Res. 2004;18:670-3.
4. Ashraf MU, Muhammad G, Hussain MA, et al. Cydonia oblonga M., A medicinal plant rich in phytonutrients for pharmaceuticals. Front Pharmacol. 2016;7:163.
5. Erturk AG, Erturk O, Ayvaz MC, et al. Screening of phytochemical, antimicrobial and antioxidant activities in extracts of some fruits and vegetables consumed in Turkey. CBU J Sci. 2018;14:81-92.
6. Stojanović BT, Mitić SS, Stojanović GS, et al. Phenolic profiles and metal ions analyses of pulp and peel of fruits and seeds of quince (Cydonia oblonga Mill.). Food Chem. 2017;232:466-75.
7. Yaqeen Z, Naqvi NH, Sohail T, et al. Screening of solvent dependent antibacterial activity of Prunus Domestica. Pak J Pharm Sci. 2013;26:409-14.
8. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondiaspinnata. BMC Complement Altern Med. 2008;8:63.
9. Iqbal L, Lateef L, Ali S, et al. Antioxidant activities of tetraketones derived from 5, 5 dimethylcyclohexane-1, 3- dione. J Chem Soc Pak. 2007;29:51-4.
10. Tappel AL. Method of Enzymology, Vol 5. Academic Press. New York, 1962, 539-42.
11. Ahmad M, Imran H, Yaqeen Z, et al. Pharmacological profile of Salvadora Persica. Pak J Pharm Sci. 2011;24:323-30.
12. Rehman A, Fatima N, Imran H, et al. Nutritional and toxicological evaluation of Grewiaasiatica (Phalsa) powder used as a summer drink. J Chem Soc Pak. 2013;35:320-3.
13. Wojdylo A, Oszmianski J, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105:940-9.
14. Chen X, Sood S, Yang CS, et al. Five lipoxygenase pathway of arachidonic acid metabolism in carcinogenesis and cancer chemoprevention. Curr Cancer Drug Targets. 2006;6:613-22.