RESEARCH ARTICLE

Indigenous vegetables of family Cucurbitaceae of Azad Kashmir: A key emphasis on their pharmacological potential

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Abstract

The antioxidant capacity of extracts of different parts of Cucurbitaceae vegetables was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino bis (ethyl benzothiazoline 6)-sulphonic acid) methods. Total phenolic content (TPC) and total flavonoid content (TFC) were also determined. The correlation of TPC, TFC, DPPH, and ABTS in different extracts of Cucurbitaceae vegetables was analyzed. The peel extracts of studied vegetables had the highest TPC, (C. grandis 3.00±0.86, T. cucumerina 3.24±0.70 and C. moschata 3.12±0.06 mg gallic acid equivalent (GAE) g⁻¹ DW) and TFC (C. grandis 18.96±1.5, T. cucumerina 13.92±1.41 and C. moschata 15.31±0.97 mg rutin equivalent (RE) g⁻¹ DW). The maximum antioxidant potential was obtained by the ABTS method in peel extracts of C. grandis (78.7%) and C. moschata (63.5%) while in pulp extract of T. cucumerina (50.1%) at 10 μg/mL. The percent radical scavenging activity (% RSA) by the DPPH method found maximum for peel and pulp of C. grandis (45.15 and 45.15%, respectively) and peel of T. cucumerina (45.15%) and C. moschata (34.15%). The EC50 obtained in the ABTS method was 0.54 and 7.15 μg/mL for C. grandis and C. moschata, respectively while 0.81 μg/mL for the pulp of T. cucumerina compared to standard ascorbic acid (1.05 μg/mL). The EC50 calculated in the DPPH method was 11.78 μg/mL, 13.34 μg/mL and 21.00 μg/mL for C. grandis, T. cucumerina, and C. moschata peel respectively compared to the standard Butylated hydroxytoluene (BHT). Among each variable, the correlation between ABTS and TPC provided the highest positive correlation (r = 0.998, p<0.05) in peel extracts.

1. Introduction

Intake of natural phenolic antioxidants in the diet has a positive correlation with reduced cancer mortality and heart diseases, and longer life expectancy [1–6]. These antioxidants are responsible to nurture immune function, inhibiting malignant transformation, reduce DNA
damage and lipid peroxidation [7]. Recently, growing attention has been given to screening out the natural sources of antioxidants like fruits and vegetables compared with synthetic materials [3,6,8–10]. Synthetic antioxidants like butylated hydroxytoluene (BHT) are reported to be carcinogenic and have various side effects [11].

Diseased people need to intake proper foods with functional activities for the fulfillment of their energy and nutritional needs [12]. Vegetables are the cheapest and most easily available source of nutrition which contain bioactive components including minerals, dietary fiber, and vitamins (A, C & E) as well as non-nutritive phytochemicals (carotenoids, alkaloids, terpenoids, bioactive peptides, phenolic compounds, and flavonoids) which reduce the risk of cardiovascular diseases, cancer, obesity, and diabetes [3]. Phenols, flavonoids, and vitamins were reported to contain antioxidant properties [13]. Various researchers have reported the antioxidant properties of vegetables due to the presence of phenolic content in them. However, the extent of antioxidant properties may be affected by many factors including the degree of ripeness, climatic, storage, and geographical conditions [14].

Cucurbitaceae is a large family of plants, with 130 genera and 800 species distributed in the tropical and sub-tropical regions of the world. This family is cultivated all around the world in a variety of environmental conditions having global pharmacological and dietary importance. The main producers of Cucurbitaceae are Turkey, China, India, and the USA [15]. The majority of the plants of Cucurbitaceae has medicinal importance and are used as a medicine for the remedy for ages, urinary ailments, intestinal worms, high blood pressure, kidney stone, headaches, abdominal tumors, fever, diarrhea, and skin allergies [16].

*Cucurbita moschata* is a species of the Cucurbitaceae family cultivated all around the world. It is commonly called squash or pumpkin. The pulp of *Cucurbita moschata* provides a cheaper and good source of food, having health benefits. The seeds of squash contain approximately 15.9 mg/100 g of total tocopherols, and a sufficient quantity of linoleic acid and L-tryptophan, which are largely used for the treatment of depression [17]. *Trichosanthes cucumerina* is another species widely distributed in the tropical and subtropical regions. It is commonly called snake guard, while the *Coccinia grandis* is species that grows only in the tropical region and is called ivy gourd. These two species are eaten as vegetables containing a large amount of antioxidants, anti-hypoglycemic agents, and immune systems modulators. Traditionally, these vegetables were largely used to treat leprosy, fever, asthma, bronchitis, scabies, and jaundice [18]. The main chemical constituents present in the Cucurbitaceae family are phytochemicals. The other commonly occurring compounds are carbohydrates, triterpenes, sterols, alkaloids, α-carotene, β-carotene, and lutein zeaxanthin [19].

