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Comparative evaluation of total phenolic content, total flavonoids content and antioxidants activity in Skin & Pulp extracts of Cucurbita maxima

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Abstract

The aim of this study was to determine the total phenolic content, total flavonoids content, and antioxidant activity of various extracts of pumpkin Cucurbita maxima skin and pulp. The various solvents used for extraction were acetone, methanol, ethanol and water. The total phenolic content in pulp extracts ranged from 9.72 ± 0.37 to 106.92 ±0.47 mg GAE/g of extract, while in skin extracts ranged from 55.91 ± 5.67 to 336.19 ± 0.89 mg GAE/g of extract. The total flavonoids content varied from 0.13 ± 0.01 to 2.21 ±0.06 mg CE/g of extract in pulp extracts and from 1.17 ± 0.08 to 4.64 ± 0.02 mg CE/g of extract in skin extracts. Antioxidant activity of extracts was expressed as percentage of 2, 2-diphenyl-1-picrylhydrazyl (DPPH), IC50 values (mg/ml), and radicals inhibition. The IC50 values in pulp ranged from 1.55 to 4.01 mg /ml and from 1.12 to 67.64 mg/ml in skin. The highest antioxidant scavenging activity was obtained in water skin extract (lowest IC50 value of 1.12 mg/ml). The Cucurbita maxima skin extracts contains impressively higher total phenolic and total flavonoids contents when compared to pulp extracts. The present study revealed that the skin followed by the pulp of pumpkin Cucurbita maxima could be used as an excellent natural source of antioxidant.

Keywords: *Phenolic content, flavonoids content, antioxidants activity, Skin, Pulp, extracts Cucurbita maxima*

1. Introduction

Antioxidants are molecules that eliminate the oxidation of other molecules. Antioxidant hypothesis has been based on neutralizing the oxidative damage resulting from the action of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These species are usually produced in vivo both for specific metabolic reasons or following exposure to exogenous factors [1-3]. Accumulation of ROS and RNS lead them to react with biological macromolecules such as lipids, protein, and carbohydrates, and nucleic acids. High levels of certain reactive species can contribute to cell damage and development of diseases [4]. Moreover, when a greater imbalance occurs in favor of the ROS, oxidative stress develops. Oxidative stress is a phenomenon which was related to the development of many pathological conditions [1]. Pathologies where ROS were identified as fundamental factors include cardiovascular disease, diabetes, rheumatoid arthritis, cancer, neurodegenerative disorders and inflammatory bowel diseases [5, 6], and the use of exogenous antioxidants was proposed for their treatment [7]. Phenolic compounds are widely distributed in plant kingdom, and considered as dietary antioxidant [8]. Chemically, they are compounds with an aromatic ring linked to one or more hydroxyl groups. They possess structures from different moieties; therefore, they have different classes such as simple phenols, benzoic acids, hydroxycinnamic acids, coumarins, naftoquinones, stilbenoids, flavonoids, and condensed tannins [9]. In addition, phenolic compounds have been received much attention on their effective antioxidant properties, they serve as electron donor, (ROS) and (RNS) scavenger and power reducer [10].

Cucurbita maxima (pumpkin) belongs to the family Cucurbitaceae. It is consist of approximately 90 genera with 700 species, mainly in tropical and subtropical regions [11]. Pumpkin is widely cultivated for use as vegetable and medicine such as antidiabetic, anti-inflammatery, antitumor, antihypertensive, and antibacterial agents [12]. In addition to that, pumpkins are considered valuable vegetables since they have higher carotenoid content and low energy value [13]. It is also an excellent source of fiber, vitamin E, vitamin C, manganese, magnesium and potassium. Moreover, pumpkins contain vitamins B1, B3, B5 and B6 [14]. To our knowledge, there is no published data until now concerning evaluation of antioxidant properties of pumpkin C. maxima skin. Thus, this study aims to evaluate and compare the total phenolic and flavonoid contents and antioxidant activity of skin and pulp using various extracts of pumpkin C. maxima in order to explore novel potential sources for natural antioxidants in food and pharmaceutical formulations.

2. Materials and methods

2.1. Experimental section

2.1.1. Plant Material

Fresh pumpkin C. maxima was purchased from local market in Al-Medina, KSA. Pumpkin C. maxima was washed by distilled water to be ready for use.

