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Antioxidant Capacity, Total Phenol Contents and Phytochemical Screening of *Citrullus colocynthis* Crust, Pulp and Seeds Extracts

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Abstract: In this study, the phytochemical screening of *Citrullus colocynthis* (*C. colocynthis*) crust, pulp, and seed extracts was studied to evaluate and compare their antioxidant capacities and total phenol contents. *C. colocynthis* fruit, including crust, pulp, and seed, was extracted using n-hexan, methanol, and 1, 1, 2 trichloroethane, respectively, using a soxhlet. Phytochemical screening, antioxidant capacity using the Ferric-Bipyridine Reducing Capacity of total antioxidants (FBRC) and Ferric Reducing Antioxidant Power (FRAP) methods, and total phenol content were assessed to estimate their effect on the antioxidant capacity of *C. colocynthis*. The results showed that the seed extract showed negative results for all tests except fixed oil and fats, protein, and amino acids, while the pulp extract showed positive results for most tests. The crust extract showed positive results for reducing sugar, tannins, and saponins. The total phenol content of Yemeni *C. colocynthi*s demonstrated the highest value compared to *C. colocynthis* from different areas. Antioxidant capacity showed the highest values for pulp and the lowest values for crust according to FBRC and FRAP methods. Based on these results, antioxidant capacity and total phenol content values showed that pulp has the highest values, while crust has the lowest values.

Keywords: *Citrullus colocynthis*, Ferric-Bipyridine Assay, Phenolic Compounds, Phytochemical, Total Antioxidant Activity

Introduction

Antioxidants are substances that play a major role in protecting against the damages caused by free radicals. The use of the plant as a source of remedies to treat many diseases dates back to prehistoric times and people on all continents have this ancient tradition. (Bnyan *et al*., 2013; Rao *et al*., 2012). Before people realized the presence of microbes, the search for agents for the treatment of infectious diseases began. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful. Nowadays, medicinal plants receive attention from research centers because of their special importance in the safety of communities (Benariba *et al*., 2013; Hany and Neelam 2020). In the last three decades, antioxidant-based drug formulations for the prevention and treatment of complex diseases, like stroke, atherosclerosis, Alzheimer's disease, diabetes, and cancer have appeared. As a result, natural antioxidants were interested in a lot of research. Flavonoids and phenolic compounds are widely distributed in plants and have been reported to exert multiple biological effects, including antioxidant, anti-inflammatory, anticarcinogenic, etc., (Kumar *et al*., 2008). The presence of various complex chemical substances which differ in compositions that occur as secondary metabolites cause the therapeutical attributes of medicinal plants. The basic raw materials for original pharmaceuticals, flavors, and industries of cosmetics and perfumery are provided by a large group of economically important medicinal and aromatic plants (Gurudeeban *et al*., 2010; Shahla *et al*., 2010).

C. colocynthis is a desert plant that grows in sandy arid soils belonging to the family of *Cucurbitaceae* (Alshammary and Ibrahim, 2014; Pravin, 2013). In English language, it is known as bitter apple and in Arabic is Hindal (Marzouk *et al*., 2011), Abujahl watermelon in Persian (Hajar *et al*., 2012) Koloquinthe in German and coloquinte in French (Al-Snafi, 2016; Teixeira and Hussain, 2017). *C. colocynthis* is a medicinal plant that grows widely in Egypt, Sudan, and many other African counties (Shawkey *et al*., 2014; Kapoor *et al*., 2020; Dash *et al*., 2015). Fruits are rounded 7-9 cm in diameter,

green and white, striped, and become yellow when ripe (Shaikh *et al*., 2016). It is filled with a soft, white pulp, in which are imbedded numerous seeds (Nessa and Khan, 2014; Saberi *et al*., 2011). All parts of the fruit are often used for the treatment of different diseases, whereas some specific parts of the fruit are used for a specific target. For example, the pulp and seed extract of *C. colocynthis* are used for the treatment of constipation and diabetes (Arora and Sen, 2014; Hajar *et al*., 2012). The reproductive organs are traditionally used in Yemeni folk medicine for treating many diseases such as rheumatism, hypertension, and various contagious diseases (Zahra *et al*., 2012). There is a lot of chemical compound in *C. colocynthis* fruit such as protein, carbohydrate, tannins, saponins, separated amino acid, phenolics, flavonoids, terpenoids, flavone glucosides, alkaloids, steroids, anthranol, saponarin, cucurbitacins, trace elements, cardic glycoloids, and many other chemical groups. It possessed antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, gastrointestinal, reproductive, protective, and many other pharmacological effects (Al-Snafi, 2016).

