Original Article: Antioxidant Activity of Parsley Leaves Extract Containing Apigenin Obtained by Ultrasonic Extraction

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Abstract

The antioxidant activity of parsley extract obtained by ultrasonic-assisted extraction method was evaluated using DPPH radical scavenging method. A high content of total polyphenols (50.82±1.8mg gallic acid equivalent) GAE)/g dried parsley powder), total flavonoids (42.6±1.2 mg quercetin/g dried parsley powder), apigenin (9.48±0.11 mg/g dried parsley powder), and antioxidant activity (27±0.9% at 100 ppm) were quantified in parsley leaves.The extract was purified by column chromatographymethod, and the apigenin purity was determined to be 86% using the HPLC method. The antioxidant activity of the unpurified and purified extracts containing the same amount of apigenin was 39.7±0.9 and 38.1±0.4%, respectively. This indicates that the antioxidant properties of the parsley leave extract were mostly attributed to the presence of apigenin whose antioxidant activity was well demonstrated.

Keywords: Antioxidant activity, Apigenin, Flavonoids, Parsley, Polyphenolic compounds

Introduction

Oxidative stress and free radicals, including peroxyl, alkoxyl, superoxide anion, and hydroxyl, cause damage to DNA, tissues, and cell membranes by attacking biomolecules such as nucleic acid, carbohydrates, lipids, and proteins, and result in cell death by apoptosis (Tan *et al*., 2018).Therefore, they are responsible for causing many diseases, including atherosclerosis, mild cognitive impairment, Parkinson's, Alzheimer's, neural disorders, cardiovascular disease, ulcerative colitis, ageing, cancer, and alcohol-induced liver disease (Alam *et al*., 2013). Generally, antioxidants (natural and synthetic) are involved in the defense mechanism of the human body against the pathologies associated with free radicals' attack. They can either inhibit or delay the oxidation processes occurring under the influence of reactive oxygen species or atmospheric oxygen(Pisoschi and Negulescu, 2011).Furthermore, antioxidants in food can protect oils and lipids against oxidative degradation. In fact, the addition of antioxidants to foods can retard toxic oxidation products formation, control rancidity development, extend product shelf-life, and maintain nutritional quality (Yashin *et al*., 2017). The use of synthetic antioxidants, because of safety concerns, is becoming limited. In contrast, the use of natural antioxidants, which are obtained from edible materials available in the nature, such as plants, herbs, and spices, is increasing considerably.

Plants, vegetables, and fruits contain a wide range of bioactive natural compounds among which are polyphenols, one of the largest group of phytochemicals. Polyphenol compounds are plant secondary metabolites, and so far, more than a thousand molecules of them have been identified in plants (Zhou *et al*., 2019). Multiple biological effects of plants, including their antioxidant activity, commonly are attributed to the presence of these compounds. In general, polyphenols in various plants (edible and non-edible) are divided into two major categories: flavonoids and non-flavonoids (Stagos, 2020).Flavonoids consist of a pyrene ring and two benzene rings, and according to their molecular structures, are divided into six classes: flavonols, is

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of lavones, flavanols, flavones, flavanones, and anthocyanidins. In addition to antioxidant activity, flavonoids have numerous biological activities such as anticoagulant, anticancer, antibacterial, antitumor, anti-inflammatory, anticancirogenic, antiallergenic, vasodilatory and immune-stimulating effects (Trinh *et al*., 2021).Polyphenols and flavonoids could not be synthesized naturally in the human body and can be obtained from plants through diets. Therefore, besides food applications, vegetables and plants have long been used in folklore medicines as effective components(Nguyen *et al*., 2020).

Parsley (*Petroselinum crispum* L.) is one of the most widely known herbaceous species as a food and medicinal plant. Parsley leaves are used for treating diseases such as skin disease, kidney stones, cardiac disease, prostatitis, eczema, hypertension, diabetes, anaemia, urinary tract diseases, nose bleeding, and baldness (Poureini *et al*., 2020). Many of the mentioned therapeutic activities of parsley leaves are due to the anti-inflammatory and antioxidant properties of parsley leaves, which are attributed to the presence of some bioactive compounds in this plant. Flavonoids are one of the main bioactive compound groups detected in parsley leaves, and apigenin (Fig. 1) is a major flavonoid in this plant classified under the flavone group (Cvetanović *et al.*, 2017).