Various methods are commonly used to measure the antioxidant activities of vegetables, including 2, 2-diphenyl-1-picrylhydrazyl radicals (DPPH) method, ferric reducing antioxidant power (FRAP) method, 2, 2’-azino-bis (3-ethylbenzthio-zoline-6)-sulphonic acid (ABTS) method, and oxygen radical absorbance capacity assay (ORAC) method [19]. Of all these methods, the most convenient and independent of expensive equipment are the DPPH and ABTS methods, thus commonly used.

The present work aimed to investigate the phenolic, flavonoid content, and antioxidant potential of various parts (peel, pulp, seeds) of some Cucurbitaceae vegetables that are commonly consumed and locally grown in Muzaffarabad, Azad Jammu & Kashmir Pakistan for their phytochemical and antioxidant behavior. Phytochemicals present in these vegetables play a protective role from oxidative stress and various other related diseases. The present study will provide information about the medicinal importance and use of studied vegetables highlighting their antioxidant properties.
2. Material and methods

2.1 Chemicals and reagents

Folinciocalteu’s reagent, quercetin, gallic acid, phosphate buffer, 5, 6-Diphenyl-3-(2-pyridyl)-1, 2, 4-triazine-4', 4''-disulfonic acid sodium salt, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), methanol, butylated hydroxytoluene (BHT), and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid was obtained from Fluka (Switzerland) while sodium carbonate, sodium nitrite, aluminum chloride, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Millipore System (Millipore, Bedford, MA, USA) and used throughout the experiments. All the used standards were stored in dark at -20°C.

2.2 Sample collection

Three locally grown Cucurbitaceae vegetables including Cucurbita moschata (Voucher No. UR-01) Trichosanthes cucumerina (Voucher No. UR-02) and Coccinia grandis (Voucher No. UR-03) were collected from the capital city Muzaffarabad of Azad Jammu and Kashmir state, Pakistan. Voucher specimens were identified by Dr. Tariq Habib, Assistant Professor, Department of Botany, University of Azad Jammu, and Kashmir (UAJ&K), Muzaffarabad, and were deposited in the herbarium of the Department of Botany, UAJ & K, Muzaffarabad.

2.3 Sample preparation

The collected fresh vegetables were washed with distilled water and the required parts including peel, pulp, and seeds were separated and ground into fine particles by using a mechanical grinder. To extract the antioxidant materials from the samples of vegetables, a reported method was applied with slight modification [3]. Briefly, a specific amount of sample (10.0 g) was treated with 100 mL distilled water in a shaking water bath for 30 minutes at 37°C and 100 rpm. The blended mixture was centrifuged at 4, 200 rpm for 30 minutes. The supernatant was collected, and the solvent was evaporated at room temperature to dryness. The obtained residue was weighed to calculate the extractive yield and stored in an airtight jar at -20°C for further use. The solid aqueous extracts were dissolved in distilled deionized water at a concentration of 10 mg/mL for experimental purposes.

2.4 Total phenolic content determination

TPC present in different parts of vegetables was determined by using the Folin-Ciocalteu method [20]. Briefly, 500 μL of the sample extract was mixed with Folin-Ciocalteu reagent (200 μL) and then mixed with 20% solution of sodium carbonate solution (1mL) after 3 minutes. The solution was incubated for one hour in the dark which turned into deep blue coloration. The absorbance of this mixture solution was monitored at 765 nm by using a blank solution prepared under the same protocol except for the extract solution. TPC was calculated from the trend line of standard gallic acid (0.05–0.25 mg/mL) and expressed in mg of GAE/g dry weight of the extract.

2.5 Determination of total flavonoid content

The TFC in vegetable samples was quantified according to the reported method [21]. The sample solution consisting of extract (1 mL), distilled water (2 mL), and 5% sodium nitrite (0.15 mL) was incubated at room temperature for 6 minutes. Aluminum chloride (10%, 0.15 mL) and sodium hydroxide (4%, 2 mL) were added to the sample solution followed by incubating
again for 6 minutes at room temperature. The final volume of the sample solution was made up to 10 mL by addition of ultrapure water, mixed thoroughly, and kept for 15 minutes at room temperature. Absorbance was recorded at 510 nm using UV-spectrophotometer (Shimadzu UV 1800) against the blank solution prepared under the same protocol except for the extract solution.

The total flavonoid content present in the peel, pulp, and seeds of Cucurbitaceae vegetables was calculated from the linear equation of quercetin (0.066–0.0166 mg/mL) taken as a standard and the results were represented as mg QE/g dry weight of the extract.