2.1.2. Apparatus

UV –VIS double beam spectrophotometer (Cintra 6 GBC) and 1 cm glass cells were used for all absorbance measurements, Sonicator bath (2510, Branson).

2.1.3. Chemicals

The aluminum chloride and Folin- Ciocalteu's phenol reagent were purchased from Fluka Chemie AG. Acetone, methanol, ethanol, Catechin standard, 2,2-diphenyl-1-picrylhydrazyl and Gallic acid standard, were obtained from Sigma Chemicals Co. All chemicals were of analytical grade.

2.1.4. Preparation of plant extracts

Plant material (20 g of pulp or skin) was shaped to small pieces (2-5 mm), grinded with pestle and mortar in 100 ml of each solvent (water, methanol, ethanol, and acetone), mixed, macerated for 2 h at room temperature, and then stored at 4◦C in a refrigerator for 48 h. The macerated solutions were sonicated for 30 min, and filtered. The filtrates were collected. Acetone, methanol and ethanol and water extracts were evaporated to dryness. Then each dried extract was dissolved in 100 ml of the same solvent, stored in a refrigerator at 4 ◦C, and then used as a sample extract.

2.2. Determination of total phenolic content (TPC):

The total phenolic content was evaluated using Folin–Ciocalteu's method [15, 16, 17]. A 2.0 ml extract was added to 2.5 ml of diluted Folin-Ciocalteu reagent (1:10, V/V), 2.0 ml sodium carbonate (Na_2CO_3), and 3.5 ml of water. The mixture was then incubated for 5 min in water bath at 50°C, and cooled for 5 min. The absorbance was then measured at 760 nm with a UV-Vis Spectrophotometer. All samples and readings were prepared and measured in triplicate, and the TPC were then expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g extract) that were derived from a calibration curve of Gallic Acid.

2.3. Estimation of total flavonoids content (TFC):

Total flavonoids content was estimated following a method described previously using catechin as standard [18]. In a 10.0 ml test tube, 3.0 ml of distilled water, and 0.3 ml of $NaNO_2$ (0.5 M) were mixed with 2 mL of extracts solution . After 5 min, 0.3 ml of $AlCl_3.6H_2O$ (0.3 M) was added to the mixture. After 6 min, 2 ml of NaOH (1 mM) and 2.4 ml of distilled water were added to the mixture. The absorbance of the mixture solution was measured at 510 nm. The content of flavonoids was expressed in terms of Catechin equivalent /g of extract (mg CE/g extract). This process was performed in triplicate for each extract.

2.4. Determination of antioxidant activity using DPPH- scavenging assay

Antioxidant potential of pumpkin C. maxima pulp and skin was estimated based on their scavenging activity to 2, 2-diphenyl-1-picryl-hydrazyl. DPPH is stable free radical that accepts hydrogen radical or an electron to become stable diamagnetic molecule. It is used to estimate the anti- oxidant activity of several natural compounds [19]. The percentage radical scavenging activity was determined accordingly [15, 18]. DPPH methanolic solution (0.1 mM) was prepared and kept in amber colored bottle to protect from sunlight. A 1.0 ml of as-prepared solution was mixed with 1.0 ml of diluted extract solution. Then, the mixture was incubated during 30min in darkness at room temperature, and its absorbance was measured at 517 nm. Percentage of inhibition was determined using the equation 1 (control simple contained all the reagents except the extract). While, the IC50 values were determined from the % inhibition versus concentration plot [20].

$$\%\ Inhibition = \left(\frac{A\ of\ control - A\ of\ sample}{A\ of\ control}\right) x100 \quad (1)$$

Where: A control - is the absorbance of the control reaction. A sample - is the absorbance of the extract sample.

3. Results and Discussions

3.1. Total Phenolic Content of the extracts

The total phenolic contents (TPC) in the examined C. maxima extracts (pulp, and skin) using the Folin-Ciocalteu method was estimasted in terms of Gallic acid equivalent (GAE) (the obtained standard curve equation: y = 0.0006x+

0.0012, R^2 = 0.9932). The values obtained for the total phenols content for pulp and skin are expressed as mg of GAE/g of extract as shown in tables 1 and 2 respectively.