The uses of plants in folklore medicine are vast, and the scientific literature on the antioxidant activity of plants is very infrequent. The present study is thus conducted to estimate the phytochemical screening showed that the glycosides, flavonoids, tannin, and sterols have been presented in *C. colocynthis*, total antioxidant activity, and Total Phenolic Content (TPC). Furthermore, the ratios of phenolic compounds to total compounds which acts as antioxidants for *C. colocynthis* (crust, pulp, and seeds) extracts were calculated.

Materials and Methods

Plant Materials

The fruits of *C. colocynthis* (*Cucurbitaceae*) were collected from the Hamdan area near the city of Sana'a, Yemen in December 2019. The harvested fruits of *C. colocynthis* were washed well in fresh water and dried in the shade.

Extraction of Plant Material for Chemical Studies

C. colocynthis fruits were prepared by segregating the seeds and crust from the pulp and then all were powdered mechanically into fine particles. The crust (30 g), the pulp (14 g), and the seeds (45 g) powder were extracted with n-hexane, methanol, and 1, 1, 2 trichloroethane, respectively using a Soxhlet apparatus for 6 h. All extracts were filtered then the remanent of the solvent was evaporated using a rotary evaporator under reduced pressure.

Preliminary Phytochemical Screening of C. Colocynthis

Benariba *et al*. (2013); Rao *et al*. (2012); Uma and Sekar (2014)).

Standard methods used to perform phytochemical screening were.

Reducing Sugars Test (Fehlings Test)

1 mL of each extract solution was added to the boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

Terpenoides Test (Salkowski Test)

1 mL of chloroform was added to 0.1 mL of each extract solution. Concentrated sulfuric acid (a few drops) was added carefully to form a layer. The brown appearance indicates the presence of terpenoids.

Flavonoids Test

0.5 mL methanol solution, was treated with 1 mL of each extract solution. This mixture was warmed and magnesium metal was added. To this solution, 5-6 drops of concentrated hydrochloride acid were added and observation of the red color indicated the presence of flavonoids.

Tannins Test

10 mL of distilled water was boiled with about 0.1 mL of the extract solution in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and brownish green color observation was for tannins.

Saponins Test

To 0.1 mL of extract solution in a test tube, 1 mL of distilled water was added. The solution was shaken well and strong continuous foam was noted. 3 Drops of olive oil were mixed with the foam and shaken well. An emulsion formation was observed.

Alkaloids Test

When a few drops of Mayer's reagents (potassium mercuric iodide) are added to the alkaloid solution, white yellowish precipitate forms. The neutral or slightly acidic solution is used with Mayer's regent to form precipitates for most alkaloids.

Phytosterols Test (A. Salkowski Test)

To 1 mL of the extract solution 1 mL of chloroform was added and treated with 0.5 mL of Conc. Sulfuric acid. The solution was shaken and allowed to stand. The appearance of yellow color indicates the presence of triterpenes.

Detection of Protein and Amino Acid

A. Xanthoprotein test: 1 mL of extract solution was treated with 0.5 mL of Conc. Nitric acid. The formation of yellow color refers to the presence of proteins.

B. Ninhydrin test: 1 mL of extract solution, 0.25% w/v ninhydrin reagent was added and boiled for several minutes. The formation of a blue color indicates the presence of amino acids.

Detection of Fixed Oils and Fats

Spot test: Two pressed filter papers were penetrated by a small quantity of the extract. An oil spot on the study indicates the presence of fixed oil.