2.6 Antioxidant activities by ABTS method

The ABTS•+ free radical scavenging assay was used to determine the antioxidant potential of extracts [22]. The ABTS•+ free radicals in the ABTS stock solution were generated by mixing potassium persulphate (2.5 mM) and ABTS (7 mM) solution (1:1, v/v) and let to stand at room temperature for 24 hours. The solution was diluted until the absorbance (Ao) of 0.90±0.04 was obtained at 734 nm. Extracts in a range of concentrations (2.0–10.0 μg/mL) were added in ABTS•+ free radical solution and absorbance of sample solution (Ai) was measured. The percentage radical scavenging activity (% RSA) was calculated by using Eq 1, and half-maximal effective concentration (EC50 μg/mL) was calculated by trend line equation taking ascorbic acid as a standard.

\[
\% \text{RSA} = \left( \frac{\text{Ao} - \text{Ai}}{\text{Ao}} \right) \times 100
\]  

Where Ao is the absorbance of the blank solution, Ai is the absorbance of the sample solution.

2.7 Analysis of antioxidant activities by DPPH method

DPPH assay [23] was performed where different concentrations of the extracts (2–10 μg/mL) were added to 1 mL of a 0.008% methanol solution of DPPH. This solution was incubated for 30 minutes at room temperature, and UV absorbance was taken at 517 nm against a blank solution kept in the reference compartment. The control was prepared using the same protocol as described above without any extract. The BHT was used as a positive control. The percentage of DPPH free radical scavenging potential of extracts was calculated using the following equation.

\[
\text{DPPH radical scavenging potential (\%) = } (1 - \frac{A_s}{A_b}) \times 100
\]

Where \(A_b\) is the absorbance of control having all reagents except the extract and \(A_s\) is the absorbance of the test.

2.8 Statistical analysis

All experiments were performed in triplicates and the data were expressed as the mean ± standard error (SD) of three independent results. One-way analysis of variance (ANOVA) with a statistical significance level set at \(p < 0.05\) correlations between the total phenolic, flavonoid content, and antioxidant capacities were made using the Pearson procedure \((p < 0.01)\).

3. Results and discussion

3.1 Extraction yield

The percentage yield of aqueous extracts from the peel, pulp, and seeds of studied Cucurbitaceae vegetables (photographic images are included in Fig 1) were shown in Table 1. The
extractive yield varied among all vegetable parts where the peel extracts provided the maximum percentage yield i.e., *C. grandis* (36.80%), *T. cucumerina* (43.87%), and *C. moschata* (29.31%). Reported literature suggests that extractive yield may vary from plant to plant and depends upon the nature of secondary metabolites [24–27].

### 3.2 Evaluation of total phenolic content

The phenolic content present in plants is responsible to reduce the reactive oxygen [O] via an H atom donated by the phenolic OH group and electrons transfer from phenoxide anions [28]. The polyol enzymes that are necessary for the nutrition and health of humans are also affected by polyphenols [29]. The TPC present in different species of the Cucurbitaceae family was determined by using a standard gallic acid calibration curve and results (expressed in mg GAE/g of dry extract weight) were presented in Table 2. Khatana et al reported that the TPC in the peel of *C. moschata* extract was 6.4±0.1 mg GAE/g DW [30], which was higher than our results (3.12±0.06 mg GAE/g DW) while in the same study the total phenolic content in pulp extract was less (2.5±0.3 mg GAE/g DW) than our results (2.95±0.04 mg GAE/g DW). KondhARE and Lade reported the total phenolic composition of the aqueous extracts of *C. grandis* fruit (8.2±0.2 mg GAE/g DW) [31], which were found to be in close agreement with the sum of the phenolic content of peel, pulp, and seed extracts (8.56±2.57 mg GAE/g DW) found in the current study. Higher values of phenolic content (8.18±1.56 mg GAE/g DW) were found in *T. cucumerina* peel, pulp, and seed extracts collectively when compared to *T. cucumerina* fruit aqueous extract (4.64±0.3mg GAE/g DW) [32]. The differences in the phenolic composition of the same species reported in different studies could be due to the variation in growing

| English name of vegetable | Scientific name of vegetable | Part used | % Yield of extracts |
|---------------------------|-------------------------------|-----------|---------------------|
| Ivy Gourd                 | *Coccinia grandis*            | Peel      | 36.80               |
|                           |                               | Pulp      | 13.70               |
|                           |                               | Seed      | 6.22                |
| Snake Gourd               | *Trichosanthes cucumerina*    | Peel      | 43.87               |
|                           |                               | Pulp      | 22.43               |
|                           |                               | Seed      | 25.60               |
| Butternut squash          | *Cucurbita moschata*          | Peel      | 29.31               |
|                           |                               | Pulp      | 25.56               |
|                           |                               | Seed      | 12.42               |

Table 1. The percentage yield of extracts from the peel, pulp, and seeds of studied Cucurbitaceae vegetables.
Table 2. TPC and TFC in the aqueous extracts of various parts of studied *Cucurbitaceae* vegetables.

