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Antioxidant activity of Butternut squash Skin: Effect of different extracting solvents

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Abstract

The goal of this work is to study the effect of different extracting solvents on antioxidant activity, total phenolic content (TPC) and total flavonoids content (TFC) of Butternut squash skin extracts. The solvents used for extraction were acetone, methanol, and water. The TPC was estimated using Folin-Ciocalteu method and their amounts in the extracts varied from 150.09 ± 4.89 to 359.61 ± 5.77 mg GAE/ g of extract. The TFC was determined in all extracts by colorimetric $AlCl_3$ method and their amounts varied from 0.79 ± 0.01 to 1.18 ± 0.03 mg CE/g of extract. Antioxidant activity was estimated using DPPH method. For all extracts, the antioxidant activity was expressed as percent of inhibition and IC_{50} values ranged from 0.21 to 1.00 mg/l.

The study showed that TPC, TFC, and antioxidant activity depends on the solvent used for extraction. Interestingly, skin extracts of Butternut squash showed higher total phenolic content and higher antioxidant activities among all examined extracts.Butternut squash skin is a new precious source of phenolic compounds that can be considered as a natural antioxidant of high value for different applications.

Keywords: *Antioxidants activity, Phenolic content, flavonoids content, Skin, extracts, Butternut squash*

1. Introduction

Pumpkin is a crop plant of high economic importance in the last decade. It was cultivated as edible fruit for many years. A large range of cultivars is available, containing those grown to produce many types of pumpkin fruits such as marrows and zucchinis. The common name pumpkin is used for more than one species of Cucurbita plant such as, C. pepo, C. maxima, C. argyrosperma, and C. moschata (Butternut squash) according to the shape and texture of their stems [1, 2]. This plant is wealthy of polyunsaturated fatty acids, proteins, trace elements, such as zinc and antioxidant vitamins such as carotenoids and tocopherol [3-5]. It is also used in traditional systems of medicine [2]. Antioxidants are important for maintaining health and well-being, as they can deactivate, or stabilize free radicals before they attack cells. They are substances that inhibit oxidation, and have the ability to counteracting the damaging effects of oxidation in body. In fact, they help to scavenge free radicals within the human body. Free radicals have an unpaired electron, which permit them to neutralize themselves by capturing and seeking out electrons from other substances [6-11]. If the production of free radicals is excessive or antioxidants are unavailable in the body, damage and oxidative stress damage can occur [12, 13]. Several studies reported that free radicals have been implicated in the pathogenesis of many diseases such as, cancer, diabetes mellitus, arthritis, atherosclerosis and neurodegenerative diseases as well as ageing process [14-18]. An increased consummation of food rich in natural antioxidants leads to lower risks of degenerative diseases, especially cancer and cardiovascular diseases [19]. Thus, several plants have been largely studied to evolve natural antioxidants for cosmetic, food, and other applications [20]. The major classes of chemicals plant are alkaloids, terpenoids, and phenolic metabolites [21]. In fact, the phenolic compounds are very important for dietary applications [22, 23]. They include polyphenols (condensed and hydrolyzable tannins), flavonoids, and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) [24-26]. These compounds have been used as antioxidants by humans and protect fruits, vegetables, and plants from oxidative damage. Several studies reported that pumpkin extracts showed a promising antioxidant activity, which could have many health benefits, especially for diabetics, pre-diabetics and vascular injury individuals [14, 15, 27, 28]. However, the skin of Butternut squash pumpkin variety was not yet explored for its antioxidant properties. Our goal is therefore to study the effect of different extracting solvent on TPC, TFC and antioxidant activity of Butternut squash skin extracts and to explore the relationship between TPC, TFC, and antioxidant activity.

2. Materials and methods

2.1. Experimental section

2.1.1. Plant Material

Fresh Pumpkin Squash originated from Al-Medina, KSA was obtained from a supermarket in AlMedina, KSA, during the month of February. Pumpkin Squash was washed by distilled water to be ready for use.