Table 1. Total phenolic and flavonoids contents in various pulp extracts of C. maxima

Type of Sample extract (Pulp)	TPC [1] (mg GAE/g extract)	TFC [1] (mg CE/g extract)
MeOH	**9.72 ± 0.37**	**0.96 ± 0.01**
EtOH	**17.68± 0.13**	**2.21 ± 0.06**
Acetone	**38.82 ± 0.69**	**1.21 ± 0.01**
Water	**106.92 ±0.47**	**0.13 ± 0.01**

1 Each value is the average of three replicates ± SD

Table 2. Total phenolic and flavonoids contents in various skin extracts of C. maxima

Type of Sample extract (Skin)	TPC [1] (mg GAE/g extract)	TFC[1] (mg CE/g extract)
MeOH	**55.91 ± 5.67**	**3.11 ± 0. 02**
EtOH	**311.71± 2.14**	**4.64 ± 0.02**
Acetone	**336.19 ± 0.89**	**1.17 ± 0.08**
Water	**181.17 ± 0.50**	**1.62 ± 0.06**

1 Each value is the average of three replicates ± SD

The TPC in the examined pulp extracts of C. maxima ranged from 9.72 ± 0.37 to 106.92 ±0.47 mg GAE/g of extract (table 1). The water pulp extract contained highest TPC followed by acetone, ethanol and methanol extracts where, the total phenolic content were 106.92, 38.82, 17.68 and 9.72 mg GAE/g extract respectively. While, the TPC in the examined skin extracts ranged from 55.91 ± 5.67 to 336.19 ± 0.89 mg GAE/g of extract (table 2). The highest TPC was measured in acetone and ethanol extracts of C. maxima skin. Acetone exhibited high total phenolic content followed by ethanol, methanol and water, where the total phenolic content were respectively 336.19, 311.71, 181.17 and 55.91 mg GAE/g extract. Therefore, the results indicate that the TPC in the C. maxima skin extracts is significantly higher than that obtained in pulp extracts regardless of the solvent used in the extraction. Several studied reported that different extracting solvents influenced different yields of TPC. In fact, Mohsen et al. [21] revealed that phenolic compounds in corn tassels can be extracted with ethanol and methanol better than others solvents as result of their polarity and good solubility for phenolic components [22, 23]. In other study, for TPC estimation from henna stem (Lawsonia inermis), it was been found that 40% acetone extraction at room temperature yield about 47.97 ± 0.71 mg GAE/ g extract [24]. Moreover, Addai et al. [25] reported that extraction solvent had significant effects on antioxidant activity, TPC, and TFC when they studied the effect of extraction solvents on the phenolic content and antioxidant properties of two papaya cultivars. They reported that methanol 50% showed the highest antioxidant activity in both cultivars of papaya fruit followed significantly with acetone 50%, whereas the TPC and TFC in the two papaya fruit varieties showed significant difference (the highest TPC of papaya cultivars was in acetone extract 39.81 ± 0.93 mg GAE/g extract). In addition, Ahin et al. [26] revealed that the water extract of Prunella grandiflora has the highest total phenol content (24.63 ± 0.55 mg GAE/g extract).

Furthermore, recently, Chekroone et al. [27] have found that the most important amount of total polyphenols was determined in butanolic extract of B. dioica roots (541.78 mg/g extract) while they obtained lowest values in C. colocynthis fruit aqueous extract (219.58 mg/g extract). Therefore, this work is in accordance with the previous studies reported above revealing that different extracting solvents influenced TPC yields. However, in C. maxima skin the values of TPC obtained in different solvent extracts were significantly higher than those estimated in C. maxima pulp and many other plants as reported above [24, 25, 26].