Determination of Antioxidant Capacity

Ferric-Bipyridine Reducing Capacity of Total Antioxidants (FBRC)

1.0 mL (0.01 m $FeCl_3$) solution was mixed with, 1.0 mL bipyridine (0.1%) then 2.0 mL 0.3 m acetate buffer (pH 4) was added. 50 µL of 10% essential oil solution was added to this mixture and diluted to 10 mL with deionized water. All samples were measured against a blank (1.0 mL $FeCl_3$, 2.0 mL acetate buffer (pH 4), 1 mL bipyridine, and 6 mL deionized water) at 535 nm. The results were expressed in g gallic acid and ascorbic acid equivalent per g of extract for each part of the fruit (g GAE or AAE/g extract). The experiments were carried out in triplicate (Naji *et al*. 2020).

Ferric Reducing Antioxidant Power (FRAP)

FRAP reagent was freshly prepared with a mixing of 10:1:1 (v:v:v) for 300 mmol/L sodium acetate trihydrate in glacial acetic acid buffer (pH = 3.6); 10 mmol/L TPTZ in 40 mmol/L HCl; and 20 mmol/L $FeCl_3$ respectively. Gallic acid and ascorbic acid were used for a standard curve. 50 µL of 10% essential oil will react with 2 mL of FRAP reagent shown above then the volume made up to 10 mL. The assay was performed at 37°C (pH = 3.6) After 10 min of incubation at room temperature, the absorbance at 593 nm was read (Benzie and Strain 1996). The results were expressed in g gallic acid and ascorbic acid equivalent per g of extract (g GAE/g of extract). The experiments were carried out in triplicate.

Total Phenol Contents (TPCs)

The Folin-Ciocalteu Aliquot method was used to evaluate the total phenol contents in the crust, pulp, and seed extracts. 0.05 mL of crust, pulp, and seed extract (1.0 mg/mL) were mixed with 2.0 mL of a 20 g/L sodium carbonate solution then 1 mL of the Folin-Ciocalteu reagent (1:10) Folin-Ciocalteu: deionized water was added. After 30 min incubation, the absorbance (750 nm) was measured at room temperature. Results were expressed as mg Gallic Acid Equivalent (GAE) and Ascorbic Acid Equivalent (AAE) per g of extracts (Benariba *et al*. 2013).

Calculation of the Ratio of TPCs to all Antioxidant Capacity

Use the following relationship to calculate the ratio of TPC to antioxidant compounds:

% TPCs = (TPCs/FBRC) *100
% TPCs = (TPCs/FRAP) *100

Statistical Analysis

Triplicates of each sample were used for statistical analysis and data are reported as mean ± SD. Pearson correlation and regression analysis were carried out using the SPSS statistical program to study the relationship between antioxidant activity and total phenol content. The values were also subjected to the analysis of variance and mean values were compared by Prism 6 174 software (GraphPad, San Diego, CA, USA). Differences at $p<0.05$ were considered to be significant.

Results

Qualitative Phytochemical Screening

Phytochemical screening is often used to identify the major components present in a given plant sample. Traditionally at various nativities in different parts of the world, *C. colocynthis (L.)* Schrad. has been used as a medicinal plant. In the current study of phytochemical compounds, antioxidant capacity and total phenol content for *C. colocynthis* were investigated.

Table 1 provides some qualitative phytochemical screening of crust, pulp, and seed extract solutions. Flavonoids were determined in all parts of *C. colocynthis*, the result obtained indicated some variation in their quantities. Pulp extract showed a positive result for terpenoids, tannins, saponins, alkaloids, and phytosterols while the crust extract showed a positive result for reducing sugar, tannins, and saponins. The seed extract showed negative results in all tests except fixed oil and fats, protein, and amino acids.

Antioxidant Capacity

Aromatic plants and their essential oils are a good source of natural antioxidants. The antioxidant capacity of these compounds is considered by their high redox properties and chemical structure, which can be responsible for the neutralization of free radicals, chelation of transition metals, and quenching singlet and triplet oxygen by delocalization or decomposition of peroxides (Brewer, 2011).

Table 1: Phytochemical screening of *Citrulluscolocynthis* crust, pulp, and seeds

Phytochemical constituents	Crust	Pulp	Seeds
Reducing sugar	+	-	-
Terpenoids	-	+	-
Flavonoids	+	+	+
Tannins	+	+	-
Saponins	+	+	-
Alkaloids	-	+	-
Phyto sterols	-	+	-
Protein and amino acids	-	-	+
Fixed oil and fats	-	-	+

+: Positive test; –: Negative test

FBRC and FRAP methods were used to quantify antioxidant capacity. Higher FBRC and FRAP values mean higher antioxidant capacity because FBRC and FRAP values are based on the reduction of ferric ions to ferrous ions by antioxidant compounds. The antioxidant capacity of *C. colocynthis* crust, pulp, and seeds are given in Table 2.

