Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2023.473.484

Research Article
Phytochemistry and Antioxidant Activity of *Citrullus colocynthis* (L.) Fruit Tissues: Correlation to Soil Macronutrients

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Abstract

Background and Objective: *Citrullus colocynthis* is a wild plant that grows in many places in the world and the antioxidant activity varies by geographic origin. Profiling of the antioxidant activity, total phenolic content (TP) and total flavonoid content (TF) of the peel, pulp and seed tissues of the dry fruit were investigated using various *in vitro* assays. **Materials and Methods:** Fruit samples grown in clayey and sandy soils were collected from five places. The ferric reducing antioxidant power (FRAP) and the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assays were used to measure antioxidant activity. In all sites, the peel tissue exhibited the highest total antioxidant activity followed by the seeds and finally the pulp. **Results:** The scavenging activity for clayey and sandy soils ranged from 52.0 to 88.0% and from 39.0 to 82.6%, respectively. The clayey fruits showed higher DPPH activity than the sandy ones with a difference in the range of 2.0-14.0%. The Folin-Ciocalteu and aluminum chloride spectrophotometry assays, respectively, were employed to determine the TP and TF. The clayey and sandy soils showed TP in the range 3.38-4.07 and 2.48-3.67 mg gallic acid equivalent g^{-1} of dry weight of the whole fruit. Also, the TF was 0.72-0.95 and 0.56-0.77 mg quercetin equivalent g^{-1}, respectively. The correlation matrix (CM) showed a significant correlation between the DPPH, TP and TF with nitrogen content in the soil. Cluster analysis was also applied for fruit discrimination. **Conclusion:** The ecological discrimination of the fruit origin was affected by the contents of the macronutrients and the type of the soil.

Key words: Antioxidant activity, *C. colocynthis* fruit, clayey and sandy soils, correlation matrix, cluster analysis

Citation: Alsharari, S.F., 2023. Phytochemistry and antioxidant activity of *Citrullus colocynthis* (L.) fruit tissues: Correlation to soil macronutrients. Asian J. Plant Sci., 21: 473-483.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The antioxidant activity of numerous medicinal plants used in conventional medicine throughout the world was examined. The Cucurbitaceae family is now being extensively studied for their antioxidant activity among many plant species which were historically used as antidiabetic remedies[1]. *Citrullus colocynthis (C. colocynthis*) or bitter fruit is among the most important genus in Cucurbitaceae that includes 118 genera spread all over the globe, many of them are edible while others are ornamental or beneficial plants[2]. It grows in tropical, subtropical, arid deserts and temperate areas[3]. This plant is widely naturalized in the Arabian deserts, Pakistan, India and Southern islands[4]. Pharmacological action corresponds to the content of this species secondary metabolites, mainly cucurbitacins, saponins, flavonoids, polyphenols, terpenoids and alkaloids[5]. *Citrullus colocynthis* rich in oil and protein and its flesh contains alkaloid compounds, saponins, glycosides, colocynthis, elaterin, elatericin B, dihydro-elatericin B, A glycoside photo-citrullus, fixed oil, ether-soluble resin, chloroform, gum, pectin acid (pectin), albuminoids, calcium phosphate, magnesium, lignin and water[6]. The *C. colocynthis* widely used to treat diabetes, cancers, hemorrhoids inflammation and infectious diseases[7]. In medicinal and food plants, phenolic and flavonoid compounds constitute significant bioactive components are a secondary metabolic by-product of the pentose phosphate, shikimate and phenylpropanoid pathways with one or more hydroxyl group on the aromatic ring.

They doing as antioxidants and free radical scavengers, helping to combat oxidative stress and its detrimental effects[8]. They act as antioxidants and free radical scavengers and help to manage oxidative stress and its harmful effects, there by preventing and regulating many terrible diseases and aging prematurely[9]. Antioxidant activity was determined in plant extract by various methods such as the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging activity assay[1], ferric reducing antioxidant power (FRAP) assay[10], Trolox equivalent antioxidant capacity (TEAC or ABTS) and reducing power (RP) assays[11]. Total phenolic content is determined by the Folin-Ciocalteu (F-C) assay[12], total polyphenol content assay[13], Prussian blue assay[14] and fast blue BB assay[15]. However, the F-C assay is the most common method applied for the determination of total phenols. Flavonoids are usually determined by the aluminium chloride colorimetric assay[16]. Formation of phenolic compounds and flavonoids in plants is extremely subjective by environmental factors such as average temperature, rain fall and soil elements[17].

The Kingdom of Saudi Arabia (KSA) is characterized by its heterogeneous soil and climatic conditions and variation in environmental conditions, including topography, geomorphology, climate and soil. These environmental changes give the country unique ecological habitats, vegetation zones and as a result, the region's rich flora. The country has a broad range of ecosystems and biodiversity suitable for numerous medicinal and aromatic plant species with different properties creditable to further study[18]. Bukhari *et al.*[19] studied the autecological effect on *C. colocynthis* species in KSA and they proposed the geographical distribution of the species and concluded that comprehensive taxonomic studies were the basic tools for identifying the different species and placing them in their proper positions. Al-Ghamdi[20] investigated the ecological correlation between the different physicochemical properties of soil with each other and their effect on the extracted and powdered materials of *C. colocynthis* leaves for potential insecticidal properties. Arid climates in KSA are known to activate the production of high concentrations of secondary plant phytochemicals. This presumably made these plants more capable of fighting their natural enemies such as insects, herbivores and diseases[21]. Another work was made on the cryopreservation of wild bitter apple (*Citrullus colocynthis* L.) seeds collected from Madinah and Riyadh Regions in KSA by keeping them in liquid nitrogen for utilization in future breeding programs for abiotic stress tolerance[22]. A cytological profiling was used to characterize the native Saudi Arabian plant *C. colocynthis* by using a library of known small molecules with assigned modes of action as reference compounds[23]. Hazardous of the *C. colocynthis* warned against its use and banned by the Food and Drug Administration (FDA)[24]. It leads to very serious side effects and may cause death, so it is advised not to use it unless you consult a doctor. The use of *C. colocynthis*, cramps, diarrhea, vomiting and shortness of breath, may cause acute colon inflammation if the bitter melon plant is swallowed and may cause internal bleeding that leads to death. The plant fruit should not be used permanently, by children, pregnant women or people with weak immunity. Finally, this plant is too poisonous and you must so avoid it.