3.2. Total flavonoids Contents of the extracts

Flavonoids are considered as the most important natural phenols. They have a broad spectrum of biological and chemical activities including radical scavenging properties [28]. The content of flavonoids in various extracts of C. maxima (skin and pulp) was evaluated using spectrophotometric method with aluminum chloride using catechin as standard (the obtained standard curve equation: $y = 0.053\ x - 0.003$, $R^2 = 0.997$). The content of flavonoids was expressed in terms of Catechin equivalent (mg of CE /g of extract). The total flavonoids content (TFC) in pulp extracts of C. maxima ranged from 0.13 ± 0.01 to 2.21 ± 0.06 mg of CE/g of extract (table 1). The highest amount of flavonoids was obtained in ethanol extract followed by acetone, methanol and water extracts. While, the TFC in skin extracts C. maxima was found ranging from 1.17 ± 0.08 to 4.64 ± 0.02 mg CE /g of extract (table 2). The highest amount of flavonoids in skin C. maxima was obtained in ethanol extract followed by methanol, water, and acetone extract. The flavonoids content is higher in skin compared to pulp for the different extracts. The best total flavonoids content in C. maxima pulp and skin was obtained using ethanol as solvent of extraction. Several studies reported that the higher estimated total flavonoids is obtained using ethanol extract. In fact, Patel et al. [29] reported that the leaves of Tephrosia purpurea Linn. (Leguminosae) have a TFC in ethanolic extract of 1.56 ± 0.12 Quercetin QE/g of extract). Also, Enujiugha et al. [30] revealed that the Raw African Yam Bean (Sphenostylis stenocarpa) has a high total flavonoids content in 70% ethanol extract 0.223 ± 0.002 (mg CE/ g of extract). Furthermore, Ghasemi et al. [31] estimated the total flavonoids content (based on colorimetric $AlCl_3$ method) of 13 citrus methanol extracts tissues and peels and reported that it ranged from 0.3 to 31.1 mg quercetin equivalent/g of extract.

3.3. DPPH- scavenging assay of extracts of Cucurbita maxima pumpkin pulp and skin

The antioxidant activity was estimated using the stable DPPH radical. The method consisted of spectrophotometric measurement of the intensity of the color change in solution depending on the amount of DPPH [15]. Free radical scavenging effects results were defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% (IC50). The highest antioxidant capacity was indicated by the lowest IC50 values [32]. Figures 1 and 2 shows the increase of radical scavenging activity of each pulp and skin extracts which was deduced from the increase of percent of inhibition as the extract concentration increases. In fact, the DPPH radical scavenging activity (IC50) was different for each pulp extracts of C. maxima, and varies in the range of 1.55 to 4.01 (mg/ml). While, it ranged between 1.12 to 67.64 (mg/ml) for skin extracts of C. maxima.

As presented in Figure 3, the IC50 values show the following decreasing order for pulp extracts: acetone extract (1.55 mg/ml) < methanol extract (2.20 mg/ml) < water extract (3.06 mg/ml) < ethanol extract (4.01 mg/ml). While, for skin extracts the obtained IC50 values are as follow: water extract (1.12 mg/ml) < ethanol extract (3.83 mg/ml) < methanol extract (11.58 mg/ml) < acetone extract (67.64 mg/ml).

Among all skin extracts, water extract of C. maxima exhibited the highest antioxidant activity followed by ethanol, methanol and acetone extract. While, by comparing pulp extracts, acetone extract exhibited the highest antioxidant activity followed by water, ethanol and methanol extract.

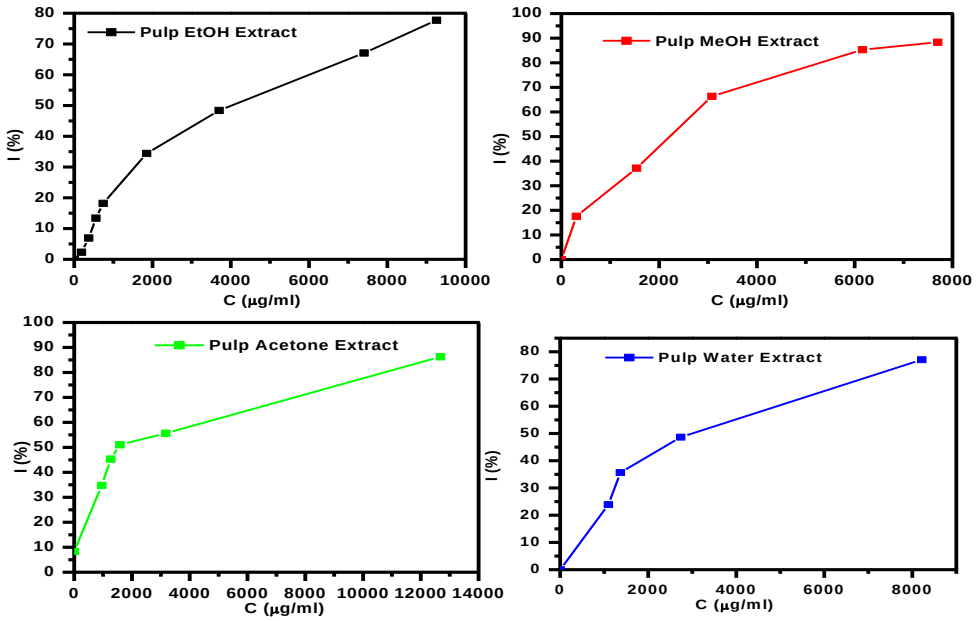

Figure 1. DPPH radical-scavenging activity of C. maxima pulp in different extracts (Values are expressed as mean ± SD of three replicates).