The results indicate that the pulp showed the highest total antioxidant capacity (0.3±0.09 g AAE and 0.37±0.1 g GAE)/g extract for the FBRC method and (0.94±0.07 g AAE and 0.2±0.02 g GAE)/g for the FRAP method, while the cross crust showed the lowest value (0.04±0.006 g AAE and 2.04±0.29 g GAE)/g for the FBRC method and (0.03±0.004 g AAE and 0.01±0.001 g GAE)/g for FRAP method.

Total Phenol Contents (TPCs)

For most studies, Phenolic compounds can be reasonably determined in a number of selected extracts making them one of the major groups of compounds that act as primary antioxidants (Abdelfadel *et al*., 2015). In experimental studies of three parts of *C. colocynthis* fruits, the total phenol content and extraction yields are given in Table 3. The crust gives the lowest extract yield, while the highest yeild is obtained for the pulp.

Extraction yields ranged from 0.67% for the crust and 26.4% for the pulp. The total phenol compounds contents (Table 3) presented in the analyzed extracts were calculated using ascorbic and gallic acids as standard compounds. The highest values were obtained for pulp (0.21±0.06 g AAE and 0.044±0.01 g GAE)/g of the extract while the crust showed the lowest total phenol values (0.04±0.001 g AAE and 0.007±0.001g GAE)/g of extract. The highest phenol contents were incorporated into the pulp extract. Whereas, crust extract has the lowest amount of phenol contents. This result is in line with that reported by Nessa and Khan (2014).

Correlation Between Antioxidant Activity (FRBC and FRAP) and Total Phenol Contents

Correlation analysis was used to determine the relationships between different antioxidant capacities and TPCs. An analysis of the correlation between TPCs and antioxidant activity was promised for the two methods used to evaluate the reduction in the efficacy of the three parts of *C. colocynthis* and R^2 values calculated.

Figure 1 shows the correlation coefficient between TPCs, FBRC, and TPCs, FRAP for crust extract expressed as AAE and GAE, respectively. The data obtained illustrate the high correlation between TPCs and two antioxidant methods (FBRC and FRAP). The correlation coefficient values were (0.9921 and 0.993) AAE and (0.9561 and 0.9462) GAE for FBRC and FRAP, respectively.

The relationship between antioxidant capacity and total phenol contents was found to be the highest values of seed extract (R^2 = 0.9678, 0.946) AAE and (0.9975, 0.970) GAE for FBRC and FRAP, respectively (Fig. 2).

As can be seen from Fig. 3, there was a small correlation between TPCs and the antioxidant methods (FBRC and FRAP) for the pulp extract (R^2 = 0.8309, 0.975) AAE and (0.791, 0.884) GAE for each FBRC and FRAP.

Calculation of the Ratio of TPCs to all Antioxidant Capacity

Because all phenolic compounds are antioxidants but not all antioxidants are phenols, the ratio of TPCs to the number of total phenols that acts as an antioxidant is calculated.

Figures 4a and 5a showed that the number of antioxidants and TPCs was highest in the pulp, while the lowest value was in the crust. The ratio of TPCs in the different parts of the fruit was estimated. The crust part had the highest value, whereas the seeds extract had the lowest value (Fig. 4b and 5b).

Table 2: FBRC and FRAP values are expressed as (g AAE and GAE /g of *Citrulluscolocynthis* fruit extracts (crust, pulp, and seeds)

	FBRC		FRAP	
Part of fruit	g AAE/g extract	g GAE/g extract	g AAE/g extract	g GAE/g extract
Crust	0.04±0.0060	0.01±0.0010	0.03±0.004	0.01±0.001
Pulp	0.30±0.0900	0.10±0.0100	0.45±0.050	0.26±0.020
Seeds	0.13±0.0060	0.04±0.0010	0.16±0.006	0.04±0.001

The results are means ± SD (n = 3).
AAE/ascorbic acid equivalent, GAE/gallic acid equivalent