To the best of our knowledge, there is no previous work that has investigated the detailed antioxidant activity of this fruit from various Saudi origins which makes it of great importance. This is due to ecological factors that are expected to affect the amount of phenolic compounds in the fruit and there by, the antioxidant activity. The present study aimed to evaluate the antioxidant activity of all parts of the *C. colocynthis* fruit collected from various places as a

comparative study of the ecological effect. Four analytical methods were used namely total polyphenols assay, flavonoids assay, free radical scavenging action assay ferric reducing antioxidant powerassay (FRAP) and [2,2-diphenyl-1-picrylhydrazy lradical (DPPH)].

MATERIALS AND METHODS

Study area: The study was made in 2020 from June to December.

Chemicals: The DPPH (1,1-diphenyl-2-picrylhydrazyl) (Sigma-Aldrich, Morocco, United States America), Potassium ferricyanide [$K_3Fe(CN)_6$] (Panreac Co., Barcelona, Spain), Aluminum chloride ($AlCl_3$) (Loba Chemie, Mumbai and India), sodium tungstate Na_2WO_4 dehydrate. The $2H_2O$ (BDH chemical Ltd., England, United Kingdom), Sodium molybdate dihydrate ($NaMoO_4$. $2H_2O$) (Panreac Co.), Ferric chloride, ($FeCl_3$) (Techno Pharmchem, Delhi, India), gallic acid and quercetin were from El-Nasar Pharmaceutical Chemicals Co. Cairo, Egypt, the ascorbic acid was from BDH chemical Ltd., Dorset, United Kingdom, the orthophosphoric acid was from Techno Pharmchem, the hydrochloric acid was from CDH Co., New Delhi, India, the lithium sulfate hydrate was from Loba Chemie, Mumbai, India, the sulfuric acid (CDH), sodium phosphate were from Techno Pharmchem, the ammonium molybdate was from Suvachem Co. Maharashtra, India). Sodium carbonate (Na_2CO_3) (Loba Chemie), sodium hydroxide (NaOH) (Techno Pharmchem), sodium nitrite ($NaNO_2$) (Panreac), HPLC grade methanol was from Fisher Scientific, Loughborough, UK were of analytical grade.

Instruments: A Cintra 1010 double-beam UV-Vis spectrophotometer functional with Cintra 2.4 software (GBC scientific equipment, Braeside, Australia) was used for measurements in all assays. The soil samples were digested using a digestion system model top wave from Analytik Jena AG Company (Jena, Germany The double-distilled water was used during the study was from a purification systematic Hamilton Glass Ltd., Margate, United Kingdom. The extract was filtered using qualitative filter paper No. 101 (Dorsan Filtration SL Co., Barcelona, Spain).

Reagents: The DPPH solution (0.3% w/v) was prepared in methanol. Potassium ferricyanide [$K_3Fe(CN)_6$] solution at a concentration of (1.0% w/v) was prepared by dissolving 1.0 g of the reagent in 100 mL double-distilled water. Ferric chloride (0.1%, w/v) solution was obtained by dissolving 100 mg of $FeCl_3$ in 100 mL water. Dissolving 10 g of sodium tungstate with 2.5 g of sodium molybdate were added to a round bottom bottle in 70 mL of distilled water to create a F-C reagent, 10 mL of hydrochloric acids and 5 mL of orthophosphoric acid were added and the mixture was refluxed for 10 hrs. After that, 15 g of lithium sulfate was added and the mixture and heated for a further 15 min. After cooling, the mixture was eventually completed with double distilled water to 100 mL. Aluminum chloride solution (3.0%, w/v) was prepared by dissolving 3.0 g of $AlCl_3$ in 100 mL water. Sodium nitrite (5%, w/v) was prepared in double distilled water. Sodium hydroxide solution (1.0 mol L^{-1}) was prepared by dissolving 4.0 g of the reagent in 100 mL double-distilled water. Standard gallic acid, ascorbic acid and quercetin solutions of 2.0 mg mL^{-1} were made by adding 0.25 g of the substance in the least amount of methanol and then completing the solution with double distilled water up to 250 mL.

Sampling sites: The *C. colocynthis* fruits were freshly collected from five places distributed around the capital Riyadh, Kingdom of Saudi Arabia. These regions were Al-Quwayiyah (Q1 and Q2), Ath Thumamah (A1 and A2), Thadiq (T1 and T2) and Al Uyaynah (U1 and U2) and Rawdat Al Khafs (R1 and R2). The numbers 1 and 2 were designated for the clayey and sandy soils, respectively. Al-Quwayiyah is located approximately 180 km from Riyadh, to the west. The eastern side is a broad flat desert with a sedimentary composition and various valleys. Al Thumama is located to the northeast of Riyadh, just 80 km from the city center with diverse geographical nature. It includes approximately 195 species of plants. Thadiq is a governorate located in the northwestern part of the Riyadh Region. It is characterized by green areas and fertile soil. The Al Uyaynah is a village located on Wadi Hanifa, in the Al-Arid area. It has fertile soil and abundant water and its great valleys and reefs. Rawdat Al-Khafs is a flat sandy plain, covered with shamrocks and it does not have large trees, surrounded by sand from the west and the mountains from the east and poured into valleys, the most important of which is an inspiring reef. All these sites are rich in the naturally growing *C. colocynthis*. Samples were collected during the first ten days of June, 2020. The growing land of the collected fruit was clay or sand type. The collected fruits were naturally dried and fresh ones were taken in plastic and the fresh ones. In the laboratory, the fresh fruits were cleaned and dried at room temperature.

Analysis of the soil macronutrients: Nitrogen, phosphorus and potassium: Using color-based reagents, the growing soil nutrient analysis was carried out to determine the concentration of three main soil macronutrients, nitrogen, phosphorus and potassium. Understanding the concentration of the three main macronutrients in soils will alert environmental scientists to know the nutrient deficiency or excess in soils used to support plant growth and provide an overall overview of an ecosystem's basic biogeochemical cycles. In this analysis, nitrogen or phosphorus is reacted to a reagent and produces colored products. On the other hand, potassium concentration can be determined by forming precipitates with sodium tetraphenylborate or by flame photometry. In this work, the macronutrients were analyzed at the Department of Soil and Drinking Water Analysis in the Faculty of Agriculture, King Saud University (Riyadh, Saudi Arabia). Total nitrogen was determined using Kjeldahl method using a 1.0 g dry soil (0.15 mm mesh size) and extractable phosphorus was determined using the bicarbonate method using a 2.5 g dry soil sample[25]. The available potassium content in the soil was analyzed using the flame photometer method[26]. Results obtained are expressed in mg kg^{-1}.