2.1.2. Chemicals

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

2.1.3. Preparation of plant extracts

The pumpkin squash skin (20 g) was shaped to small pieces (2.5 mm), grinded with pestle and mortar, then extracted by maceration during 2h with 100 ml of different solvents (acetone, methanol and boiled water). Each mixtures was left for 48 h in refrigerator, then sonicated with Sonicator bath (2510, Branson) for 30 min and filtered. The extraction

was performed three times until complete extraction and followed by a collection of the filtrates. Acetone, methanol and ethanol and water extracts were evaporated to dryness. Then the 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. Phytochemical Analysis

2.2.1.Estimation of total phenolic content (TPC)

The TPC was evaluated using Folin–Ciocalteu's method [29-31]. A 2.0 ml extract was added to 2.5 ml of diluted FolinCiocalteu 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 (Cintra 6 GBC). All samples were prepared and measured three times, and the TPC was expressed as mg Gallic acid equivalents per gram of extract (mg GAE/g extract).

2.2.2. Estimation of total flavonoids content (TFC)

The TFC was estimated following a method described previously using catechin as standard [32]. In a 10.0 ml test tube, 2 mL of extracts solution was mixed with 0.3 ml of $NaNO_2$ (0.5 M), and 3.0 ml of distilled water. After 5 min, the mixture was added to 0.3 ml of $AlCl_3.6H_2O$ (0.3 M). Then, after 6 min, the mixture was added to 2 ml of NaOH (1 mM) and 2.4 ml of distilled water. The absorbance of the mixture solution was measured at 510 nm. The TFC was expressed in terms of Catechin equivalent /g of extract (mg CE/g extract). This process was performed in triplicate for each extract.

2.2.3Estimation of antioxidant activity using DPPH- scavenging assay

Antioxidant potential of skin extracts of Butternut squashwas estimated based on their scavenging activity to DPPH. In fact, DPPH is a stable free radical that accepts an electron to become stable diamagnetic molecule or hydrogen radical. It is used to estimate the antioxidant activity of several natural compounds [33]. The percentage radical scavenging activity was determined accordingly [29, 32]. 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 30 min at ambient temperature in darkness, and then the absorbance was measured at 517 nm.The scavenging effect was estimated using the following equation:

$$\% \, inhibition = \frac{(A_0 - A_1)}{A_0} * 100$$

Where

A_0: the absorbance of control reaction.

A_1: the absorbance of mixture containing the extract.

The IC_{50} values were determined from the % inhibition versus concentration plot [34].

3.Results and Discussions

3.1.Estimation of total phenolic content

The TPC was determined in water, methanol and acetone extracts of Butternut squash skin by the FolinCiocalteu method, as described in the literature for estimation of phenolic compounds [29, 31, 35]. The phenolic compounds react with FolinCiocalteu reagent and leads to the appearance of a blue color complex, which absorbs radiation and permits quantification. Gallic acid was used as a standard compound to perform the standard curve equation (y = 0.0006x + 0.0012, R^2=0.9932, where y is absorbance at 765 nm).

Table 1. Total phenolic contents in various skin extracts of Butternut squash

Type of Sample extract	TPC [1] (mg GAE/g extract)
Acetone	359.61 ± 5.77
MeOH	150.09 ± 4.89
Water	225.09 ± 1.45

1 Each value represents the average of three replicates ± SD

The TPC in Butternut squash skin extracts ranged from 150.09 ± 4.89 to 359.61 ± 5.77 mg GAE/g as presented in Table 1. The acetone extract contained highest TPC followed by water, and methanol extracts where, the total phenolic content were 359.61, 225.09, 150.09 mg GAE/g extract respectively. Therefore, the results indicate that the TPC in the Butternut squash skin extracts is significantly depending on the solvent used in the extraction. Similar results were reported by several studies, which revealed that different extracting solvents influenced the yields of TPC [26, 36-39]. In fact, Milan et al. [26] revealed that the TPC in plant extracts of Marrubiumperegrinum L depends on the type of extract and the polarity of solvent used in extraction. The highest TPC was estimated in methanolic (49.27 mg GAE/g extract), acetone (48.72 mg GAE/g extract) and water (46.780 mg GAE/g extract) extracts. On the other hand, Min et al reported [39] that the TPC in methanol, ethyl acetate and petroleum ether extracts of Hibiscus tiliaceus wood were respectively 23.09, 30.18, and 11.90 mg GAE/g of extract. In addition, in our previous work [35], Cucurbita. maxima skin extracted with various solvent (acetone, methanol, ethanol and water) were evaluated for their antioxidant properties. Acetone exhibited high TPC followed by ethanol, methanol and water, where the TPC were respectively 336.19, 311.71, 181.17 and 55.91 mg GAE/g extract. Therefore, this work is in accordance with the previous studies reported above revealing that the solvents used for extraction influenced the TPC yields. Furthermore, the values of TPC estimated in Butternut squash skin extracts using different solvent were significantly higher than those estimated in many other plants [26, 39, 40].

