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Assessment of total phenolic and flavonoid contents of selected fruits and vegetables

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This work was conceptualized with the goal to investigate different fruits and vegetables for their comparative investigation of total phenolic and total flavonoid contents. The total phenolic content of 9 fruits and 12 vegetables used in the current study was determined by Folin-Ciocalteau assay. In addition, total flavonoid content was identified through catechin and aluminum colorimetric analysis. The ratio among the phenolic and flavonoid contents of fruits and vegetables extracts were also analyzed. Our results showed that methanolic extract of *Citrullus lanatus* had higher contents of phenolics and flavonoids (215±1.24 mg GAE/100 g and 73±0.81 mg CE/100 g) than other fruits. Moreover, maturity process of fruits from unripened to fully ripened stage showed significant increase in the total phenolic and flavonoid contents. Fruits under study had shown flavonoids/phenolics ratio of 0.32, which indicates that these fruits contained about 32% of flavonoid contents. Among vegetables, the greatest value of phenolic contents was observed in *Capsicum annuum* (213±1.24 mg GAE/100 g), and total flavonoid content in *Raphanus sativus* (45±1.24 mg CE/100 g). Vegetables showed lower ratios of flavonoids/phenolics (0.11-0.2) indicating lesser total flavonoid content (11-20%) as compared with fruits. The obtained results indicate that fruits and vegetables could be attributed to a potential source of natural phenolics and flavonoids in the pharmaceutical and food industry. Moreover, the antioxidant activities of these selected fruits and vegetables should also be determined in order to explore their beneficial effects against the prevention and management of disorders caused by oxidative stress.

Keywords: Flavonoids, Fruits, Phenolics, Vegetables

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World health organization (WHO) recommends that each individual should take at least 400 g of fruits and vegetables on daily basis to maintain good health[1]. Fruits and vegetables are proficient in delivering health benefits in addition to fulfilling the physiological needs. The daily intake of fruits and vegetables also confers considerable benefits to human health as functional foods[2,3]. Though fruits and vegetables account for only a modest constituent in our everyday food consumption, their benefits contribute significantly to the human health[4,5]. The intake of vegetables and fruits in the regular diet provides antioxidants and phytochemicals, which have significant defensive effects against several life-threatening diseases including stroke, pulmonary and coronary heart diseases and different cancer types[6,7]. The contributory factors include the existence of carotenoids, polyphenols, vitamins and flavonoids. The benefits associated with foods and the increasing consumer's awareness for healthy foods emphasize upon the investigation of natural alternatives for the food industry[5,8,9].

Plant secondary metabolites contain phenolic compounds, while their presence in fruits and vegetables are frequently reported as important total phenolic content with different characterized functions and numerous proposed beneficial effects[8,10]. Phenolic contents are largely correlated

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favorably against inflammation, aging, viral infections, fatigue, hypoxia, immune dysfunction, radiation hazards, oxidative stress, hypoglycemia and carcinogenesis[8,10,11]. Phenolic substances belong to the category of phytonutrients that exhibit huge antioxidant activities. They can be divided into simple phenols, flavonoids, hydroxycinnamic acid derivatives and phenolic acids. Despite, several studies have reported that some phenolic substances have ability to act as potent antioxidant components[5,12,13]. Among the polyphenolics, flavonoids commonly occur as glycoside forms, whereas hydroxycinnamic acids are generally present in ester forms. Recently, flavonoids have received considerable interest regarding their health-beneficial properties including those against obesity-related disorders such a cardiovascular diseases, metabolic syndrome, Type 2 diabetes and cancer[11,14]. The polyphenolic molecules possess anti-inflammatory action and free radical scavenging activity, which may contribute to reduce blood lipids and glucose, inhibit hydrolytic and oxidative enzymes, thereby improving human immunity[15]. Due to potential health benefits, polyphenolic compounds have achieved a considerable value in human health. Furthermore, they also influence the sensory qualities such as taste, color, and flavor in the fruits, vegetables, and beverages[16]. Moreover, concentrations of phenolic content and secondary metabolites of fruits and vegetables are affected by various factors such as irrigation, soil sensitivity and variation of climatic conditions[8].