Sampling of soil and *C. colocynthis* fruit: Two kinds of soils (clayey and sandy) at the five sites (Q, A, T, U and R) compromise a total of 10 sampling locations. For each location, 5.0 soil portions or fruits were collected with total size of 50 each. The soil samples were taken directly from the surrounding parts in the vicinity of the plant root. About 1.0 kg soil was collected at each plant with a total size of 5.0 kg from either the clayey or sandy soil. The soil macronutrient analysis was performed to determine the concentration of three major soil macronutrients.

In conjunction with taking the soil sample, a naturally dried *C. colocynthis* fruits were collected with at least 5.0 PCs from each place. The collected fruits were picked while being attached to the plant. The fruit without any crushing or pitting as taken. The weight of the fruit ranged from 15.8-20.5 g with an average weight of 18.1±1.6 g. The measurement of fruit length was made on the polar axis, i.e. between the apex and the end of the stem. The average axis length was 5.0±0.6 cm. Air-dried fruit from each location was divided into the peel, the pulp and the seeds (Fig. 1). The average weight was 4.7±0.5, 2.3±0.4 and 11.1±0.7 for peel, pulp and seeds, respectively. All dried fruits from the distinctive site were sorted into the peel, pulp and seeds and grouped together. The ultimate amount from each tissue was completely homogenized. The peel and the pulp were ground first in a porcelain mortar into a fine powder before taking the representative sample. While in the case of seeds, the total amount was mixed by mechanical stirring and then the sample was taken for grinding. The final powdered samples were kept in cleaned glass bottles for the subsequent extraction.

Extraction of phenolics and flavonoids: The phenolic and flavonoid compounds in the peel, pulp or seed of the *C. colocynthis* fruit were extracted according to the reported method[27], with minor modification. An accurately weighed portion of 10.0 g of the powdered sample was weighed on a digital balance with an accuracy of 0.001 g. The sample was infused in 50 mL of 70% methanol and distilled water at room temperature for 72 hrs over a magnetic stirrer. After that, the mixture was passed through Dorsan No. 101 filter paper. The clear extract was evaporated and the average amount of 0.4±0.07, 1.2±0.03 and 0.15±0.01 g of the dried extract was obtained for the peel, pulp and seed, respectively equivalent to a yield of 4, 12 and 1.5%. The dried extract was stored in airtight screw-capped dark glass bottles at 4°C till further use. A definite concentration of the dried extract at a concentration of 2.0 mg mL^{-1} was prepared in methanol and analyzed.

Free radical scavenging activity (DPPH) assay: The free radical scavenging activity of the ethanolic extracts of the *C. colocynthis* fruit was determined using DPPH assay

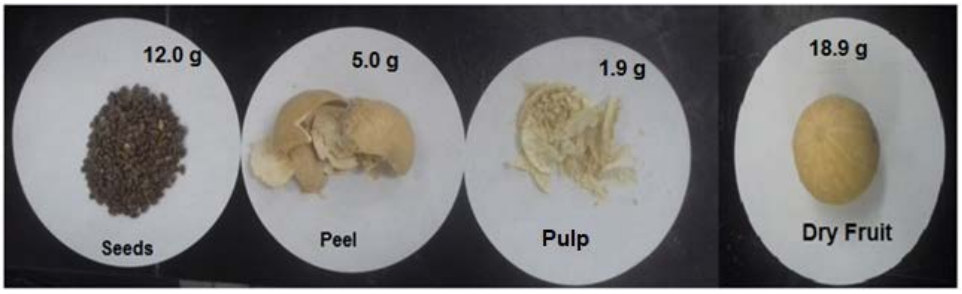

Fig. 1: Weight fractionation of a dried *C. colocynthis* fruit of 20.4 g into its peel, pulp and seeds

according to the reported method[1]. Briefly, 1.0 mL of the extract (2.0 mg mL^{-1}) was added to 1.95 mL of 0.5% (w/v) DPPH solution. The mixture was strongly shaken and incubated in the darkness for 30 min. The absorbance (A) of the samples and the control sample (ascorbic acid was used as antioxidant reference) was measured at 515 nm and the scavenging activity was expressed as the percentage in inhibition as follow:

$$\text{Free radical scavenging activity (\%)} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100$$

Ferric reducing activity power (FRAP) assay: The FRAP test was used to measure the reducing power of ferric iron to the ferrous form in the presence of antioxidant components. The reducing power of the *C. colocynthis* fruit extract was evaluated according to the previous method[10] using ascorbic acid (AA) as a standard. To 1 mL of extract solution (2.0 mg mL^{-1}), 2.5 mL of 0.2 mol L^{-1} phosphate buffer (pH 6.6) and 2.5 mL of (1%, w/v) $K_3Fe(CN)_6$ were added. The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of (1%, w/v) $FeCl_3$ solution. The absorbance was measured at 700 nm against a blank sample and ascorbic acid was used as a reference standard. The absorbance increased by increasing the reducing power of the extract. Finally, results were calculated using ascorbic as a positive standard at a concentration of 2.0 mg L^{-1}.