3.2. Estimation of total flavonoids content

The TFC of various Butternut squash skin extracts was obtained using spectrophotometric method with aluminum chloride. In fact,a complex was formed between the hydroxyl groups of flavones and flavonols, the carbonyl and aluminum ion that gives a yellow color [41]. Catechin was used as a standard compound to perform the standard curve equation ($y = 0.053x + 0.0038$, $R^2 = 0.9976$, where y is absorbance at 765 nm). The results of estimated TFC are shown in Table 2.

Table 2. Total flavonoids contents in various skin extracts of Butternut squash skin extracts

Type of Sample extract	TFC [1] (mg CE/g extract)
Acetone	1.16 ± 0.08
MeOH	0.79 ± 0.01
Water	1.18 ± 0.03

1 Each value represents the average of three replicates ± SD

The results show that the TFC in skin extracts of Butternut squash skin extracts depended of solvent used for extraction and ranged from 0.79 ± 0.01 to 1.18 ± 0.03 mg CE /g of extract (table 2). The greatest amount of flavonoids was obtained in water extract (1.18 mg CE /g) followed by acetone (1.16 mg CE /g), and methanol extract (0.79 mg CE /g). Several studies reported that the variation in the recovery of flavonoids and phenolic from natural products may be influenced by the type of plant material, the chemical nature of the extractable compounds and the polarity and effectiveness of extraction solvents to solubilize such compounds [39, 42]. In fact, the TPC and TFC in water, methanol, acetone, ethyl acetate and petroleum ether extracts of different plant parts of Teucriummontanum L. var. montanum (leaves, flowers and stems) were evaluated and varied from 8.33 to 169.06 mg GA/g. and 3.96 to 88.31 mg RU/g respectively [43]. The high TFC was obtained in acetone and some methanolicand ethyl acetate extracts. The antioxidant activity analyzed using DPPH reagent showed that T. montanum is a good source of phenolic compounds and a valuable natural antioxidant. Furthermore, hot water extracts of 13 natural spices and herbs such as caraway, oregano, and rosemary were investigated for their TFC, TPC, and antioxidant activity [44]. The TFC of the spices ranged between 3.38 µg QE/g for coriander to 324.08 µg QE/g for thyme. The results showed that the high antioxidant activity was obtained at hot water extract of several spices. On the other hand, in our previous work [35], the TFC of different extracts of Cucurbita maxima skin were determined and the highest amount was estimated in ethanol (4.64mg CE /g of extracts) followed by methanol (3.11mg CE /g of extracts), water (1.62mg CE /g of extracts), and acetone (1.17 mg CE /g of extracts) extracts. The present study revealed that the TFC of Butternut squash skin extracts depended of solvent used for extraction, which is in accordance with the different previous studies as presented above.