Human food consists of proteins, carbohydrates, lipids and vitamins, which are adversely affected by reactive oxygen species (ROS)[17]. However, antioxidants inhibit the oxidation in a biological system from reactive radicals such as ROS, which could play an important role in mediating the pharmacological effects[18,19]. In contrast, ROS retain hydrogen peroxide, hydroxyl radical, superoxide radical anion and singlet oxygen, which are normally generated during the physiological and metabolic activities. ROS have been considered to be associated with several diseases including neurodegenerative diseases, aging, cardiovascular diseases, inflammation, cancer, immune system disorders and atherosclerosis[11,16,20]. Polyphenols such as phenolic acids and flavonoids, which are daily consumed in our diets have shown antioxidant properties by radical-forming enzymes, chelating metal ions, quenching free radicals and inhibiting lipid oxidation[16,18,21]. However, several studies indicating numerous plants including fruits and vegetables showed the occurrence of antioxidant and phenolic compounds. For example *Allium sativum*, *Agaricus campestris*, *Spinacia oleracea*, *Brassica oleracea*, *Brassica oleracea* var. *sabellica*, *Brassica oleracea* var. *italica*, *Allium cepa*, *Brassica oleracea* var. *capitata*, *Phaseolus vulgaris*, *Beta vulgaris*, *Zea mays*, *Citrus reticulata*, *Piper nigrum*, *Solanum melongena*, and *Cucumis sativus* are rich sources of phenolic compounds and antioxidants[1,2,22-26].

However, limited information is available about the content of phenolics in common food stuffs of plant origin and their antioxidant properties. In recent years, research has been done on vegetables and fruits due to their biologically beneficial effects on the health emanating from antioxidant properties of phytochemicals[23]. In Pakistan, plants have been used as essential source of therapeutics for various disorders such as oxidative stress for several decades in the past. Thus, this preliminary study was conducted to evaluate total phenolic and total flavonoid contents from endemic plants grown in the district Dera Ismail Khan (D I Khan), Khyber Pakhtunkhwa (KP), Pakistan, thereby providing information about the usefulness of these plants in reducing the risks of various diseases.

Materials and methods

Chemicals

Folin-Ciocalteu reagents (FCR), catechin, aluminum chloride, gallic acid and dimethyl sulfoxide (DMSO) were obtained from Acros Chem. Co. The remaining chemicals and solvents used were of standard analytical grades.

Ethnomedicinal data collection

The present study was conducted in the district D I Khan, KP, Pakistan. A total of 200 local informants were interviewed using semi-structured questionnaire method in the study area. In each field survey, participants were chosen randomly, except for key informants and Traditional Health Practitioners (THPs). Key informants are those having knowledge about edible food, localities as well as the experience in using them. THPs were selected based upon their experience and expertise in medicinal uses of plants. During field work, information was documented by showing fresh specimens, about the local name of plants, plant parts used, medicinal uses and disease treated. In most cases, data were cross-checked among informants by showing the fresh specimens to verify the authenticity of the selected fruits and vegetables of the study area[27].

Plant material and preparation of extract

The vegetables and fruits (edible part only) were shade-dried, then crushed and ground into fine power using mortar and pestle. Powered samples were extracted with 100 mL of 80% (v/v) methanol in ultrasonic bath for 1.5 h. The extraction process was carried out three times to maximize the yield. The extracts were filtered by Whatman No. 2 filter paper and samples were dried using a rotary vacuum evaporator. All the extracted samples were stored at -20°C until further analysis.