Determination of total phenolics: The total phenolic (TP) content of the obtained extracts from all parts of the *C. colocynthis* fruit was quantified by Folin-Ciocalteu (F-C) method[12] using gallic acid (GA) as standard. For analysis of the sample, a 2.0 mL volume of the mixture was mixed with 2.0 mL of the F-C agent. The solutions were mixed and incubated for one minute. After that the samples were then added to 5 mL of 20% of Sodium carbonate (Na_2CO_3) solution and the volume was made up to 50 mL. After incubation for 60 min, the absorbance was tested at 765 nm. For every sample, the data presented are mean values mg gallic acid equivalents per dry weight (GAE mg g^{-1} dw) of triplicate trials. The standard gallic acid calibration curve obtained with the F-C method is:

$$A = 0.043+0.002\ C\ (\text{mg GA } L^{-1}),\ R = 0.994$$

Determination of total flavonoids: Total flavonoid (TF) content was determined by the aluminum chloride colorimetric method[28] using quercetin (Q) as a standard. An aliquot of 1.0 mL of the hydro-methanolic extract sample was mixed to 4 mL of water in a 25 mL volumetric flask. Then, equal volumes (0.3 mL) of sodium nitrite and aluminum chloride were added after passage of 5 min and the sample was incubated for 6 min at room temperature. After that, a 1.0 mL aliquot of 1 mol L^{-1} sodium hydroxide was added to the mixture and the final volume of the solution was directly made with 10 mL of distilled water. The absorbance of the resultant mixture was tested at 510 nm using UV-Vis spectrophotometer against the blank. All the measurements were repeated in triplicates for precision and the flavonoid content expressed in quercetin equivalent per dry weight (QE mg g^{-1} dw) and reported as the Mean±SD. The typical standard curve for flavonoid determination is represented by:

$$A = 0.119+0.0062C\ (\text{mg Q } L^{-1}),\ R^2 = 0.988$$

Statistical analysis: The experimental results of this study are expressed as Mean±SD of three replicate measurements. Obtained results were analyzed by the Analysis of Variance (ANOVA) procedures. The diagrams and correlation coefficients were performed using the SPSS statistics V15.0 software. The pearson's test was calculated for the correlation between DPPH, FRAP, TP, TF and the macronutrients in the soil (nitrogen, phosphorous and potassium) using the statistical software with a level of significance established at $p<0.05$.

RESULTS AND DISCUSSION

Macronutrients in of the growing soil: The amount of the macronutrients (nitrogen, phosphorus and potassium) in the growing soils of the collected samples was compiled in Table 1. Nitrogen content ranged from 60.5 to 6.2 mg kg^{-1}. It was found maximum in the sites U1 and Q1. The least amount was found in the site R2. The increased content of nitrogen can be due to the high content of organic matter which able to fix nitrogen. The phosphorus content ranged from 1.0 to 12.0 mg kg^{-1}. The site T2 showed the maximum value and the least amount was observed in R2. The high phosphorus content might be attributed to organic wastes which increase the organic phosphorus to the soil through decomposition. Exchangeable potassium content ranged from 39 to 450 mg kg^{-1}. Again, the site Q1 was the maximum value and R2 was the least content. Very clear that the two sites Q1 and Q2 are distinguished from the rest of the sites. Importantly, due to the lack of sufficient application of fertilizers, the content of these elements may lose a considerable amount with time. This can affect the growing efficiency in the plant from one site to another.

Table 1: Analysis of soil macronutrients (nitrogen, phosphorus and potassium) in the studied sites

	Studied site[a]									
Element	Q1	Q2	A1	A2	T1	T2	U1	U2	R1	R2
Nitrogen (mg kg^{-1})	55.5	33.6	24.6	9	16.2	8.4	60.5	33	20.7	6.2
Phosphorus (mg kg^{-1})	4	4	3	7	10	12	9	4	6 1	
Potassium (mg kg^{-1})	450	132	54	66	78	37	107	77	73	39

[a]Site: Al-Quwayiyah (Q), Ath Thumamah (A), Thadiq (T), Al Uyaynah (U) and Rawdat Al- Khafs (R), Numbers 1 and 2 designated for clayey and sandy soils, respectively. Results are expressed in mg kg^{-1}

Table 2: DPPH antioxidant radical scavenging activity of the methanolic extracts of peel, pulp and seed of *C. colocynthis* fruits from various regions of KSA

	DPPH scavenging activity (%)[b]		
Sample site[a]	Peel	Pulp	Seeds
Q1	88.0±1.3	77.0±1.0	81.3±0.6
Q2	80.0±0.1	68.0±1.7	74.5±0.3
A1	81.0±1.2	71.1±0.5	78.0±1.1
A2	73.5±0.5	63.0±1.6	67.5±0.3
T1	67.0±1.2	52.0±0.9	53.0±0.3
T2	61.4±1.1	48.0±1.2	51.0±0.2
U1	86.7±1.0	73.0±1.3	82.4±0.8
U2	82.6±0.5	66.5±0.3	75.0±1.9
R1	70.0±0.5	53.0±1.0	55.8±1.4
R2	58.2±0.4	39.0±1.4	46.0±1.1

[a]Sites: Al-Quwayiyah (Q), Ath Thumamah (A), Thadiq (T), Al Uyaynah (U) and Rawdat Al- Khafs (R), Numbers 1 and 2 designated for clayey and sandy soils, respectively, Results determined as Mean±SD for triplicate measurements and [b]Methanol extract concentration (2.0 mg mL^{-1}) against standard ascorbic acid solution of 0.05 mg mL^{-1}

Antioxidant activity

DPPH free radical scavenging assay: The value of the antioxidant activity was quantified by the DPPH method and was represented as the scavenging activity (%). The scavenging activity measures the hydrogen donating ability of the extract to DPPH. It was evaluated at a specific concentration of the dry extract and the corresponding dry weight for each fruit tissue. Results obtained for the studied 10 samples were shown in Table 2. The percentage of scavenging activity of the hydro-methanolic extract ranged from 58.2 to 88.0%, from 39.0 to 77.0% and from 46.0 to 82.4% in peel, pulp and seeds, respectively. In methanolic extracts, comparable scavenging activity of 88.0% at concentration of 2.5 mg mL^{-1} was reported by Kumar *et al.*[14] and was 74.5% at 2.0 mg mL^{-1} in the seed extract[2]. Clearly, the peel tissue exhibited the highest scavenging activity which indicates higher amount of reducing phenolic and flavonoid contents. The seeds exhibited a lower scavenging activity than the peel. The least amount was obtained in the pulp tissue.