Table 3: Yield of extracted substances and TPCs values were expressed as (g AAE and GAE/g in fruit extracts (crust, pulp, and seeds)

	The yield of extracts			Total Phenols Contents (TPCs)	
Part of fruit	Dry weight (g)	Extract yield	Percentage of yield %	g AAE/g extract	g GAE/g extract
Crust	30	0.2 g	0.67	0.04±0.001	0.007±0.0010
Pulp	14	3.7 g	26.40	0.21±0.060	0.044±0.0100
Seeds	45	4 mL	8.90	0.05±0.001	0.010±0.0010

The results are means ± SD (n = 3)
AAE/ascorbic acid equivalent, GAE/gallic acid equivalent

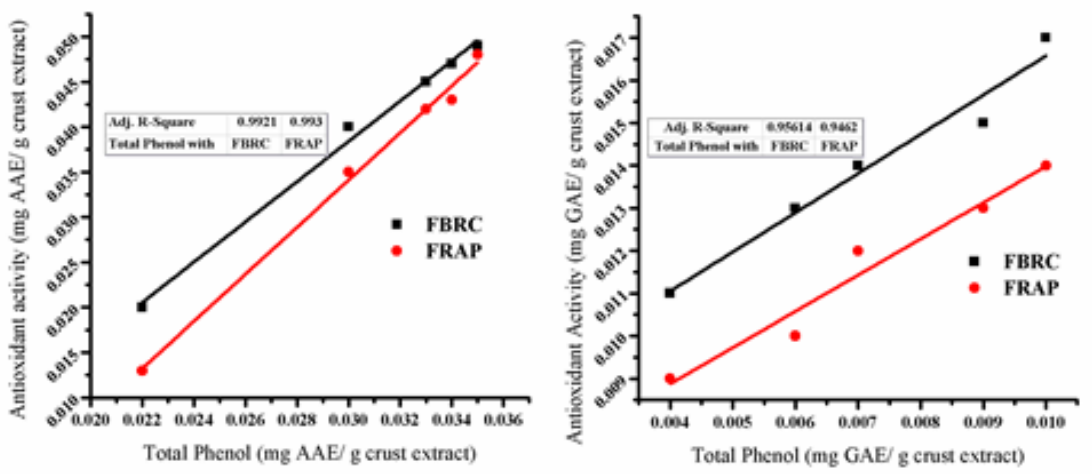

Fig. 1: Linear correlation between the number of total phenol contents and total antioxidant activity using (FBRC and FRAP) methods for crust extract express as AAE and GAE respectively

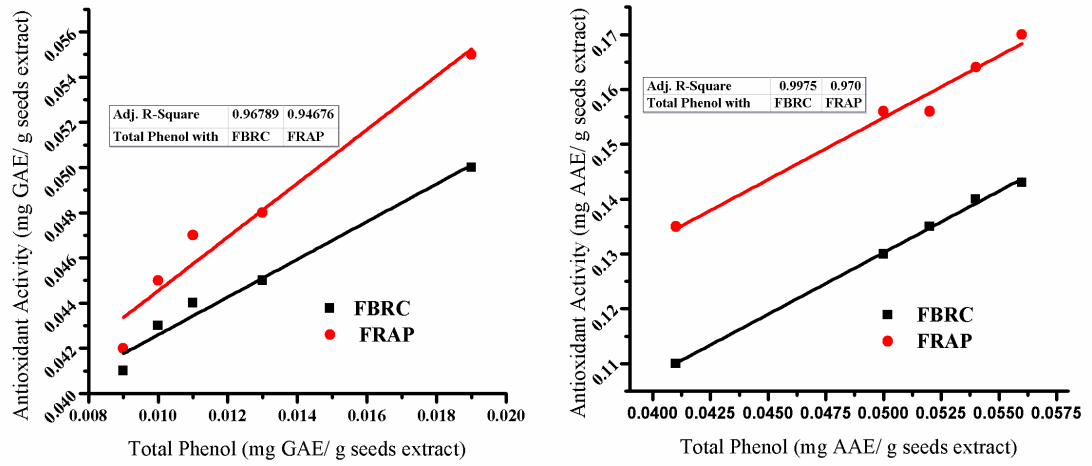

Fig. 2: Linear correlation between the number of total phenol contents and the total antioxidant activity using (FRBC and FRAP) methods for seeds extract express as AAE and GAE respectively