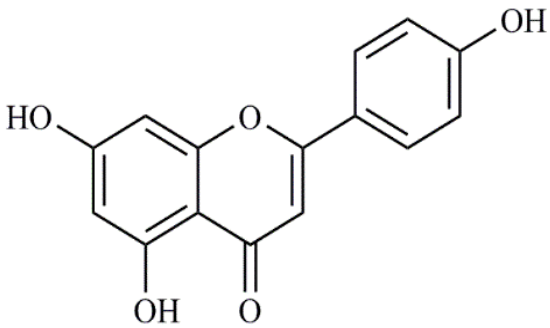

Fig. 1 Chemical structure of apigenin

A study on medicinal plants starts with the extraction of bioactive compounds, and extractionis one of the most important steps in separating the bioactive compounds from plant materials. Traditional extraction methods (maceration, decoction, Soxhlet, and heat reflux) have many disadvantages, such as requiring high energy and long extraction time, which are commonly used at the small manufacturing enterprise and research setting level. In contrast, modern extraction methods, including supercritical extraction, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical fluid extraction, etc., due to their significant advantages such as consumption of less solvent and energy and short extraction time, have been considered for increasing the extraction yield at a lower cost (Azwanida, 2015).

Hitherto, some investigations have been undertaken for thee xtraction of parsley extract using traditional extraction methods to evaluate its antioxidant properties. In this regard, Wong *et al*. (2006) used the heat reflux method using water as a solvent to investigate the antioxidant activity of parsley leaves extract. The total polyphenols and antioxidant activity of the obtained extract were 89.3 mg caffeic acid/100 g fresh weight and 6%, respectively. Juhaimi and Ghafoor (2011)evaluated the antioxidant activity of parsley leaves using the maceration method by distilled water as a solvent. The total polyphenol and antioxidant activity of the obtained extract were 1.2 mg of gallic acid equivalents/100 ml and 32%, respectively. In another investigation, Epifanioa *et al*. (2020) used a decoction method by distilled water for the extraction of parsley leaves extract to characterize and evaluate the antioxidant activity of the extract. The total polyphenol, total flavonoids, and antioxidant activity of the obtained extract were 12.49 mg gallic acid equivalent (GAE)/g of parsley extract, 15.05 mg of quercetin equivalents/g of parsley extract, and 15.50%, respectively.

Despite many advantages of modern extraction methods, so far, few report shave been published on the properties of parsley leaves extracts obtained by modern extraction methods, and the reported antioxidant activities are low. The content of polyphenolic and flavonoids in the obtained plant extract isdirectly associated with their antioxidant activities. Therefore, the extraction method can be effective in the extraction yield of bioactive compounds and, consequently the antioxidant properties of the extracts. In this study, parsley leaves extract was obtained by UAE method. In order to isolate the apigenin, the extract was purified by column chromatography method. The antioxidant activity of the extract and the purified extract was investigated, and the results were compared.

Materials and Methods

Parsley (*P. crispum* L.) was acquired from a grocery store located in Mazandaran, Iran. Apigenin was provided by Santa Cruz Biotechnology Inc. (California, USA).2,2-Diphenyl-1-picrylhydrazyl (DPPH), $NaNO_2$, gallic acid, ethanol, and methanol were obtained from Sigma-Aldrich (Saint Louis, USA). Folin-Ciocalteu reagent, aluminum chloride hexahydrate, sodium hydroxide, and sodium carbonate were provided by Merck (Darmstadt, Germany).

Ultrasonic-Assisted extraction

Parsley leaves extract, which contains a significant amount of apigenin, was extracted by ultrasonic extraction technique according to our previous study(Poureini *et al*., 2020). In brief, parsley leaves were isolated, dried, and ground to obtain a uniform powder; 0.5 g of powder mixed with ethanol: water solution (80:20 (v/v)) was subjected to ultrasonic extraction. The extraction was performed using an Elmasonicultrasonic bath (P30H, Germany) for 30 min at 40°C, and ultrasonic power of 90%. To determine the amount of apigenin, the obtained extracts were analyzed using a UV–vis spectrophotometer (Analytik Jena AG, SPEKOL 1500, Germany). Moreover, to purify the extract, the column chromatography method was used with methanol, hexane, and chloroform as solvents to create different polarities. The purity of apigenin was determined to be 86% using HPLC analyses. For this purpose, an HPLC apparatus (Smartline, Knauer, Berlin, Germany) equipped with Spherisorb 80-5 ODS-2 C18 column (set at 30°C) and UV detector 2500 series was used. The mobile phase consisted of a 0.2% phosphoric acid aqueous solution and methanol (42:58, v/v) and used at a flow rate of1 ml/min.