3.3.DPPH radical scavenging assay

DPPH method was used in large scale to quantify polyphenols antioxidant activity [33]. Antioxidant properties, particularly radical scavenging activities, are very important due to the essential role of free radicals in foods and biological systems [45]. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability [46]. The reaction between radicals and antioxidant molecules resulting in the scavenging of the radical by hydrogen donation causes the decrease in the absorbance of DPPH radical, which is visualized as a color change from purple to yellow [47, 48]. The IC_{50} value is often used to express the concentration of extracts required to scavenge 50% of the free radicals. The antioxidant activity of different extracts of skin Butternut squash was expressed in terms of percentage of inhibition and IC_{50} values (mg/ml). The IC_{50} value is inversely proportional to the scavenging activity of the extract. The results show the increase of radical scavenging activity of skin extracts of Butternut squash as the extract concentration increases (Figure 1). Furthermore, the estimated IC_{50} values were different in extracts of Butternut squash, and were depending on the solvent used for extraction. In fact, the IC_{50} values in acetone, methanol and water extracts were 0.29 mg/ml, 0.21 mg/ml and 1.00 mg/ml, respectively (Figure 1, Table 3). According to the derived IC_{50}values, all extracts of Butternut squash skin exhibited higher scavenging activities, which can be attributed to their high polyphenols content as reported above. The highest antioxidant activity of Butternut squash skin extracts was found in methanol extract (lowest IC_{50} value). The difference of antioxidant activity between the different extracts can be explained by the presence of antioxidant compounds having different chemical characteristics and polarities, and thus different solubility in a particular solvent[49]. The obtained values of IC_{50} of Butternut squash skin extracts was lower than those reported by many studies. In fact, Mau et al. [50] reported that Antrodiacamphorata mycelia, used as an anticancer, antidote, anti-itching and hepatoprotective drug was investigated for its antioxidant activity and the IC_{50} for white and red mycelia were1.56 and 1.70 mg ml^{-1} respectively. Also, Chandrasekhar et al. [51] revealed that the IC_{50} values estimated in some polyherbal extracts, ranged from 24.1 to 186.9 µg ml^{-1}. Furthermore, Torey et al. [52] investigated the antioxidant activity of IxoracoccineaL. (Rubiaceae)

flower, leaf and stem. They reported that the corresponding IC_{50}values in methanol extracts were 6.6 mg/mL, 109.95 mg/ mLand 272.42 mg/ mL, respectively. The flower extract of IxoracoccineaL. showed highest antioxidant activity and highest TPC (210.55 ± 6.31 mg GAE/g extract). In addition, Azizah et al. [53] revealed that the methanol extracts of Pumpkin (Cucurbita moschata) cooked in different ways (boiling and stir frying for different times) exhibited high free radical scavenging activity in the range of 81.1% to 94.6% with IC_{50} of 1.41 to 1.62 mg ml^{-1}.The present study revealed that the different extracts of Butternut squash skin presented high antioxidant activity, which was demonstrated by the lowest IC_{50} values obtained in all extracts.

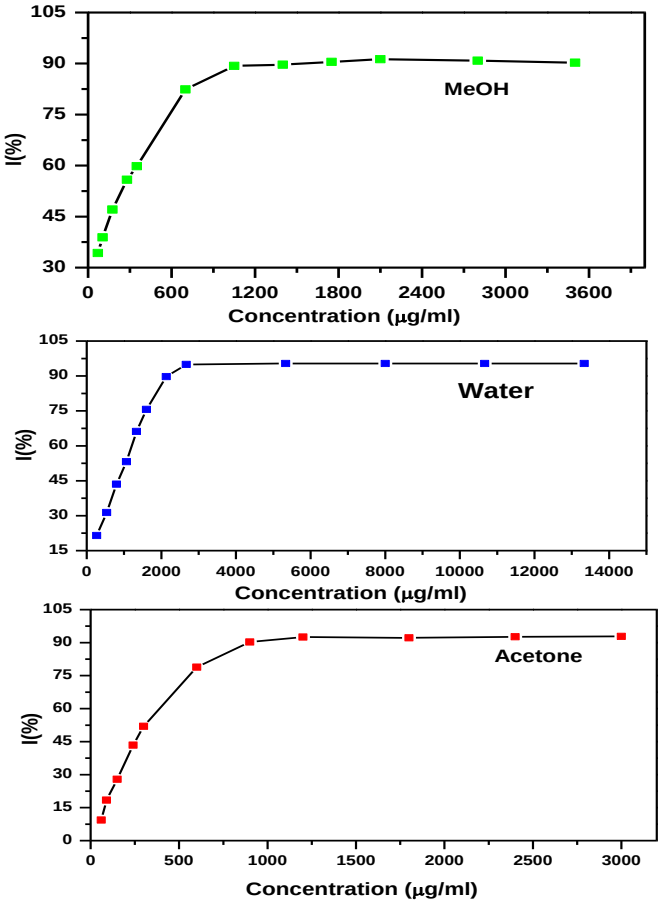

Figure 1. DPPH radical-scavenging activity of Butternut squash skin in methanol, water and acetone extracts (values are expressed as mean ± SD of three replicates).