Determination of total phenolic content

Total phenolic content of the extracts was determined by Folin-Ciocalteu method with minor modifications[28]. In brief, 1 mL of different extracts (4 mg/mL) were combined with 750 µL of Folin-Ciocalteau reagent, followed by incubation at room temperature for 5 min. The reaction mixture was then incubated at room temperature for 1.5 h in dark after the addition of 750 µL sodium carbonate solution (7.5%). Next, absorbance of the samples was recorded at 725 nm against reagent as blank using spectrophotometer (Visiscan 167, Sytronics). Gallic acid was used as standard and calibration curve was prepared at concentration ranges from 20-500 µg GA/mL. The total phenolic content was expressed as mg gallic acid equivalents per gram extract (mg GAE/g extract). The negative control was prepared by using 100 µL of DMSO instead of test sample. The measurement was performed in triplicates and results were expressed as mean ± SD.

Determination of total flavonoid content

Total flavonoid content of extracts were determined by aluminum chloride colorimetric method with minor modifications[16]. Briefly, 2 mL of various extracts (4 mg/mL) were combined with 100 µL of aluminum chloride solution (10%), followed by the addition of 100 µL potassium acetate (1 M) with 2.9 mL of dH_2O and reaction mixture was incubated at room temperature for 30 min. The absorbance of the tested samples was measured by spectrophotometer at 510 nm against reagent as blank. The calibration curve was plotted using 20-500 µg/mL of catechin as standard, and total flavonoid content was expressed as mg of catechin equivalent per gram of extract (mg CE/g extract). Whereas, the negative control was prepared using 100 µL of DMSO instead of extract. Results were repeated three times and expressed as mean±SD.

Statistical Analysis

All reaction mixtures were prepared in triplicates and three independent experiments were performed for each extract. All the data were represented as mean±SD.

Results

The ethnobotanical survey results showed that these 09 fruits and 12 vegetables had been used by local inhabitants of the research area for multiple purposes. The selected fruits and vegetables are represented by scientific names, family, vernacular names, parts used, mode of utilization and medicinal uses (Table 1). It is usually reported that antioxidant activity, phenolic and flavonoid contents of fruits and vegetables may attribute towards biological activities and health benefits. Thus, total phenolic content and total flavonoid content of 9 fruit extracts were examined in this study. The total phenolic contents of various fruit extracts were found to be in the range between 51-215 mg GAE/100 g (Table 2). *Citrullus lanatus* and *Fragaria vesca* showed the highest phenolic contents of 215±1.24 and 211± 1.69 mg GAE/100 g, respectively. Moreover, the highest flavonoid contents were determined in *Citrullus lanatus* (73±0.81 mg CE/100 g) and *Vitis vinifera* (56±1.69 mg CE/100 g). Additionally, *Musa paradisiaca, Malus pumila* and *Mangifera indica* showed a higher flavonoids/phenolics ratio of 0.34 (Table 2).

Next, we investigated the total phenolic and total flavonoid contents of *Fragaria vesca* at different stages of maturation, such as unripened, partly ripened and fully ripened. Our results indicated that fully ripened *Fragaria vesca* exhibited remarkable amount of total phenolic and total flavonoid contents as compared to the unripened stage (Table 3). Similarly, the highest total phenolic and total flavonoid contents were found at the red-ripe compared with white-ripe and white-pink-ripe stages of *Citrullus lanatus* (Table 3).

A total of 12 vegetables were investigated for total phenolic and total flavonoid contents. Results showed the highest total phenolic content in *Capsicum annuum* (213±1.24 mg GAE/100 g), however, the flavonoids/phenolics ratio was very low 0.12 (Table 4).

Table 1 — Indigenous ethnobotanical uses of fruits and vegetables of D I Khan, Pakistan.