Total Polyphenols and Flavonoids Content

Total polyphenols of parsley leaves extract were determined by the Folin-Coicalteu method based on the procedure described by Cvetanović *et al*. (2017). In brief, a mixture was prepared by mixing 1 ml of Folin-Ciocalteu reagent, 3 ml of sodium carbonate, 15.8 ml of distilled water, and 0.2 ml of the extract. The mixture absorbance was read at 750 nm. Total polyphenols content in the extract was calculated based on the standard calibration curve (y= 101.3843x+0.3626; R^2=0.9938) generated with gallic acid. The result was represented as mg of gallic acid equivalent (GAE)per g of dried parsley powder (DPP).

Moreover, for determination of the total flavonoids content in the obtained extract, the colorimetric assay was used based on the method described by Cvetanović *et al*. (2017). Briefly, 1 ml aliquots along with 3 ml $NaNO_2$ solution were mixed with aluminum chloride hexahydrate (0.3 ml) and sodium hydroxide (1 ml). Sample absorbance was spectrophotometrically measured at 510 nm to calculate the total flavonoid content from a previously obtained calibration curve with quercetin (y=11.59327x-0.02744; R^2=0.9943), as mg of quercetinper g of DPP.

All experiments and the estimation of polyphenol content in all samples were carried out in triplicate.

DPPH Radical Scavenging Capacity

The radical scavenging capacity (RSC)of the ultrasonic extracts of parsley leaves with different concentrations was specified by the DPPH method (S. Yang *et al*., 2018); this was used as a measure of antioxidant activity. The ultrasonic extracted solution of parsley leaves was dried at 40°C, and by diluting with methanol, different concentrations from 40 to 100 ppm were created. Methanolic extract solutions and the prepared methanolic purified extracts solution (50 ppm) were analyzed in parallel for comparison. For this purpose, 0.5 ml of prepared sample was mixed with methanol solution of DPPH (4.5 ml, 0.1 mM). The samples were incubated at room temperature for 30 min in dark. Then, DPPH solution without extract was applied as a control. For measuring the radical scavenging activity of the samples, their absorbance values were immediately read at 517 nm by a UV-vis spectrophotometer. To estimate the RSC, the following equation was used:

$$\text{DPPH RSC (\%)} = \left[1 - \frac{A_{sample}}{A_{control}}\right] \times 100 \quad (1)$$

where A_{sample} represents the absorbance of the extract and $A_{control}$ denotes the absorbance of the control solution.

Statistical Analysis

All experiments performed in this study were conducted in triplicate, and the quantitative data were shown as mean value±SD. Data were statistically

analyzed by One-Way ANOVA followed by Tukey's comparison test using SPSS 18.0 statistics software (IBM Corp., Armonk, New York). A value of *p<0.05 was considered a statistically significant difference.

Results and Discussion

UV-vis spectra of standard apigenin and parsley extract at different are shown in Figure 2. The recorded spectra of standard apigenin showed two characteristic peaks at 268 and 337 nm; bothpeaks were observed in the spectra of all extracts, which had different concentrations (40 to 100 ppm). By increasing the concentration of the extract, the intensity of these characteristic peaks increased, which indicates the presence of a higher amount of apigenin in the extract.

Total Polyphenols and Flavonoids Content

Total polyphenols, total flavonoids, and apigenin content of the parsley extract are represented in Table 1. Results indicate that the parsley extract contained average total polyphenols, total flavonoids, and apigenin of50.82±1.8mg GAE/g DPP, 42.6±1.2mg quercetin/g DPP, and 9.48±0.11mg/g DPP, respectively. For the sake of comparison, total polyphenols, apigenin content, and total flavonoids of parsley leaves extract obtained in this study and some other studies are summarized in Table 1.A comparison of the results indicates the high efficiency of the used method in this study to obtain a high yield of bioactive compounds.