Table 3. Antioxidant activity of various extracts of Butternut squash skin expressed in terms of IC_{50} values (mg/ml)

Type of Sample extract	**IC_{50} (mg/ml)**
Acetone	0.29
MeOH	0.21
Water	1.00

3.4.Relationship between TPC, TFC and antioxidant activities of Butternut squash skin extracts

Several studies reported that the antioxidant properties of flavonoids and phenolic acids resulted from their ability to chelate metals and quenching of singlet oxygen and redox properties [54-57].

Through the correlation analysis for TPC and TFC and antioxidant activity of Butternut squash skin extracts (Figure 2), the TPC and TFC pointed a non-significant correlation with DPPH radical scavenging activity. In fact, the coefficients of correlation were R^2=0.0049, R=0.07 and R^2= 0.37, R=0.61respectively. Our results are consistent with those found by several studies, which revealed that there is no existence of a significant relationship between TPC and TFC and DPPH radical scavenging activity [35, 58-60]. The results can be interpreted by the fact that antioxidant activity of flavonoids is influenced by their chemical structure, particularly of the distribution of hydroxyl groups [61, 62]. Only flavonoids with a certain structure, especially hydroxyl position in the molecule can show radical scavenging activity by acting as proton donating [63, 64]. In addition, Prior et al. [65] revealed that the Folin-Ciocalteau assay allow a crude estimate of the TPC present in an extract, however the free radical scavenging assay is not only specific to polyphenols. On the other hand, the TPC may not incorporate necessarily all the antioxidants that can be present in an extract [35, 59, 66]. It is also reported that various phenolic compounds respond differently in DPPH assay, depending on the number of phenolic groups they have [59, 67].

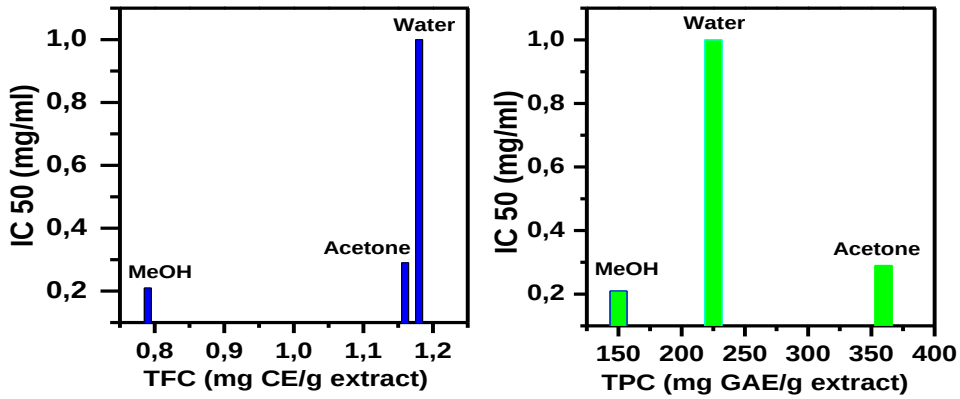

Figure 2. Relationship between TPC,TFC and antioxidant activity in various extracts of Butternut squash skin (Values are expressed as mean ± SD of three replicates).

4. Conclusion

This work consisted of the study of the effect of different extracting solvents on antioxidant activity, total phenolic content and total flavonoids content of Butternut squash skin. The TPC, TFC, and antioxidant activity of all examined extracts were different, and significantly depend on the solvent used for extraction. Interestingly, skin extracts of Butternut squash showed highest TPC and highest antioxidant activity. Among the various extracts, methanol extract exhibited highest DPPH radical scavenging activities represented by the lowest IC_{50} value. The study revealed that Butternut squash skin can be considered as a novel safe natural source of antioxidant that can be used for different important applications in natural antioxidants, functional foods, and neutraceuticals. It is of a great interest to focus the ongoing works on studying the medicinal active components of this important plant species, which can lead to the preparation of natural pharmaceutical products of high value.

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