| Vegetable Scientific name | TPC (mg GAE/g dry extract) | TFC (mg RE/g dry extract) |
|---------------------------|-----------------------------|---------------------------|
|                           | Peel | Pulp | Seed | Peel | Pulp | Seed |
| *Coccinia grandis*        | 3.00±0.86 | 2.87±0.91 | 2.69±0.83 | 18.96±1.5 | 9.50±0.90 | 7.76±0.20 |
| *Trichosanthes cucumerina* | 3.24±0.70 | 2.51±0.41 | 2.43±0.45 | 13.92±1.41 | 9.14±0.46 | 8.73±0.05 |
| *Cucurbita moschata*      | 3.12±0.06 | 2.95±0.04 | 2.88±1.6 | 15.31±0.97 | 14.38±0.73 | 9.71±0.16 |

Table 3. Antioxidant activity of aqueous extracts of various parts of studied *Cucurbitaceae* vegetables.

| Concentration (μg/mL) | Ascorbic acid | 50% RSA | 75% RSA | 90% RSA | Radicals scavenged (μg/mL) | C. grandis (9.71±0.16 mg RE/g DW) | T. cucumerina (8.73±0.05 mg RE/g DW) | C. moschata (9.71±0.16 mg RE/g DW) |
|-----------------------|---------------|---------|---------|---------|----------------------------|-----------------------------------|-------------------------------------|-------------------------------------|
| 2.0                   | 53.0±1.05     | 53.2±0.92 | 51.4±0.99 | 25.5±0.32 | 27.4±0.21 | 27.6±0.43 | 14.7±0.09 | 27.1±0.09 | 26.9±0.89 | 4.7±0.03 |
| 4.0                   | 68.0±1.08     | 60.9±0.75 | 60.8±0.82 | 27.5±0.40 | 31.0±0.25 | 35.1±0.50 | 15.2±0.02 | 37.9±0.98 | 29.7±0.92 | 10.8±0.32 |
| 6.0                   | 80.0±1.20     | 69.9±0.93 | 64.9±0.87 | 28.3±0.69 | 34.2±0.45 | 41.0±0.67 | 18.1±0.53 | 46.7±1.06 | 32.6±0.97 | 17.8±0.50 |
| 8.0                   | 92.0±1.21     | 72.1±0.15 | 69.9±0.92 | 30.2±0.72 | 38.7±0.70 | 47.4±0.58 | 19.3±0.45 | 50.3±1.21 | 38.8±1.5 | 20.9±0.65 |
| 10.0                  | 98.0±1.30     | 78.7±1.05 | 74.9±1.20 | 32.1±0.77 | 40.9±0.68 | 50.1±0.98 | 20.9±0.91 | 63.5±1.34 | 42.2±1.30 | 25.9±0.89 |

conditions, indicating that phenolic content may vary with variation in geographical region and climatic conditions.

3.3 Evaluation of total flavonoid content

Flavonoids are among the polyphenols, which gained significant interest because of their various biological effects as anti-inflammatory, antimicrobial, anticarcinogenic, antioxidant, and vasorelaxant [33]. Flavonoids have a broad spectrum of antioxidant properties particularly due to radical scavenging activity.

The highest flavonoid content was found in the peel extracts of all studied *Cucurbitaceae* vegetables (Table 2) where *C. grandis*, *T. cucumerina*, and *C. moschata* exhibited 18.96±1.5, 13.92±1.4, and 15.31±0.97 mg RE/g DW flavonoid content respectively. The lowest flavonoid content was obtained from the seed extracts, *C. grandis* (7.76±0.20 mg RE/g DW), *T. cucumerina* (8.73±0.05 mg RE/g DW), and *C. moschata* (9.71±0.16 mg RE/g DW). No reported literature was available for the total flavonoid content of any species of the *Cucurbitaceae* family.

3.4 Antioxidant activities by ABTS method

The ABTS method has been broadly used for the assessment of the antioxidant capabilities of natural products. In this method, the dark blue color of 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS**⁺**), is reduced by an antioxidant (which transfer an electron) into colorless ABTS solution. The change in concentration of ABTS**⁺** after the reduction process is measured spectrophotometrically. In the current study, the antioxidation potential of the peel, pulp, and seed extracts from three *Cucurbitaceae* vegetables was evaluated and presented in Table 3. The EC50 (concentration of extract scavenging 50% of free radicals) was calculated from the % RSA values by using the linear equation of ascorbic acid taken as a standard (Table 5).
The highest antioxidant property was presented by the peel extracts of three Cucurbitaceae vegetables. *C. grandis* and *C. moschata* exhibited the scavenging activity in the range of 53.2±0.92–78.7±1.05% and 27.1±0.89–63.5±1.34% respectively in the concentration range of 2–10 μg/mL. Whereas *T. Cucumber* in a pulp extract provided the radical scavenging activity in the range of 27.6±0.43–50.1±0.98% with the same concentrations. A moderate level of % scavenging radical activities was noted in the pulp extracts of *C. grandis* (51.4±0.99–74.4±1.20%), *C. moschata* (26.9±0.85–42.2±1.30%), and peel extracts of *T. cucumerina* (27.4±0.21–40.9±0.68%). The lowest scavenging potential was exhibited by the seed extracts of three studied samples. A similar trend was reported by Xu et al. [34] and Zhang et al. [35] where peel extract provided the maximum antioxidant potential than pulp and seeds. Variation in antioxidant potential among different fruit tissues of the same vegetable could be due to the presence of the maximum amount of phenolic and flavonoid content in the peel.