Antioxidant Activity

The antioxidant activity of parsley extract at different concentrations was measured by DPPH assay; the results of this analysis are presented in Fig. 3. As shown in Figure 3, the RSC of the extract with a concentration of 40 ppm was 8±0.3%. By increasing the extract concentration from 40 to 100 ppm, the RSC enhanced from 8±0.3 to 27±0.9%

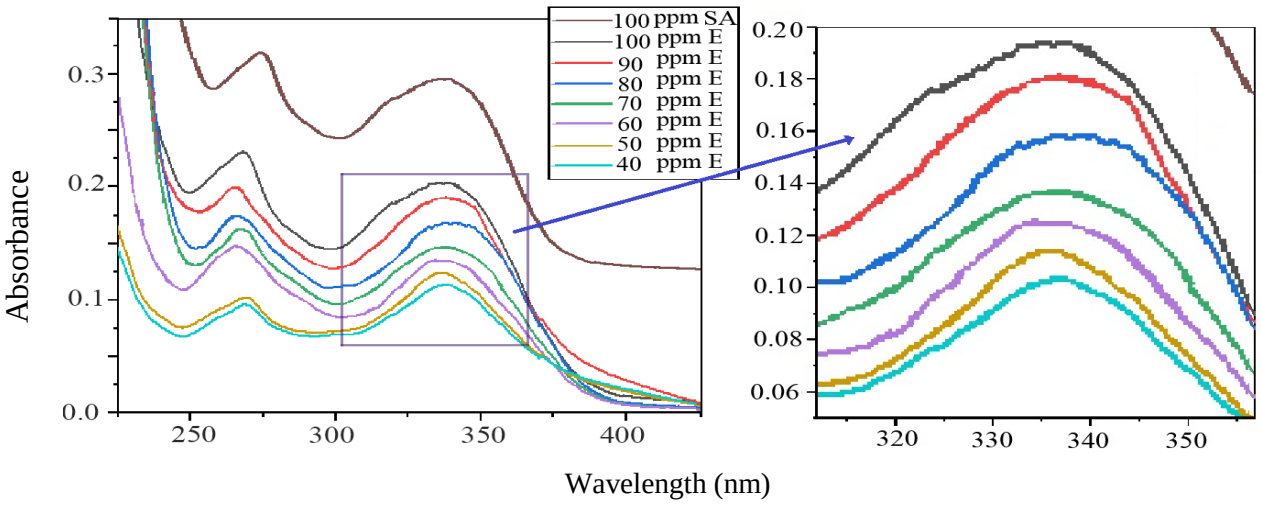

Fig. 2 UV-visible spectra of standard apigenin (SA) and parsley extract (E) with different concentrations obtained from UAE method

Table 1 Comparison between total polyphenols, total flavonoids, and apigenin content of the parsley leaves extract obtained by UAE in this study and some other studies on plants extract containing apigenin

Plant sample	Extraction method	TPC (mg GAE/g DPP)	TFC (mg Q/g DPP)	AC (mg/g DPP)	Ref.
P. crispum L.	UAE	50.82±1.8	42.6±1.2	9.48±0.11	This study
	Blanching	46.04±0.22	41.14±0.19	-	(Kaiser *et al*., 2013)
	PLE	23.00	-	-	(Luthria, 2008)
	Vortex mixing	12.80	-	-	(Luthria *et al*., 2006)
Chamomilla matricaria L.	SWE	98.78±1.09	14.72±0.4	3.33±0.32	(Cvetanović *et al.*, 2017)
Scutellaria barbata D. Don	USC-CO_2-E	-	-	2.43±0.09	(Y.-C. Yang and Wei, 2018)

TPC=total polyphenols content; TFC= total flavonoids content; AC=apigenin content; Q=quercetin; DPP= dried parsley powder; PLE=pressurized liquid extraction; SWE= subcritical water extraction; USC-CO_2-E= ultrasound-assisted supercritical CO_2 extraction