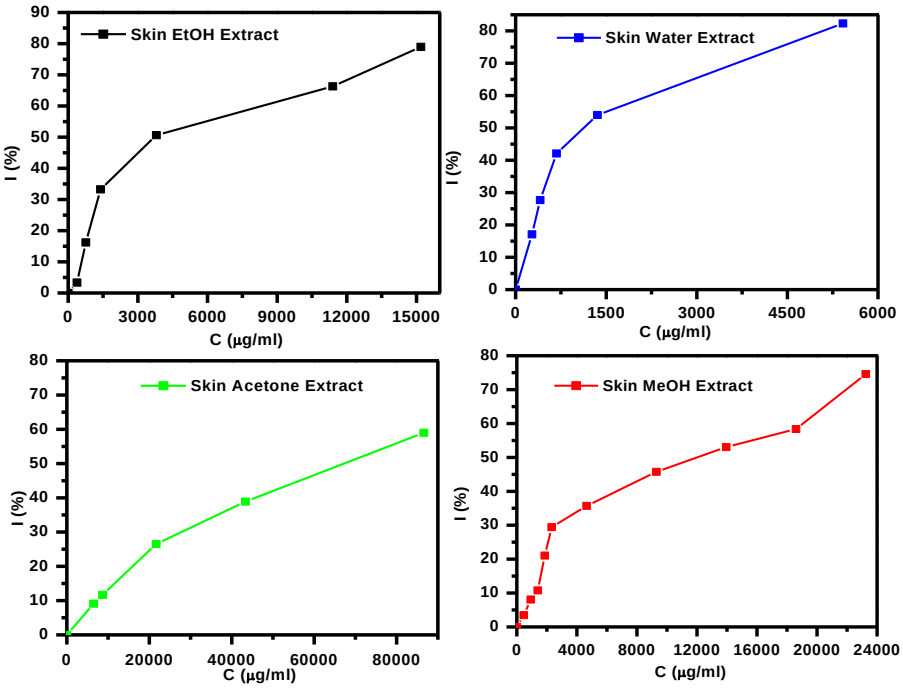

Figure 2. DPPH radical-scavenging activity of various extracts of C. maxima Skin (Values are expressed as mean ± SD of three replicates).

