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RESEARCH ARTICLE

Phytochemical and antioxidant potential of selected plants from Mianwali, Pakistan

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Abstract

Plants have been used since ancient times as an important source of biologically active substances. Specific activities of these plant extracts are generally linked to the presence of secondary metabolites together with their phenolic contents. Present study aimed at investigating the total phenolic and flavonoid contents, and antioxidant activity of selected plants from five different families. The total phenolic content was measured using Folin-Ciocalteu assay and total flavonoid content by aluminum chloride colorimetric method. The antioxidant capacity was estimated by phosphomolybdinium assay. Our findings indicates that total phenolic content for methanolic extracts ranged from 27.07 to 59.11 mg GAE/g DW, and total flavonoid content ranged from 38.37 to 124.23 mg QE/g DW, with an antioxidant activity ranging from 55.82 to 129.06 mg AAE/g DE. Following trend was shown in the assessment of total phenolic and flavonoid contents: *Rhazya stricta>Cicer arietinum>Solanum melongena>Solanum surattense>Solanum nigrum>Withania somnifera>Sisymbrium irio>Withania coagulans>Raphanus sativus>Fagonia indica>Brassica napus*. While the antioxidant capacity followed the trend: *Cicer arietinum>Solanum nigrum>Withania coagulans>Rhazya stricta>Raphanus sativus>Solanum melongena>Withania somnifera>Solanum surratense>Fagonia indica>Brassica napus>Sisymbrium irio*. It is also seen that both wild and cultivated plants have higher medicinal value, which can be linked to the phenolic and flavonoid content, and antioxidant potential. Findings of the study revealed that wild plants possess higher phenolic content compared to cultivated plants, whereas cultivated plants had higher antioxidant activity

Keywords

Antioxidant, Brassicaceae, Secondary metabolites, Solanaceae, Phenolic content

Introduction

Plant-based antioxidants play an important role in cellular stress protection from reactive oxygen species (ROS) caused by complex diseases and ageing processes (1). Natural antioxidants derived from plants and vegetables have been extensively studied in recent years, with active ingredients showing promise in the prevention of a wide range of oxidative stress and free stress disorders, as well as free radical damage (2). It is well known that oxidative

stress causes oxidation of biomolecules, which leads to degradation and death due to imbalance in the production of free radicals and other reactive species (3). Plants primary antioxidant compounds are phenols, which have an aromatic ring that allows for the stabilization and transfer of unpaired electrons in their structure, allowing for the redistribution of elements such as hydrogen atoms and electrons from their hydroxyl groups (4).

Plant polyphenols are widely regarded as one of the most important groups of plant chemicals because they are abundant in our diet and are distributed secondary metabolites in the plant kingdom. This class contains approximately 8000 bioactive compounds (5). These phenolic compounds are classified into basic and polyphenols. The basic phenols with carboxyl group, such as ferulic acid, chlorogenic acid and gallic acid are classified as phenolic acids (6). Polyphenols are made up of at least two 6-membered phenol rings linked together by a very short carbon chain. Polyphenols are flavonoids are classified into six groups: flavones (apigenin), flavonols (quercetin), flavanols (catechins), flavanones (naringenin), anthocyanins (cyanidins) and isoflavones (genistein) (6, 7). Stilbenes (resveratrol), coumarins, tennins and lignans are some other polyphenol classes. However, phenolics remain important due to their antioxidant, anti-inflammatory, antidiabetic and antimicrobial properties (5, 8, 9). The scientific community has described polyphenols as prophylactic and therapeutic agents for a variety of disorders. Polyphenols are well-known for their ability to prevent oxidative damage by scavenging free radicals, acting as reducing agents, chelated transition metals for radical formation and regulating defensive enzymes and modulating cell signaling pathways and gene expression (10). Polyphenols are the most common antioxidants isolated from higher plants. Plant polyphenols and antioxidant activity in plant products are being studied to better understand plant therapeutic properties (11). The main source of phenolic antioxidant activity is redox effects, which include reducing agents, hydrogen donors and singlet oxygen quenchers. Polyphenols with aromatic phenyl ring compounds are easily oxidized by ROS to quinines, which contributes to their free radical scavenging capacity, although lipoxygenase can be inhibited by phenolic compounds (12). Plant phytochemicals have the potential to act as antioxidants, releasing free radicals in response to oxidative stress. The controlled formation of free radicals and antioxidants protects cellular components from oxidative damage caused by ROS-containing chemical reactions (13). These ions and radicals can initiate chain reactions that produce free radicals that cause oxidative damage to DNA, proteins and lipids. This causes cellular function loss and free radical-related diseases like atherosclerosis, neuronal degeneration, ischaemia-reperfusion damage and cancer (14-17).