The high scavenging activity can be due to the *C. colocynthis* fruit tissues are rich in many reducing polyphenols compounds including phenolic acids and flavonoids. Besides, seeds and pulps, leaves and roots of *C. colocynthis* were found to contain alkaloids, sterols cucurbitacins[2]. The flavonoid compounds exist are quercetin, chemopherol, myricetin, isovitexin, isoorientin, isoorientin-3-methyl ether and C-p-hydroxybenzyl derivatives (8-C-p-hydroxybenzoyl isovitexin, 6-C-p-hydroxybenzoyl-vitetoxin and 8-C-p-hydroxybenzoyl isovitexin-4'-o-glucoside)[3].

Based on the type of soil, the clayey sites showed higher scavenging activity than sandy ones. A significant difference in DPPH radical scavenging activity was observed between the clayey and sandy soils. In clayey soil the range was 52.0-88.0% and in sandy soils ranged from 39.0-82.6%. The difference in scavenging activity in peel was 8.0, 7.5, 5.6, 4.1 and 11.8%, respectively for the sites Q, A, T, U and R, respectively. In pulp, it was 9.0, 8.1, 4.0, 6.5 and 14.0%, respectively. For seeds, it was 6.8, 10.5, 2.0, 7.4 and 9.8%, respectively. The sites Q, U and R showed the highest difference in inhibition activity in case of the peel and seeds. However, the largest difference in all tissues was remarkable in the R site which reflects the significant difference in the soil macronutrients. This conveys the significant effect of the soil composition on the formation of polyphenols.

The increase in antioxidant activity in clayey soils than the sandy lands can be due to the clay soil is a fine-grained material containing hydrous clay minerals that provide plasticity when humid[29]. Also, the presence of phyllosilicate minerals that contain a variable amount of trapped water in the mineral structure making it a good source for watering the plant during its growing period.

FRAP reducing antioxidant power assay: The extract's FARP reducing power represents its antioxidant activity, which was

determined using the reduction assay Fe^{3+} to Fe^{2+}. The extract solution's yellow color shifts to the different shades of green and blue, depending on the reducing power of the sample. Existing antioxidants in the sample result in Fe^{3+} being reduced to Fe^{2+} in the ferricyanide complex to give the Perl's Prussian blue, which is quantified at 700 nm. Determining the antioxidant activity by the FRAP allows a more accurate estimation of the plant's possible protective effect. Figure 2 showed the FRAP antioxidant capacity of the selected samples to peel, pulp and seed, expressed as FRAP value. Obviously, the FRAP value differed significantly depending on tissue evaluated in the fruit. In peel, the FRAP reducing power was the highest and ranged from 0.71-1.44, followed by the seeds in the range 0.63-1.23 and finally the pulp in the range 0.44-0.85. Results were quantified compared to ascorbic acid reducing power that was 1.67 at 2.0 mg AA mL^{-1}.

Likewise, clay soils have shown higher FRAP reducing power in all fruit tissues compared to sandy soils. The values ranged from 0.54 to 1.44 and from 0.52 to 1.00 for clayey and sandy soils, respectively. The reason could be due to the higher element concentration in the clayey than sandy soil. In addition, the clayey soil able to hold more nutrients and water than the sandy ones for longer periods during plant growth. Finally, the FRAP assay could clearly provide ecological discrimination the various kinds of *C. colocynthis* samples.

TP content by F-C assay: The amount of TPC was determined using the F-C method and expressed in gallic acid equivalents (GAE). This assay enables estimation all the flavonoids, anthocyanins and non-flavonoid phenolic compounds that present in the sample. Results obtained were compiled in Table 3. Peel exhibited the highest TP content in all samples compared to other tissues of the fruit. Its TP content ranged from 3.11 to 5.69 GAE mg g^{-1} dw. The following content was observed in the seeds within the range 3.27 to

Table 3: TP content of the peel, bulb and seed extracts of *C. colocynthis* fruits from various regions of KSA

	TP content[b] (mg g^{-1})					
Sample site[a]	Peel	Pulp	Seeds	Total TP (mg $fruit^{-1}$)	Whole fruit[c] (mg g^{-1})	Fruit weight (g)
Q1	5.42±0.13	2.98±0.30	3.73±0.41	65.8	4.06	16.2
Q2	4.51±0.32	2.65±0.10	3.36±0.08	66.9	3.57	18.7
A1	5.17±0.49	2.24±0.15	3.58±0.45	66.3	3.89	17.1
A2	4.45±0.20	2.15±0.22	3.06±0.32	63.1	3.34	18.9
T1	4.46±0.29	2.35±0.31	3.30±0.37	55.8	3.38	16.5
T2	3.85±0.24	2.07±0.19	3.71±0.30	67.5	3.27	19.4
U1	5.69±0.37	2.88±0.04	3.65±0.16	64.3	4.07	15.8
U2	5.32±0.34	2.54±0.18	3.33±0.08	75.3	3.67	20.5
R1	4.65±0.11	2.50±0.35	3.34±0.14	68.2	3.53	19.3
R2	3.11±0.18	2.00±0.26	2.27±0.21	46.5	2.48	18.6

[a]Sites: Al-Quwayiyah (Q), Ath Thumamah (A), Thadiq (T), Al Uyaynah (U) and Rawdat Al- Khafs (R), Numbers 1 and 2 are designated for clayey and sandy soils, respectively, [b]Total phenolic content mg g^{-1} dry plant tissue, measured as gallic acid equivalent (GAE), Results determined as Mean±SD for triplicate measurements (n = 3) and [c]Calculated value for the whole fruit

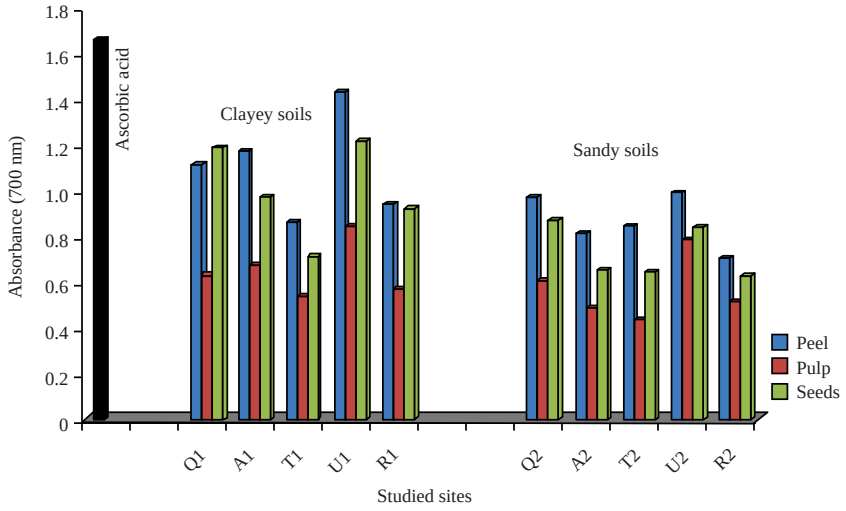

Fig. 2: Ferric reducing antioxidant power (FRAP) of the methanolic extracts of *C. colocynthis* fruit parts (peel, pulp and seeds) for the clayey and sandy soils