Scientific names	Family	Vernacular names	Part(s) Used	Mode of Utilization	Medicinal uses
Citrullus lanatus (Thunb.) Matsum. & Nakai	Cucurbitaceae	Watermelon	Fruit, leaves	Eaten in raw form, powder	Diuretic
Fragaria vesca L.	Rosaceae	Strawberry	Fruit	Eaten in raw form, powder, juice	Diarrhoea, dysentery
Cucumis melo L.	Cucurbitaceae	Melon	Fruit	Eaten in raw form, Decoction	Diuretic, blood purification
Musa paradisiaca L.	Musaceae	Banana	Fruit	Eaten in raw form, extract	Bronchitis, dysentery, ulcers
Psidium guajava L.	Myrtaceae	Guava	Fruit	Eaten in raw form, juice, extract	Diarrhoea
Vitis vinifera L.	Vitaceae	Grapes	Fruit	Eaten in raw form, juice,	Blood pressure
Malus pumila Mill.	Rosaceae	Apple	Fruit	Eaten in raw form, extract	Constipation, diarrhea
Saccharum officinarum L.	Poaceae	Sugarcane	Stem	Juice, eaten in raw form	Sore throat
Mangifera indica L.	Anacardiaceae	Mango	Fruit	Eaten in raw form, juice	Blood purification, diarrhea
Capsicum annuum L.	Solanaceae	Green pepper	Fruit	Eaten in raw form, extract	Antispasmodic, analgesic
Spinacia oleracea L.	Amaranthaceae	Spinach	Leaves	Eaten in raw form	Anemia
Raphanus sativus L.	Brassicaceae	Radish	Root, leaves	Eaten in raw form, juice	Indigestion, abdominal bloating
Allium cepa L.	Amaryllidaceae	Onion	Bulb	Eaten in raw form, cooked	Cold, fever
Solanum tuberosum L.	Solanaceae	Potato	Tuber	As vegetable, extract	Stomach ulcer
Daucus carota L.	Apiaceae	Carrot	Root	Eaten in raw form, juice	Diuretic
Solanum lycopersicum L.	Solanaceae	Tomato	Fruit	Eaten in raw form, cooked	Rheumatism
Allium sativum L.	Amaryllidaceae	Garlic	Bulb	As vegetable, juice, extract	Blood purification,
Brassica rapa L.	Brassicaceae	Turnip	Root	As vegetable, poultice	Skin disease
Phaseolus vulgaris L.	Leguminosae	Beans	Seed	As vegetable, extract	Rheumatism, arthritis
Cucumis sativus L.	Cucurbitaceae	Cucumber	Fruit	As vegetable, juice	Diuretic, dyspepsia
Brassica oleracea L.	Brassicaceae	Cauliflower	Inflorescence	As vegetable, powder	Diarrhoea, stomach, laxative

Table 2— Total phenolic and total flavonoid contents in selected fruits.

Fruits	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg CE/100 g)	Flavonoids/Phenolics ratio
Citrullus lanatus	215±1.24	73±0.81	0.34
Fragaria vesca	211±1.69	50±1.24	0.23
Cucumis melo	47±1.24	15±0.81	0.32
Musa paradisiaca (pealed)	51±1.69	18±0.94	0.35
Psidium guajava	57±1.69	17±0.81	0.30
Vitis vinifera	174±1.24	56±1.69	0.32
Malus pumila	128±1.24	45±2.49	0.35
Saccharum officinarum (juice)	123±1.63	36±0.81	0.29
Mangifera indica (pulp)	105±2.49	48±1.69	0.35

GAE, Gallic Acid Equivalents; CE, Catechin Equivalents. The results are expressed as mean ± standard deviation of triplicate samples (*n=3*).

Table 3 —Total phenolic and total flavonoid contents at different stages of *Fragaria vesca* and *Citrullus lanatus* ripeness.

		Un-ripened	Partly ripened	Fully ripened
Total phenolic content (mg GAE/100 g)	*Fragaria vesca*	95±0.81	144±0.94	211±1.24
	Citrullus lanatus	102±1.69	134±0.81	215±0.81
Total flavonoid content (mg CE/100 g)	*Fragaria vesca*	19±1.24	28±0.81	50±1.24
	Citrullus lanatus	35±0.47	42±1.24	73±0.81

The results are expressed as mean ± standard deviation of triplicate samples *(n=3)*.

Table 4— Total phenolic and total flavonoid contents in selected vegetables.