A group of researchers reported the % ABTS radical scavenging potential of *C. grandis* whole fruit extracts prepared in different solvents including petroleum ether (43.2±0.2), dichloromethane (61.0±0.5), acetone (57.4±0.4), methanol (95.8±1.0), and water (88.4±0.6) at a concentration of 100 μg/mL [31]. In the present study, almost the same results were observed by 10 μg/mL of peel and pulp extracts indicating that the *C. grandis* species in the current work has far greater antioxidant potential. This can be due to the various number of antioxidants present in the same species grown in different areas under different conditions. No study was found in the reported literature regarding the % RSA of *T. cucumerina* and *C. moschata* measured by the ABTS method.

### Table 4. The antioxidant activity of the aqueous extracts of various parts of studied Cucurbitaceae vegetables evaluated by the DPPH radical scavenging assay.

| Concentration (μg/mL) | Butylated hydroxy toluene (BHT) | Coccinia grandis | Trichosanthes cucumerina | Cucurbita moschata |
|-----------------------|-------------------------------|-----------------|-------------------------|---------------------|
|                       | Peel                          | Pulp            | Seed                    | Peel                |
|                       | 2.0                           | 53.0±1.05       | 22.46±0.21              | 18.69±0.47          |
|                       | 4.0                           | 68.0±1.08       | 27.38±0.95              | 30.69±0.55          |
|                       | 6.0                           | 80.0±1.20       | 32.3±1.02               | 32.15±1.01          |
|                       | 8.0                           | 92.0±2.11       | 39.53±1.09              | 38.38±0.65          |
|                       | 10.0                          | 98.0±1.30       | 45.15±1.10              | 45.15±1.18          |

### Table 5. Means EC50 values for ABTS++ and DPPH radical scavenging potential of the aqueous extracts of various parts of studied Cucurbitaceae vegetables.

| Sample         | Vegetable Part | EC50 for ABTS++ radical scavenging potential (μg/mL) | EC50 for DPPH radical scavenging potential (μg/mL) |
|----------------|----------------|-----------------------------------------------------|--------------------------------------------------|
| Ascorbic acid  | -              | 1.05                                                | -                                                |
| BHT            | -              | -                                                   | 7.35                                             |
| *Coccinia grandis* | Peel         | 0.54                                                | 11.78                                            |
|                 | Pulp           | 0.81                                                | 12.10                                            |
|                 | Seed           | 32.76                                               | 13.51                                            |
| *Trichosanthes cucumerina* | Peel     | 14.96                                               | 13.34                                            |
|                 | Pulp           | 9.40                                                | 14.62                                            |
|                 | Seed           | 45.22                                               | 17.80                                            |
| *Cucurbita moschata* | Peel     | 7.15                                                | 21.0                                             |
|                 | Pulp           | 14.04                                               | 22.41                                            |
|                 | Seed           | 18.09                                               | 23.41                                            |

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3.5 Antioxidant activities by DPPH method

The antioxidant ability of any material can be evaluated by various methods, in which the DPPH essay is very simple and rapid. This method is frequently used to assess the ability of a material to scavenge the free radical and provides valuable information about hydrogen donors compounds [36]. Free electron generated by DPPH reagent show absorption peak at 517 nm [37]. However, when the odd electron of the DPPH reagent is reduced by the H atom of any hydrogen donor substance, the purple color of DPPH fades to yellow. The color change is the indication of the generation of reduced DPPH [38]. The extent of decolorization of DPPH indicates the radical-scavenging potential of the antioxidant. The resulting decolorization is stoichiometric with respect to the number of electrons captured. Results of percent scavenging activity and the obtained EC50 values were presented in Tables 4 and 5 respectively.