Table 4: TF content of the peel, pulp and seed methanolic extracts of *C. colocynthis* fruits from various regions of KSA

	TF content					
Sample site[a]	Peel	Pulp	Seeds	Total TF (mg fruit^{-1})	Whole fruit (mg g^{-1})	Fruit weight (g)
Q1	1.30±0.10	0.72±0.02	0.80±0.06	14.72	0.91	16.2
Q2	1.10±0.04	0.58±0.01	0.75±0.02	14.36	0.77	18.7
A1	0.90±0.05	0.63±0.04	0.81±0.05	13.61	0.80	17.1
A2	0.76±0.06	0.45±0.02	0.68±0.01	11.60	0.61	18.9
T1	0.97±0.03	0.47±0.05	0.64±0.01	12.10	0.72	16.5
T2	0.90±0.08	0.40±0.01	0.53±0.06	11.70	0.60	19.4
U1	1.23±0.11	0.66±0.04	0.87±0.03	15.04	0.95	15.8
U2	0.88±0.05	0.48±0.02	0.73±0.04	14.21	0.68	20.5
R1	1.02±0.04	0.51±0.03	0.69±0.02	14.19	0.74	19.3
R2	0.63±0.05	0.32±0.01	0.45±0.04	10.53	0.56	18.6

[a]Sites: Al-Quwayiyah (Q), Ath Thumamah (A), Thadiq (T), Al Uyaynah (U) and Rawdat Al- Khafs (R), Numbers 1 and 2 are designated for clayey and sandy soils, respectively, [b]Total flavonoid content mg g^{-1} dry plant tissue, measured as quercetin equivalent (QE) and Results determined as Mean±SD for triplicate measurements (n = 3)

3.73 GAE mg g^{-1} dw. Finally, the pulp showed the least amount of TP in the range 2.00-2.98 GAE mg g^{-1} dw. The order of TP in clayey soils was maximum in the site Q1 followed by T1, A1, U1 and finally R1. The whole TP in a single fruit varied from 46.5-75.3 GAE mg^{-1} fruit dry weight. These values are comparable to that reported by Chekroun *et al.*[1]. Also, the weight of TP for each gram of the fruit was in the range 2.48-4.07 GAE mg g^{-1} which is slightly higher than the result of 3.07 GAE mg g^{-1} obtained by Hussain *et al.*[30]. This could be due to the methanolic extract used in this work afforded higher TP content than the ethanolic extract used in this reported work. Also, the ecological conditions in Saudi Arabia are characterized by sunny weather which assists the formation of phenolic compounds with a higher extent than in plants growing in areas of less exposure to the sun.

Type of soil extensively affected the TP content. As can be observed, the clayey soils had higher TP content than the sandy soils. In clayey soils, the TP varied from 4.46-5.69, 2.24-2.98 and 3.30-4.73 GAE mg g^{-1}dw for peel, pulp and seeds, respectively. The higher TP content in clayey soils reflects the role played by the existing elements in the growing land which assist the formation of TPs secondary metabolites in the plant organs. In sandy soils, the TP content varied in the range 3.11-4.51, 2.00-2.65 and 2.27-3.71 GAE mg g^{-1} dw, respectively. The site T2 showed the highest TP value followed by A2 and then Q2. On the other hand, the sites with the lowest TP content were R2 followed by U2.

TF content: The amount of TFs was determined using the aluminium chloride method and expressed in quercetin equivalents (QE). This assay enables the estimation of all flavonoids in the extract based on the flavonoid-aluminium complex. The results obtained were presented in Table 4. Similar to TPs, the order of TF in the tissue was peel>seed>pulp. It was ranged from 0.63 to 1.3, 0.32 to 0.72 and 0.45 to 0.87 and QE mg g^{-1} dw for the peel, pulp and seed, respectively. The calculated TF for the whole fruit varied from 10.53-15.04 QE mg fruit^{-1} dry weight corresponding to an amount ranged from 0.56-0.95QE mg g^{-1} that are slightly higher than the resultobtained[30] for Pakistani flora and showed the major flavonoids estimated in the fruit were quercetin, catechin, myricetin and kaempferol.

The TF content varied remarkably according to the type of soil. The clayey soils revealed higher TF content than the sandy ones. The descending order of TF content in the clayey soils was Q1, U1, R1, T1 and A1. The values of TFs varied from 0.90-1.3, 0.47-0.72 and 0.64-0.87 QE mg g^{-1} dw for peel, pulp and seeds, respectively. In sandy soils, the decreasing order was Q2, T2, U2, A2 and R2. The TF content ranged from 0.63-1.10, 0.32-0.58 and 0.45-0.75 QE mg g^{-1} dw for peel, pulp and seeds, respectively. Identically to TPs, the higher content of nutrients in the clayey soils making them better the growing land and higher TF content in the plant organs.