Vegetables	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg CE/100 g)	Flavonoids/Phenolics ratio
Capsicum annuum	213±1.24	25±1.63	0.12
Spinacia oleracea	145±1.69	29±1.24	0.2
Raphanus sativus	142±0.81	45±1.24	0.31
Allium cepa	137±1.24	17±2.16	0.12
Solanum tuberosum	91±1.24	18±0.47	0.19
Daucus carota	89±1.24	15±1.24	0.17
Solanum lycopersicum	85±2.35	11±0.94	0.13
Allium sativum	82±2.44	12±1.69	0.15
Brassica rapa	80±0.81	14±0.81	0.17
Phaseolus vulgaris	58±0.45	12±1.24	0.20
Cucumis sativus	45±0.81	9±0.94	0.20
Brassica oleracea	34±1.24	6±0.47	0.17

The results are expressed as mean ± standard deviation of triplicate samples (*n=3*).

Subsequently, highest total flavonoid content was detected in *Raphanus* sativus (45±1.24 mg CE/100 g) and *Spinacia oleracea* (29±1.24 mg CE/100 g) with flavonoids/phenolics ratio of 0.31 and 0.2, respectively (Table 4).

Discussion

Phenolics and flavonoids are essential fruit constituents which exhibit antioxidant activity by preventing the conversion of hydroperoxides into free radicals or inactivating lipid free radicals[29]. The current study was focused on total phenolic and total flavonoid contents in 9 fruits and 12 vegetables cultivated in the area of district D I Khan. The present study showed total phenolic contents ranging from 47±1.24 to 215±1.24 mg GAE/100 g with the highest levels in *Citrullus lanatus* and *Fragaria vesca* using a standard curve of gallic acid (R^2=0.9896). Whereas, the total flavonoid content ranged from 15±0.81 to 73±0.81 mg CE/100 g, and the highest flavonoid contents were found in *Citrullus lanatus*, *Vitis vinifera* and *Fragaria vesca*, using standard curve of catechin (R^2=0.9762). The lowest total phenolic and total flavonoid contents were observed in the *Cucumis melo*. Therefore, these results suggest that *Citrullus lanatus*, *Vitis vinifera* and *Fragaria vesca* display the highest phenolic and flavonoid contents.

Studies have shown that the variety and developmental stages (ripening) of fruits affect the levels of phenolics and flavonoids in different parts of fruits[24,29]. It has been reported that Cavendish variety (*Musa paradisiaca L*, *cv cavendshii*) of *Musa paradisiaca* showed greater values of phenolics and flavonoids than that of Dream variety (*Musa acuminata colla*. *AAA, cv Berangan*). Moreover, the highest phenolic and flavonoid levels were reported in peeled and ripened *Musa paradisiaca* in comparison with pulp and unripened *Musa paradisiaca*[29]. In this study, we have also found a significant induction of phenolics and flavonoids during the process of fruit development. Our findings indicated that total phenolic and total flavonoid contents were elevated in *Fragaria vesca* during the developmental process towards maturity (unripened to ripened stage). Additionally, our results demonstrated that ripened stage of *Fragaria vesca* exhibited twice the amount of total phenolic and total flavonoid contents when compared with unripened stage (Table 3), as reported previously by others[24]. Likewise, total phenolic and total flavonoid contents were determined at three different ripening stages (white, white-pink and red) of *Citrullus lanatus*, the red-ripe stage showing twice higher the phenolic and flavonoid contents than white-ripe stage (Table 3). In *Fragaria vesca* and *Citrullus lanatus,* the highest amounts of phenolics and flavonoids at ripened stage of development could possibly be explained by the accumulation of anthocyanins and flavonols in red-colored fruits[30]. Previous reports have demonstrated that total phenolic content increased during the development of*Momordica charanita*[31]. As an illustration, it was shown that total phenolic and flavonoid contents in various fruits, such as *Manilkara hexandra*[32], *Prunus avium*[33] and *Morinda citrifolia*[34] have been attributed to their maturation stages. However, white-colored fruits demonstrated an inverse trend for total phenolic and flavonoid contents. These values were decreased in the ripening process of fruit development; *Musa paradisiaca* and *Agaricus campestris* are the typical examples of this trend[19,29]. Our results indicated that the white-colored fruits such as *Cucumis melo*, *Musa paradisiaca* and *Psidium guajava*, retain low total phenolic (47±1.24 to 57±1.69 mg GAE/100 g) and flavonoid contents (15±0.81 to 18±0.94 mg CE/100 g) as compared to red-colored fruits (Table 2). *Vitis vinifera*, *Malus pumila*, *Saccharum officinarum* and *Mangifera indica* contained total phenolics ranging from 105±2.49 to 174±1.24 mg GAE/100 g and total flavonoids from 36±0.81 to 56±1.69 mg CE/100 g. All the fruits studied had almost same flavonoids/phenolics ratio (average=0.32) indicating around 32% of flavonoid contents.