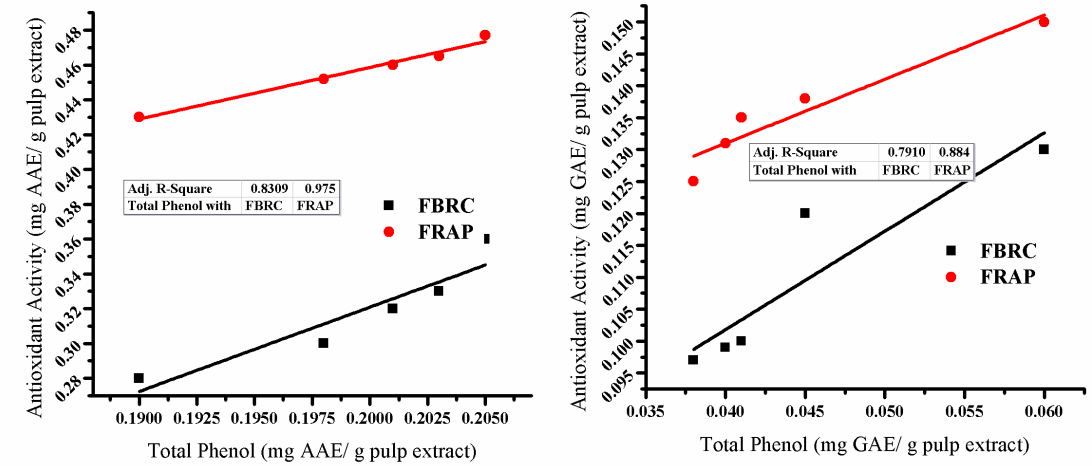

Fig. 3: Linear correlations between the number of total phenol contents and the total antioxidant activity using (FBRC and FRAP) methods for the pulp extract expressed as AAE and GAE respectively

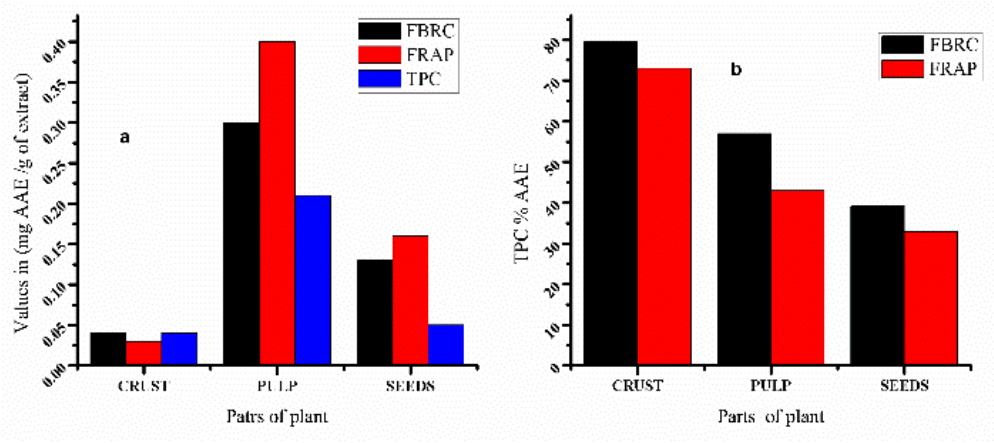

Fig. 4: (a) Amount of total phenol contents and total antioxidant activity using (FRBC and FRAP) methods; (b) the ratio of TPC to (FRBC and FRAP) for the three-part fruit extract expressed as AAE