Correlation matrix (CM)

Clayey soils: A correlation matrix (CM) analysis was performed on DPPH, FRAP, TP, TF and the macronutrient content of nitrogen, phosphorus and potassium. Values of Pearson correlation coefficients at 2-tailed one-way ANOVA were listed in Table 5. In peel, the results revealed excellent correlations between the TF contents and the DPPH scavenging assays (r = 0.9641) and moderate positive correlation to FRAP assays (r = 0.4902) and the TP content (r = 0.6859). The TP content showed a lower correlation to DPPH (r = 0.5174) and an excellent correlation to FRAP (r = 0.9355). The moderate correlation between TP and TF

Table 5: Correlation matrix for DPPH, FRAP, TP, TF and the macronutrients (N, P and K) in the clayey soils (Q1, A1, T1, U1 and R1)

Tissue	Correlation	DPPH	FRAP	TPC	TFC	N	P
Peel	FRAP	0.3597					
	TPC	0.5174	0.9355**				
	TFC	0.964**	0.4902	0.6859			
	N	0.8117*	0.7941	0.9164*	0.9135*		
	P	0.2506	-0.0753	-0.2535	0.0485	-0.0373	
	K	0.6670	0.0784	0.4192	0.7734	0.6093	-0.3592
Pulp	FRAP	0.5819					
	TPC	0.5712	0.4865				
	TFC	0.8893**	0.6519	0.6814			
	N	0.7725	0.7668	0.9149**	0.8496		
	P	-0.2824	0.0525	0.0453	-0.5212	-0.0373	
	K	0.6377	-0.0057	0.7441	0.6679	0.6093	-0.3592
Seeds	FRAP	0.8709*					
	TPC	0.5928	0.8938*				
	TFC	0.7347**	0.8613*	0.8529*			
	N	0.8859*	0.9392*	0.8412	0.7788		
	P	0.1383	-0.3104	-0.4933	-0.2657	-0.0373	
	K	0.2930	0.5514	0.6159	0.1571	0.6093	-0.3592

DPPH: Reducing scavenging activity assay, FRAP: Ferric reducing antioxidant power assay, TPC: Total phenolic content and TFC: Total flavonoid content and *Significant at p<0.01, **Significant at p<0.001

assay indicated that flavonoids are the dominating phenolic group in the peel tissue of *C. colocynthis*. Furthermore, the positive and meaningful correlation between FRAP assay and TP content in peel implies that the FRAP value depends on the content of TPs and partially contributes to the antioxidant ability by the synergistic effect imposed by the level and type of TPs. In pulp, the TF correlation was strong to DPPH (r = 0.8893) and moderate to FRAP (r = 0.6519) and TP (r = 0.5712). The moderately positive and non-significant correlations with TP found in the pulp were associated with non-polyphenolic compounds such as vitamin antioxidants and other unquantified phenolics and/or the relationship between these compounds and major phenolics synergistic effects between them may contribute to their antioxidant capacity[31]. In seeds, strong and significant correlations to DPPH (r = 0.7347), FRAP (r = 0.8613) and TP (r = 0.8529) which indicated the TF were the major polyphenolic compounds contributing to the antioxidant capacity.

Nitrogen content in the soil showed a strong and positive correlation to DPPH scavenging activity in the peel (r = 0.8859), pulp (r = 0.9392) and seeds (r = 0.8412). This conveys the role played this element in supporting the growing and formation of the polyphenolic compounds as secondary metabolites.

Phosphorous showed very weak correlation to DPPH (r = 0.1383) and negative correlation to FRAP (r = -0.3104), TP (r = -0.4933) and TF (r = -0.2657). This negative correlation indicated that, the lower is the value of the phosphorous content in the soil, the higher is the TP content.

Potassium showed positive weak correlation to DPPH (r = 0.2930), moderate to FRAP (r = 0.5514), moderate to TP (r = 0.6159) and very weak to TF (r = 0.1571). This relevant correlation proved that this element takes part as an assistant factor during the growing stages of the plant but less important than nitrogen.

Sandy soils: Results obtained for the CM in sandy soils were presented in Table 6. In peel, TP are strongly and significantly correlated to DPPH (r = 0.9498) and FRAP (r = 0.7952). On the other hand, the TF significantly correlated to DPPH scavenging assays (r = 0.5363) and strongly correlated to FRAP assays (r = 0.9357) and the TP content (r = 0.5381). The adequate correlations indicate that flavonoids are the principal phenolic compounds in the tissue. In pulp, the TF correlation was strong to DPPH (r = 0.8837) and moderate to FRAP (r = 0.3400) and TP (r = 0.8588). This correlation was slightly less than that for the clayey soils which may impose the less content of TF in pulp of the fruit grown in the sandy soil compared to that obtained in clayey type. In seeds, strong and significant correlations to DPPH (r = 0.9868), FRAP (r = 0.8549) and moderate to TP (r = 0.4683) indicated the TF contributes effectively to the total antioxidant capacity. Despite this, the amount of TF in seeds of clayey origin may be higher than that from sandy origin which can be concluded from the lower correlation between TF and TP in the latter.

Similar to clayey soil, nitrogen showed a strong and positive correlation in all parts of the fruit to DPPH scavenging activity (r = 0.9012-0.9868), FRAP (r = 0.8426-0.9248) and TF (r = 0.7445-0.8178). Phosphorous showed either very weak positive to negative correlations (except with TP in seeds)which indicated less dependence or inverse proportion to the formation of TP and TF in the plant.