Additionally, 12 vegetables were investigated for total phenolic and total flavonoid contents. Our results showed high values for total phenolic content in *Capsicum annuum*, indicating that this vegetable is rich in hydroxycinnamic acids. *Spinacia oleracea*, *Raphanus sativus* and *Allium cepa* showed comparatively low phenolic contents (137±1.24 to 145±1.69 mg GAE/100 g), while the values for other vegetables such as *Solanum tuberosum*, *Daucus carota*, *Solanum lycopersicum*, *Allium sativum* and *Brassica rapa* ranged from 80±0.81 to 91±1.24 mg GAE/100 g. However, lower phenolic contents were identified in *Phaseolus vulgaris*, *Cucumis sativus* and *Brassica oleracea* (34±1.24 to 58±0.45 mg GAE/100 g). On the other hand, high total flavonoid content (45±1.24 mg CE/100 g) was detected only in *Raphanus sativus* with flavonoids/phenolics ratio of 0.31. As compared to fruits, vegetables had smaller flavonoids/phenolics ratios (0.11 to 0.2), indicating less amounts of total flavonoids (11 to 20%) with the exception of *Raphanus sativus* (31%). Thus, our study revealed that total flavonoids in the fruits are much higher than vegetables, which is in accordance with the previous findings[35-39].

It is understood that fruits and vegetables contain phenolic acids and flavonoids as secondary metabolites and have much importance due to their antioxidant activity. Important phenolic acids are hydroxycinnamic acids, coumaric acids, hydroxyl benzoic acids, gallic acids and vanillic acids, while important flavonoids include catechin, rutin and quercetin[40-43]. The correlation between phenolic substances and antioxidant property was not studied here. It is known that the phenolic substances have different actions towards Folin-reagent. It is also worth mentioning that the antioxidant activity of phenolic compounds depends on their chemical structure[44]. Thus, the exact mechanism of antioxidant activity cannot be explained simply through determination of total phenolic contents. Therefore, further studies should be conducted in order to correlate the phenolic and flavonoid contents with the antioxidative capacities of these fruits and vegetables, thereby revealing their medicinal significance in the management of various disorders.

New finding of the study

The total phenolic and total flavonoid contents of 09 fruits and 12 vegetables were reported from the area of district D I Khan. The significance of these fruits and vegetables was evaluated on the basis of ethnic community cultivation.

Conclusion

The current study evaluates the phenolic and flavonoid contents of 09 fruits and 12 vegetables. Among fruits and vegetables, *Citrullus lanatus*, *Capsicum annuum* and *Raphanus sativus* exhibited the highest total phenolic and total flavonoid contents. Presence of substantial amounts of phenolics and flavonoids in vegetables and fruits may be responsible for their effective antioxidant potency, which is a key to fight against various diseases related to oxidative stress. Therefore, it can be concluded that these fruits and vegetables may be attributed as a potential source of natural flavonoids and phenolics in pharmaceutical as well as food industry. Additional work may be focused on the isolation and characterization of the functional components of phenolics and flavonoids as well as the antioxidant properties of these fruits and vegetables in order to identify beneficial effects on the management and treatment of different disorder associated with oxidative stress.

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Conflict of interests

None.

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