Figure 4 shows the antioxidant activityof parsley extract and the purified extract obtained fromparsley. As prior described, purification of the extract was carried out by column chromatography and based on the HPLC analysis, the purity of apigenin in this sample was 86%. Results (Fig. 4) show that the antioxidant activity of the extract containing 50 ppm apigenin and 50 ppm purified apigenin was 39.7±0.9 and 38.1±0.4, respectively. These observations demonstrate that the antioxidant activityof parsley extracts isattributed to the presence of apigenin. Previous studies have also reported the antioxidant activity of apigenin(Romanova *et al*., 2001). Apigenin imparts a protective effect and reduces inflammation by increasing the cellular antioxidant activity (such as superoxide dismutase, catalase, and glutathione content) and reducing lipid peroxidation (Ali *et al*., 2017).

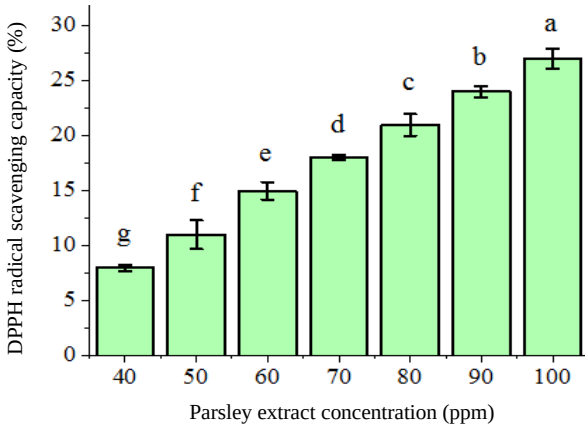

Fig. 3 Antioxidant activities (DPPH radical scavenging) of parsley leaves extracts at different concentrations obtained from UAE method, and different letters above bars(a–g) showsignificant differences(P<0.05)

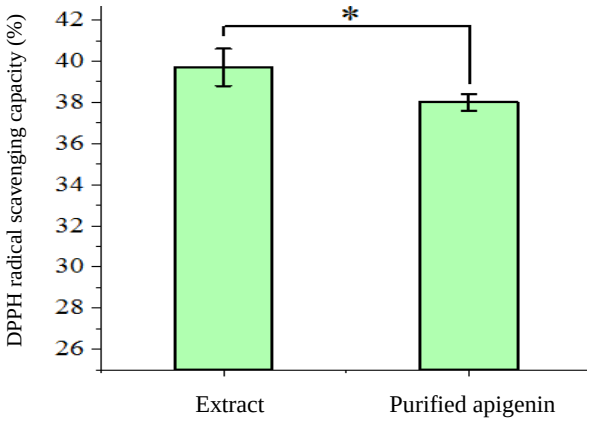

Fig. 4 Antioxidant activity (DPPH radical scavenging) of extract and purified extract of parsley leaves containing the same concentration of apigenin (50 ppm) (n=3, mean±SD,* p<0.05)

A slight increase in the antioxidant activity of the unpurified extract could be due to a synergistic effect between apigenin and other compounds with antioxidant properties in the extract.

Conclusions

In the present study, the antioxidant activity of the extract obtained from parsley leave using UAE method was analyzed. According to the results, the total polyphenols, total flavonoids, and total apigenin contents of the parsley leaves extracts were 50.82±1.8 mg GAE/g DPP, 42.6±1.2 mg quercetin/g DPP, and 9.48±0.11 mg/g DPP, respectively. Also,the antioxidant activity of the extract at 100 ppm was 27±0.9%. The antioxidant activity of the purified apigenin was compared with that of unpurified extract containing the same amount of apigenin. The antioxidant activity of the unpurified extract (39.7±0.9%) was slightly different from purified apigenin, with antioxidant activity of 38.1± 0.4%; therefore, it is most likely that the antioxidant activity of the parsley extract originates from its apigenin content.

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References

Al-Juhaimi, F. and Ghafoor, K. 2011. Total phenols and antioxidant activities of leaf and stem extracts from coriander, mint and parsley grown in Saudi Arabia. Pakistan Journal of Botany, 43: 2235-2237.

Alam, M.N., Bristi, N.J. and Rafiquzzaman, M. 2013. Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi pharmaceutical Journal, 21: 143-152.