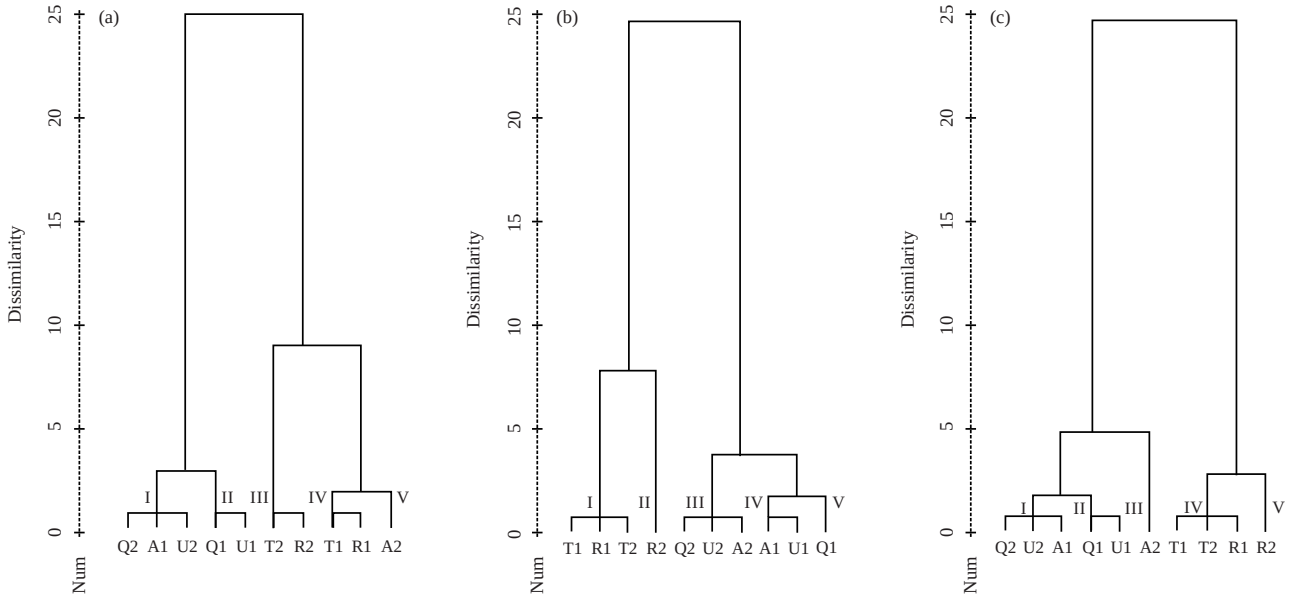

Fig. 3: Dendrograms for cluster analysis of the antioxidant activity assays of *C. colocynthis* fruit tissues from various sites in Saudi Arabia, (a) Peel, (b) Pulp and (c) Seeds

Table 6: Correlation matrix for DPPH, FRAP, TP, TF and the macronutrients (N, P and K) in the sandy soils (Q2, A2, T2, U2 and R2)

Tissue	Correlation	DPPH	FRAP	TPC	TFC	N	P
Peel	FRAP	0.7941					
	TPC	0.9498*	0.7952				
	TFC	0.5363*	0.9357*	0.5381			
	N	0.9012*	0.8836*	0.7736	0.7445		
	P	-0.2224	0.1533	0.0610	0.2605	-0.2956	
	K	0.6724	0.8121	0.5612	0.7865	0.8302	-0.2874
Pulp	FRAP	0.6868*					
	TPC	0.9736**	0.7661				
	TFC	0.8837*	0.3400	0.8588			
	N	0.9285*	0.8426	0.9863**	0.7769		
	P	-0.2132	-0.4933	-0.2554	-0.1022	-0.2956	
	K	0.9355*	0.4789	0.9032*	0.9731**	0.8302	-0.2874
Seeds	FRAP	0.7956					
	TPC	0.3305	0.6958				
	TFC	0.9868**	0.8549*	0.4683			
	N	0.8160	0.9248**	0.3898	0.8178		
	P	-0.2126	0.0772	0.7640	-0.0711	-0.2956	
	K	0.8727	0.7320	0.2805	0.8321	0.8302	-0.2874

DPPH: Reducing scavenging activity assay; FRAP: Ferric reducing antioxidant power assay, TPC: Total phenolic content and TFC: Total flavonoid content, *Significant at p<0.05 and **Significant at p<0.01

Potassium, in contrary to the clayey soil, showed moderate-strong positive correlation to DPPH (r = 0.6724-0.9355), FRAP (r = 0.4789-0.8121), weak-strong to TP (r = 0.2805-0.9032) and strong to TF (r = 0.7865-0.9731). A plausible explanation can be due to the more dependence of the plant on potassium during the growing period in sandy soil than in clayey one. This may reflect the deficient of soil macronutrient in sands thus the plant tends to consume all available nutrients.

Cluster analysis (CA): To obtain a classification of the studied sampling sites based on the quantified antioxidant activity, the values for the *in-vitro* analytical assays of the *C. colocynthis* tissues were subjected to cluster analysis. The dendrograms obtained for the cluster analysis based on the DPPH, FRAP, TP and TF assays were shown in Fig. 3.

Grouping of the samples (sites) were presented in terms of their similarity based on the Euclidean distance. Exactly consistent grouping of the sampling sites depended

strongly on the kind of the plant tissue. Based on the fruit part, all sampling sites were classified into five clusters (I, II, III, IV and V) at a dissimilarity level of 2.0. In the peel dendrogram (Fig. 3a),cluster I include three samples (Q2, A1 and U2) and cluster II grouped two samples (Q1 and U1). Each of clusters III and IV grouped two samples T2 and R2 and T1 and R1, respectively. Finally, one sample (A2) was found in cluster V. In the pulp dendrogram (Fig. 3b),cluster I grouped three sites (T1, R1 and T2), cluster II included a single sites (R2) and three samples (Q2, U2 and A2) were grouped in cluster III. Cluster IV grouped A1 and U1 while the distinct site Q1 was found in cluster V. The seed dendrogram (Fig. 3c) has grouped (Q2, U2 and A1) in cluster I, (Q1 and U1) in cluster II and the sites (T1, T2 and R1) in cluster IV. The sites A2 and R2 were not grouped and presented singly in the clusters III and V, respectively. Generally, the site R2 was not grouped in the pulp and seed dendrograms which reflects its high difference to the reaming samples which was clear from the lower antioxidant activity obtained from this sampling location. Also, site A2 was not grouped in peel and seed dendrograms, conveying the dissimilarity of these sites. Finally, the three sites Q2, U2 and A1 were found in cluster I in both of the peel and seed tissues which indicates comparative soil content or ecological factors in these areas.

CONCLUSION

The present work demonstrated the dependence of antioxidant capacity assays in the tissues of *C. colocynthis* fruit including DPPH, FRAP, TP and TF on the variation of the growing site and the soil macronutrients. Of the five examined clayey and sandy soil sites, the order of antioxidant activity, TP and TF were peel>seed>pulp. Nitrogen content was found strongly correlated to antioxidant activity. Statistical analysis of the antioxidant activity and soil macronutrient data using the correlation matrix and cluster analysis identified the samples successfully at their respective growing sites. Finally, the study gives information helping to discriminate the effect of growing soils and nutritional factors on the polyphenolic formations in *C. colocynthis* parts.

SIGNIFICANCE STATEMENT

The present study emphasizes the antioxidant activity of *Citrullus colocynthis* plant tissues based on its phenolic and flavonoid content. Also, the level of soil macronutrients has a significant effect on the antioxidant activity properties of the plant. The application of correlation matrix and cluster analysis assisted in understanding the ecological effect of the diversity of antioxidant activity based on the studied locations. This can improve the nutritional and medicinal use of this plant in the region.

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