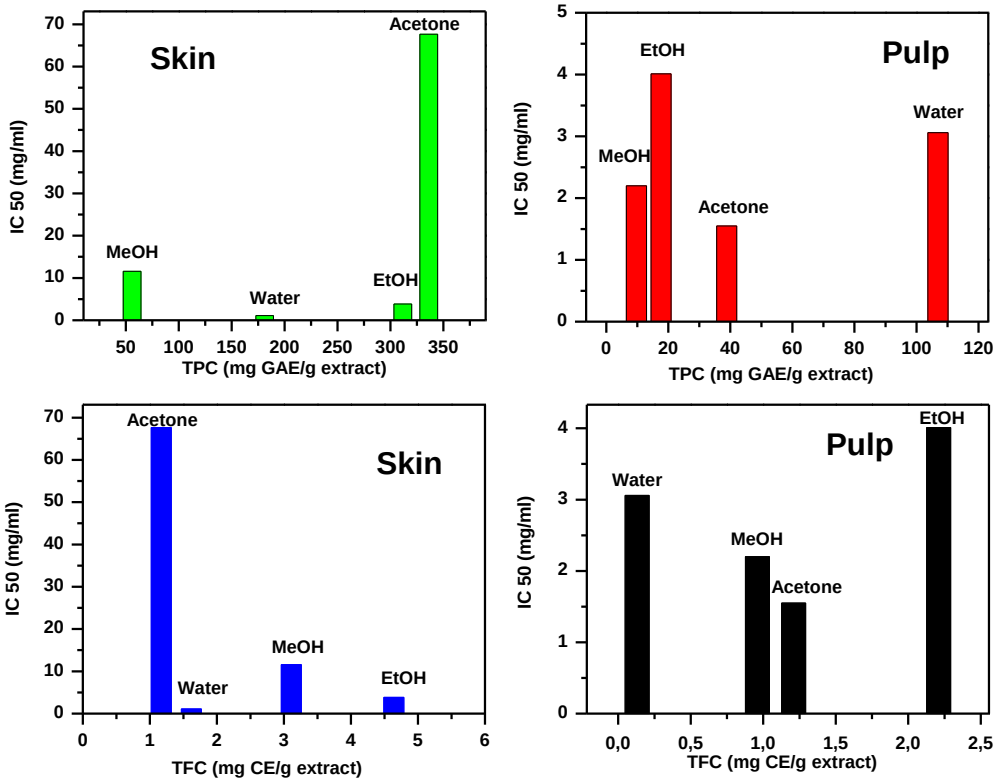

Figure 3. Relationship between antioxidant activity in different extracts of C. maxima pulp and skin and total phenols and flavonoids contents (Values are expressed as mean ± SD of three replicates).

All C. maxima skin and pulp extracts presented an interesting scavenging activity. The water extract of skin C. maxima exhibited the highest antioxidant activity (the lowest IC50 values of 1.12 mg/ml), comparatively to all C. maxima extracts. Ooomah et al. [33] reported in the study of phenolic and antioxidant activity of various extracts of lentil and pea hulls (acetone, ethanol, water and hot water extracts), that the most potent phenolic antioxidant was estimated in water extract of red and green lentil hulls, followed by those extracted with ethanol, acetone, and hot water. Furthermore, Tatiya et al. [34] have studied the effect of various solvents such as water, acetone (70%), methanol (50%) and ethanol (50%) on the total polyphenol content, antimicrobial and antioxidant activities of Bridelia retusa and reported that acetone extract contains the highest TPC and shows the highest antioxidant activity. The IC50 values were in the range of 61.93 to 70.46 mg/ml. Several studies has been proven that antioxidant activity of plant extracts is mainly related to the TPC in the plants [21, 27, 35-39]. In this study, the correlation between total phenolic and total flavonoids contents and radical scavenging activity of C. maxima skin and pulp extracts were investigated. The skin extracts of C, maxima have more phenolics and flavonoids contents than pulp extracts. However, as shown in Figure 3, there were a weak correlation between the total phenolic and/ or flavonoids contents and antioxidant activity in C. maxima skin and pulp extracts ($r < 0.5$). This result is in agreement with the results of several studies [40-42]. Furthermore, when 13 citrus species peels and tissues were compared for their TPC, TFC, and antioxidant activity [31], it has been found that the TPC (determined by folin Ciocalteu method) ranged from 66.5 to 396.8 mg gallic acid equivalent/g of extract and TFC (determined by colorimetric $AlCl_3$ method) ranged from 0.3 to 31.1 mg quercetin

equivalent/g of extract. There were no correlation between TPC and / or TFC and antioxidant activity in tissues and/or peels. The results obtained in the present study can be explained by the fact that scavenging action of various phenolic compounds can be affected by their spatial conformation. In fact, the epicatechin unit of phenol has been found to be more efficient than catechin [43]. Only flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity [44, 45]. In addition, the extracts contains many different compounds with distinct activities [44, 45]. Furthermore, the weak correlation can be due to the fact that total phenolic content may not incorporate all the antioxidants that can be present in an extract [46, 47].

4. Conclusion

In conclusion, the total phenolic content, the total flavonoids content and free radical scavenging activity of skin and pulp of C. maxima extracts showed differences depending on extracting solvents used and type of part of plant material. C. maxima skin contain high amounts of polyphenols and flavonoids when compared to C. maxima pulp in all tested solvent extracts. Furthermore, all skin and pulp extracts of C. maxima presented an interesting antioxidant activity while skin water extract exhibited the most significantly elevated activity among them. Data from the present study revealed that the skin of pumpkin C. maxima could be used as an excellent natural source of antioxidant. These results will serve as a precursor for further research and improvement strategies of this important plant.

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