Ali, F., Rahul, Naz, F., Jyoti, S. and Siddique, Y.H. 2017. Health functionality of apigenin: A review. International Journal of Food Properties, 20: 1197-1238.

Azwanida, N. 2015. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal and Aromatic Plants, 4: 2167-0412.

Cvetanović, A., Švarc-Gajić, J., Gašić, U., Tešić, Ž., Zengin, G., Zeković, Z. and Đurović, S. 2017. Isolation of apigenin

from subcritical water extracts: optimization of the process. The Journal of Supercritical Fluids, 120: 32-42.

De Menezes Epifanio, N.M., Cavalcanti, L.R.I., Dos Santos, K.F., Duarte, P.S.C., Kachlicki, P., Ożarowski, M., Riger, C. and de Almeida Chaves, C.J. 2020. Chemical characterization and *in vivo* antioxidant activity of parsley (*Petroselinum crispum*) aqueous extract. Food & function, 11: 5346-5356.

Kaiser, A., Carle, R. and Kammerer, D. R. 2013. Effects of blanching on polyphenol stability of innovative paste-like parsley (*Petroselinum crispum* (Mill.) Nym ex AW Hill) and marjoram (*Origanum majorana* L.) products. Food chemistry, 138: 1648-1656.

Luthria, D.L. 2008. Influence of experimental conditions on the extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes using a pressurized liquid extractor. Food Chemistry, 107: 745-752.

Luthria, D.L., Mukhopadhyay, S. and Kwansa, A.L. 2006. A systematic approach for extraction of phenolic compounds using parsley (*Petroselinum crispum*) flakes as a model substrate. Journal of the Science of Food and Agriculture, 86: 1350-1358.

Nguyen, V., Le, V., Vo, T., Bui, L., Anh, H. and Danh, V. 2020. Preliminary phytochemical screening and determination of total polyphenols and flavonoids content in the leaves of *Houttuynia cordata* Thunb.Materials Science and Engineering, 734: 062013.

Pisoschi, A.M. and Negulescu, G.P. 2011. Methods for total antioxidant activity determination: a review. Biochem Anal Biochem, 1: 106.

Poureini, F., Mohammadi, M., Najafpour, G.D. and Nikzad, M. 2020. Comparative study on the extraction of apigenin from parsley leaves (*Petroselinum crispum* L.) by ultrasonic and microwave methods. Chemical Papers, 74: 3857-3871.

Romanova, D., Vachalkova, A., Cipak, L., Ovesna, Z. and Rauko, P. 2001. Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. Neoplasma, 48: 104-107.

Stagos, D. 2020. Antioxidant Activity of Polyphenolic Plant Extracts. Antioxidants, 9:1-19.

Tan, B.L., Norhaizan, M.E., Liew, W.P.P. and Sulaiman Rahman, H. 2018. Antioxidant and oxidative stress: a mutual interplay in age-related diseases. Frontiers in pharmacology, 9: 1162.

Trinh, D., Trung, H., Nguyen, X., Tráng, N., Cao, H., Vo, A., Vu, T., Bui, C., Thanh, V. and Tran, L. 2021. Studies on the antioxidant activity of apigenin, luteolin and nevadensin using DFT. Vietnam Journal of Science and Technology, 59:1-19.

Wong, P.Y. and Kitts, D.D. 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts. Food chemistry, 97: 505-515.

Yang, S., Dai, L., Sun, C. and Gao, Y. 2018. Characterization of curcumin loaded gliadin-lecithin composite nanoparticles fabricated by antisolvent precipitation in different blending sequences. Food Hydrocolloids, 85: 185-194.

Yang, Y.C. and Wei, M.C. 2018. Development and characterization of a green procedure for apigenin extraction from *Scutellaria barbata* D. Don. Food chemistry, 252: 381-389.

Yashin, A., Yashin, Y., Xia, X. and Nemzer, B. 2017. Antioxidant activity of spices and their impact on human health: A review. Antioxidants, 6: 70.

Zhou, Y., Jiang, Z., Lu, H., Xu, Z., Tong, R., Shi, J. and Jia, G. 2019. Recent Advances of Natural Polyphenols Activators for Keap1-Nrf2 Signaling Pathway. Chemistry & biodiversity, 16: 1900400.