DPPH radical scavenging activity increased as the concentration of extracts was increased. The percent DPPH radical scavenging potential (% RSA) of peel extracts of all studied vegetables was found to be maximum which might be attributed to the greater amount of phenols and flavonoids present in peel extracts. The % RSA of the aqueous peel extracts ranged for *C. grandis* (22.46 ± 0.21–45.15 ± 1.10%), the pulp (21.61±0.84–45.15±1.08%) and seed (18.69±0.47–40.84±1.09%), *T. cucumerina* peel (28.52±0.34–45.15±1.18%), pulp (18.53±0.98–37.46±1.20%), and seed (15.3±0.95–32.15±1.15%), *C. moschata* peel (22.76±0.51–34.22±1.05%), pulp (18.3±0.22–30.65±1.11%) and seed (6.23±0.06–23.05±0.43%) at the concentration range of 2.0–10.0 μg/mL. The order for the DPPH radical scavenging activity of the tissues of all studied vegetables was peel > pulp > seed. Liyanage et al reported the DPPH radical scavenging potential of the aqueous extracts of *T. Cucumerina* fruit, leaves, and flower with the % RSA values of 10.83 ± 0.7, 3.08 ± 0.2, and 4.16 ± 0.1 respectively, at a concentration of 100 μg/mL [32].

3.6 Statistical analysis

To identify the chemical content responsible to give the antioxidant capacity to the vegetables of the Cucurbitaceae family, correlation coefficients among the TPC, TFC, and antioxidant capacity by ABTS and DPPH methods were analyzed. Table 6 compiled the Pearson correlation among EC50 of DPPH and ABTS radical scavenging activity and TPCs and TFCs.

| Correlation (r) among variable | Sample | TPC  | TFC  |
|-------------------------------|--------|------|------|
| *ABTS*                        | Peel   | 0.998** (0.030) | -0.954 *** (0.127) |
|                               | Pulp   | -0.92** (0.02)  | -0.915*** (0.058) |
|                               | Seed   | 0.260 *** (0.23) | 0.984 *** (0.876) |
| *DPPH*                        | Peel   | 0.234** (0.012) | -0.47*** (0.93)   |
|                               | Pulp   | 0.24** (0.005)  | 0.87*** (0.09)    |
|                               | Seed   | 0.66** (0.01)   | 0.959*** (0.07)   |

r, correlation coefficient; TPC, total phenolic content; TFC, total flavonoid content; DPPH, DPPH radical scavenging activity; ABTS, 2, 2'-azino-bis (3-ethylbenzthiazoline-6)-sulphonic acid method. The numbers in parentheses are p-values.

**Correlation is significant at the 0.01/0.05 level (two-tailed), ns = not significant.

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correlation as indicated with the coefficient of correlation (r) values among IC50. The higher the r-value, the higher the correlation of variables. In the case of peel extract, among each variable, the correlation between ABTS and total phenolic content showed the highest positive correlation (r = 0.998, p < 0.05) which was like the study of other researchers [39] for the cucurbit family while in the study of seed the ABTS and TPC had statistically no significant correlation. In the case of the seed sample, a moderate and positive correlation between DPPH radical scavenging capacity and TPC (r = 0.686, p < 0.05) was observed. In another report, the highest and most positive correlation exists between DPPH scavenging capacity and total flavonoid (r = 0.910, p < 0.01) for the species of the Cucurbitaceae family [40].

For peel, ABTS values were correlated with the phenolic content (r = 0.998). The TFC and ABTS in the case of peel were not significantly correlated as p values were higher than the 0.05 significance level. However, studies have been found to report a high correlation between ABTS values with the total phenolic (TP) and total flavonoid (TF) content of other Cucurbitaceae species [39]. The difference may arise due to the difference in specific solvents used. Other compounds could be involved in the antioxidant capacity apart from phenols, such as vitamin C. For pulp the ABTS have negative correlation with TPC and TFC (r = -0.92 and -0.915 respectively). In the case of seed, ABTS was not significantly correlated with the phenolic content and flavonoid content (p > 0.05). For peel, pulp, and seed, DPPH values were correlated with the phenolic content, while TFC was not significantly correlated with the EC50 value of DPPH (p > 0.05). There is no correlation between DPPH and TFC might be due to different mechanism abilities and the use of different standards. While the contradictory report also found such as the antioxidant activities of T. cucumerina well correlated with the amount of total phenolic and flavonoid contents [32]. This study indicated that phenolic compounds significantly affected all antioxidant activities while flavonoids did not contribute significantly.

4. Conclusion

To evaluate the antioxidant capacity of the sample, various methods must be used in parallel. The aqueous extracts of peel, pulp, and seed had the lowest EC50 values in the ABTS method and were considered strong antioxidants. The correlation between ABTS and TPC exhibited the highest positive correlation in peel extracts. Phenolic compounds were the major contributors to ABTS antioxidant capacity in peel extracts. Not all variables in the peel, pulp, and seed extracts from three species of Cucurbitaceae were linear. Results showed that the vegetables of the family Cucurbitaceae have a high potential for use in pharmacy and phytotherapy. It could be concluded that the studied vegetables are natural sources of phytochemicals and antioxidant substances of high biological importance. Antioxidant activities of the extracts may be attributed to these phytochemicals. The edible part of the vegetable is its pulp, but our results indicated the presence of maximum phenolic and flavonoid content in the peel of all studied vegetables based on which, we strongly recommend the use of the peel of all studied vegetables as an economical and valuable source of phytochemicals and antioxidants rather than discarded as waste material. Moreover, in view of phytochemical screening and antioxidant potential presented by these vegetables of the Cucurbitaceae family, their optimum consumption in the diet has been strongly recommended to reduce the risk of oxidative stress and its related diseases.