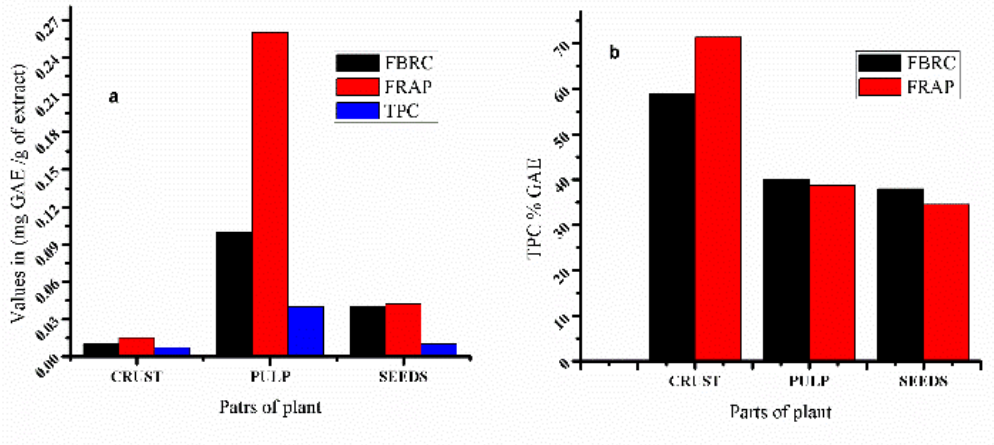

Fig. 5: (a) Amount of total phenol contents and total antioxidant activity using (FBRC and FRAP) methods; b. the ratio of TPC to (FBRC and FRAP) for three parts of fruit extract expressed as GAE

Discussion

The *C. colocynthis* phytochemical study provides analyzed information on the compounds present in all parts of the fruit. The behavior of crust, pulp, and seed extracts with different chemical agents was also investigated.

The presence of alkaloids and flavonoids in the pulp of fruit supported the reports of various authors (Uma and Sekar, 2014; Ali *et al*., 2013; Rao *et al*., 2012) which proved that alkaloids were found in all parts of the plant except the roots (Nora *et al*., 2015) as a result *C. colocynthis* is recommended for the treatment of inflammation and microbial infection as reported by Hany and Neelam (2020).

From obtained results, Yemeni *C. colocynthis* has the highest total phenols content (6.1 g GAE/100 g extract) compared with *C. colocynthis* obtained from Pune, India (3.2 g GAE/100 g extract) (Talole *et al*., 2013), Haryana, India (0.74 g GAE/100 g extract) (Kumar *et al*. 2008) and west Algeria (0.166 g GAE/100 g extract) (Benariba *et al*., 2013). As a result, *C. colocynthis* (crust, pulp, and seeds) extracts showed good antioxidant capacity values. Pulp extract had the highest antioxidant value whereas crust had the lowest value. This result asseverates that the antioxidant activities of extracts are associated with their polyphenolic content (Nessa and Khan, 2014).

The high correlation between TPCs and two antioxidant activity methods estimated (FBRC and FRAP) show that, the antioxidant activity of *C. colocynthis* (crust, pulp, and seeds) extracts appear to be largely influenced by the total phenols, these results were in agreement with previous literature (Chang *et al*., 2007; Jiang and Zhang, 2012; Marques and Perestrelo, 2007). These results indicate that plant antioxidant activity is not only influenced by phenol contents but also by another compounds such as volatile oils, diterpenes, carotenoids, and vitamins which act as secondary metabolites. This result is accepted with positive results for terpenoids phytochemical

test for pulp. Chua *et al*. (2015) suggested that the antioxidant capacity is influenced by the aggregate activity of compounds such as phenolics, peptides, organic acids, enzymes, and other minor components.

The ratio of TPCs to all antioxidant capacity indicates that the most antioxidant compounds in the crust were phenolic compounds whereas in pulp and seeds other compounds act as antioxidants besides phenolic compounds.

Conclusion

C. colocynthis fruit is a valuable source of medicinal compounds that have been traditionally used for numerous applications. Based on the results obtained in this study, it can be concluded that *C. colocynthis* fruit (crust, pulp, and seeds) extracts were showed highly effective as an antioxidant. The results indicate that the Yemeni *C. colocynthis* has the highest value of total phenol contents compared with the other areas. Consequently, Yemeni *C. colocynthis* can be used broadly in pharmacological preparations.

This, in turn, leads to a significant increase in health insurance costs and an improvement in the life quality of patients.

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Author's Contributions

Faten Hameed Thamer: Conceived and designed the experiments, performed the experiments and analyzed, wrote the paper.

Noah Thamer, Anhar Alhamzi, Naseem Al-Ansi, Sarah Al-Sadi and Abdullah Al-Shibeh: Performed the experiments and analyzed, interpreted the data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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