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Ethics statement
This research did not include any human subjects or animal experiments.

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References
1. Halliwell B. Dietary polyphenols: good, bad, or indifferent for your health?. Cardiovasc Res. 2007; 73:341–347. https://doi.org/10.1016/j.cardiores.2006.10.004 PMID: 17141749
2. Maisarah AM, Nurul Amira B, Asmah R, Fauziah O. Antioxidant analysis of different parts of Carica papaya. Int Food Res J. 2013; 20.
3. Deng GF, Lin X, Xu XR, Gao LL, Xie JF, Li HB. Antioxidant capacities and total phenolic contents of 56 vegetables. J Funct Foods. 2013; 5:260–266.
4. Lü Z, Zhang Z, Wu H, Zhou Z, Yu J. Phenolic composition and antioxidant capacities of Chinese local pummelo cultivars’ peel. Hortic Plant J. 2016; 2:133–140.
5. Santos JS, Brizola VRA, Granato D. High-throughput assay comparison and standardization for metal chelating capacity screening: A proposal and application. Food Chem. 2017; 214:515–522. https://doi.org/10.1016/j.foodchem.2016.07.091 PMID: 27507505
6. Singh B, Singh JP, Kaur A, Singh N. Phenolic composition, antioxidant potential and health benefits of citrus peel. Food Res Int. 2020; 132:109114. https://doi.org/10.1016/j.foodres.2020.109114 PMID: 32331689
7. Gropper SS, Simmons KP, Gaines A, Drawdy K, Saunders D, Ulrich P, et al. The freshman 15-a closer look. J Am Coll Health. 2009; 58:223–231. https://doi.org/10.1080/07448480903295334 PMID: 19959436
8. Deng GF, Xu XR, Guo YJ, Xia EQ, Li S, Wu S, et al. Determination of antioxidant properties and their lipophilic and hydrophilic phenolic contents in cereal grains. J Funct Foods. 2012; 4:906–914.
9. Demir S, Korukluoglu M. A comparative study about antioxidant activity and phenolic composition of cummin (Cuminum cyminum L) and coriander (Coriandrum sativum L). Indian J Tradit Knowl. 2020; 19:383–393.
10. Chatoui K, Harhar H, El Kamli T, Tabayaoui M. Chemical composition and antioxidant capacity of Lepidium sativum Seeds from four regions of Morocco. Evid-based Complement. Altem Med. 2020; 2020.
11. Namiki M. Antioxidants/antimutagens in food. Crit Rev Food Sci Nutr. 1990; 29: 273–300. https://doi.org/10.1080/10408399009527528 PMID: 2257080
12. Ulger TG, Songur AN, Cirak O, Cakiroglu FP. Role of vegetables in human nutrition and disease prevention. Vegetables-Importance of quality vegetables to human health; Intech Open; London, UK. 2018; 7–32.
13. Zou Z, Xi W, Hu Y, Nie C, Zhou Z. Antioxidant activity of Citrus fruits. Food Chem. 2016; 196:885–896. https://doi.org/10.1016/j.foodchem.2015.09.072 PMID: 26593569
14. Zargoosh Z, Ghayam M, Bacchetta G, Tavili A. Effects of ecological factors on the antioxidant potential and total phenol content of Scrophulariastriata Boiss. Sci Rep. 2019; 9: 1–15.
15. Rolnik A, Olas B. Vegetables from the Cucurbitaceae family and their products: Positive effect on human health. Nutrition. 2020; 78. 110788. https://doi.org/10.1016/j.nut.2020.110788 PMID: 32540673
16. Jamuna S, Karthika K, Paulsamy S. Phytochemical, and pharmacological properties of certain medicinally important species of Cucurbitaceae family a review. J Res Biol. 2015; 6:1835–1849.

17. Patel S, Raul A. Edible seeds from Cucurbitaceae family as potential functional foods: Immense promises, few concerns. Biomed. Pharmacother. 2017; 91:330–337. https://doi.org/10.1016/j.biopha.2017.04.090 PMID: 28463796

18. Taur DJ, Patil RY. Mast cell stabilizing, antianaphylactic, and antihistaminic activity of Coccinia grandis fruits in asthma. Chin. J. Nat. Med. 2011; 9:359–362.

19. Montesano D, Rocchetti G, Putnik P, Lucini L. Bioactive profile of pumpkin: An overview on terpenoids and their health-promoting properties. Curr Opin Food Sci, 2018; 22:81–87.

20. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic.1965; 16:144–158.

21. Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, Ismail A. Antioxidant capacities of peel, pulp, and seed fractions of Canarium odontophyllum Miq. fruit. J Biomed and Biotechnology. 2010.

22. Falkeborg M, Cheong LZ, Gianfico C, Sztukiel KM, Kristensen K, Glasius M, et al. Alginate oligosaccharides: Enzymatic preparation and antioxidant property evaluation. Food Chem. 2014; 164:185–194. https://doi.org/10.1016/j.foodchem.2014.05.053 PMID: 24996323

23. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT—Food Sci Technol. 1995; 28:25–30.

24. Kaneria M, Kanani B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pac J Trop Biomed. 2012; 2:195–202. https://doi.org/10.1016/S2221-1691(12)60041-0 PMID: 23569897

25. Zhou HC, Lin YM, Li YY, Li M, Wei SD, Chai WM, et al. Antioxidant properties of polymeric proanthocyanidins from fruit stones and pericarps of Litchi chinensis Sonn. Food Res Int. 2011; 44:613–620.

26. Rakholiya K, Chanda S. In vitro interaction of certain antimicrobial agents in combination with plant extracts against some pathogenic bacterial strains. Asian Pac J Trop Biomed.2012; 2;S1456–S1470.

27. Chanda S, Amrutiya N, Rakholiya K. Evaluation of antioxidant properties of some Indian vegetable and fruit peels by decoction extraction method. Am J Food Technol. 2013; 8:173–82.

28. Munoz-Bernal OA, Coria-Oliveros AJ, Vazquez-Flores AA, de La Rosa LA, Núñez-Gastélum JA, Rodrigo-García J, et al. Evolution of phenolic content, antioxidant capacity and phenolic profile during cold pre-fermentative maceration and subsequent fermentation of Cabernet Sauvignon Red Wine. S Afr J Enol Vitic. 2020; 41:72–82.

29. Asian HE, Beydemir Ş. Phenolic compounds: the inhibition effect on polyol pathway enzymes. Chem Biol Interact. 2017; 266:47–55. https://doi.org/10.1016/j.cibi.2017.01.021 PMID: 28153995

30. Khatana S, Jain C, Vijayvergia R. Estimation of total phenolic and flavonoid content of some cucurbit fruit peels and in-vitro evaluation of their methanolic extracts for antioxidant potential. Int J Pharm Sci & Res. 2021; 12:491–95.

31. Kondhare D, Lade H. Phytochemical profile, aldose reductase inhibitory, and antioxidant activities of Indian traditional medicinal Coccinia grandis (L.) fruit extract. 3 Biotech. 2017; 7:1–10.

32. Liyanage R, Nadeeshani H, Jayathilake C, Visvanathan R, Wimalasiri S. Comparative analysis of nutritional and bioactive properties of aerial parts of snake gourd (Trichosanthes cucumerina Linn.). Int J Food Sci. 2016. https://doi.org/10.1155/2016/8501637 PMID: 27995134

33. Chandran G. Insights on the neuromodulatory propensity of Selaginella (Sanjeevani) and its potential pharmacological applications. CNS Neurol Disord Drug Targets. 2014; 13:82–95. https://doi.org/10.2174/1871927311312660188 PMID: 24152330

34. Xu G, Liu D, Chen J, Ye X, Shi J. Composition of major flavanone glycosides and antioxidant capacity of three citrus varieties. J. Food Biochem. 2009; 33:453–469.

35. Zhang H, Yang YF, Zhou ZQ. Phenolic and flavonoid contents of mandarin (Citrus reticulata Blanco) fruit tissues and their antioxidant capacity as evaluated by DPPH and ABTS methods. J. Integ. Agric. 2018; 17:256–263.

36. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005; 53:1841–1856. https://doi.org/10.1021/jf030723c PMID: 15769103

37. Azizah AH, Ruslawa NN, Tee TS. Extraction and characterization of antioxidant from cocoa by-products. Food Chem. 1999; 64:199–202.

38. Woldegorgis AZ, Abate D, Haki GD, Ziegler GR. Antioxidant property of edible mushrooms collected from Ethiopia. Food Chem. 2014; 157:30–36. https://doi.org/10.1016/j.foodchem.2014.02.014 PMID: 24679748

39. Singh J, Singh V, Shukla SK, Rai A. Phenolic content and antioxidant capacity of selected cucurbit fruits extracted with different solvents. J Nutr Food Sci. 2016; 6:1–8.
40. Fidrianny I, Darmawati A, Sukrasn O. Antioxidant capacities from different polarities extracts of Cucurbitaceae leaves using frap, DPPH assays and correlation with phenolic, flavonoid, carotenoid content. Int J Pharm Pharm Sci. 2014; 6: 858–862.