Solanaceae family is very important to human beings. Species of this family are used for food, drugs, ornamental purposes and some play an important role in antioxidant and phytochemical analysis. The leaves of *Solanum nigrum* L. are rich in polyphenols, including phenolic acids and flavones (18). It was reported that total polyphenolic content and antioxidant activity and concentration of phenolic content and flavones compounds were determined (19). *Solanum melongena* L. commonly known as eggplant is an economically important vegetable crop. A study for turkey indicated that 26 eggplants cultivars ranged from 2664-8257 µmol Trolox/kg and total phenolic contents ranged from 615-1376 mg/kg (20). Literature on the pharmacological activity of *Solanum surattense* confirms the scientific validation of folklore claims and its traditional uses for the treatment of various diseases (21). The genus *Withania* plays an important role in the Indian ayurvedic system. Among the 23 known species of *Withania,* the two species *Withania coagulans* and *Withania somnifera* are pharmaceutical and ethnomedicinal properties (22). Brassicaceae (Cruciferae) family includes many economically important edible, industrial oilseed, vegetable and fodder crops. Members of the family Brassicaceae produce secondary metabolites that are not only family-specific but also species and genus-specific (23). *Sisymbrium irio* L. has been employed as a folk medicine remedy for inflammation, antipyretic, analgesic and antimicrobial activities. Moreover, ethanolic extracts of *S. irio* showed antioxidant activity (9). *Raphanus sativus* L. belongs to genus raphanus included in the rapa lineage according to ohylogenetic studies of Brassicaceae family. *Raphanus sativus* contain many classes of biological and phytochemicals (24). *Brassica rapa* L. have beneficial effects because they contain important phytochemicals possessing antioxidant, anticancer, antimicrobial and important secondary metabolites in Brassicaceae (25). *Fagonia indica* belongs to family Zygphyllaceae, it is distributed in Pakistan, North and East tropical Africa. *F. indica* has been reported for antioxidant, antidiabetic and phytochemical analysis (26). While *Rhazya stricta* Decne. (Apocynaceae family) is well documented phytochemical, pharmacological and toxicological properties. Several alkaloids and flavonoids have been isolated characterized (8). Similarly, *Cicer arietinum* L. from Fabaceae family has several health benefits and chief phytochemicals comprised of phenolic, flavonoids and antioxidants (27).

Previously, we have reported the phenolic and flavonoid content of vegetables and fruits from Dera Ismail Khan (28). The study aimed to determine the total phenolic and flavonoid contents, and antioxidant potential of selected plants from Solanaceae, Brassicaceae, Zygophyllaceae, Apocyanaceae and Fabaceae families.

Materials and Methods

Chemicals

Gallic acid and ascorbic acid have been bought from Sigma Aldrich Chemical Co. (St. Louis, Mo, USA). The remaining sources of the reagents, chemicals and solvents were used in standard analytical grade.

Ethnomedicinal investigation

In the current study, 180 local informants from Miawali district, Pakistan, were interviewed using semi-structured

questionnaire. The participants in this study were chosen at random, including traditional health professionals as well as key informants. The primary informants were people who had first hand knowledge of plant uses and locations. After confirmations and investigations by local traditional health practitioners, all specimens were recorded in the field work of plant selection/collection (29). Dr. Muhammad Zafar, Herbarium Botanist at Quaid-i-Azam University, Department of Plant Sciences, identified and authenticated the plants and specimens were deposited in the Herbarium of Islamabad (ISL), Quaid-i-Azam University.

Preparation of plant material and extract

The taxonomist identified the aerial sections of the plants from the Miawali. Before being ground into fine powder, the plants were washed and air-dried in the shade for ten days. The powdered material was kept at room temperature for ten days after being soaked in 500 ml of methanol. The crude methanolic extracts were filtered and the solvent solution was evaporated under reduced pressure from the filtrate using a rotary evaporator (BUCHI Rotavapor R-20 Switzerland). The extracts were preserved at -20°C for further investigation.

Total phenolic content

The total content of phenolic was determined using Folin-Ciocaltea method previously described (30, 31). In brief, the reaction system was prepared with 2.5 ml of Folin-Ciocalteu's reagent (10%) and 0.6 ml of Na_2CO_3 (20%) combined with 0.5 ml of methanol extract solution or gallic acid standard solution, followed by a reaction mixture incubated for 30 min at room temperature. Instead of sample solution, methanol was used as a blank. Absorbance was measured using spectrophotometer at a wavelength of 760 nm. As a standard, a curve was plotted using gallic acid 50-750 µg/ml (R^2=0.9995). The gallic acid equivalent per gram (GAE/g) was calculated by the graphing software standard curve equation and all the data were expressed as mg GAE/g of dry weight (DW) (32).

Determination of total flavonoid content

Total flavonoid content of extracts was determined by aluminum chloride colorimetric method with minor modifications (33). 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 quercetin as standard, and total flavonoid content was expressed as mg of quercetin equivalent per gram of extract (mg QE/g DW). Whereas, the negative control was prepared using 100 µl of DMSO instead of extract. Results were repeated three times and expressed as mean ± SD.

Phosphomolybdate assay

As previously stated, total antioxidant activity was determined using phosphomolybdenum assay (31, 34). An aliquot of 0.3 ml from each of the tested samples was mixed in 1 ml of reaction mixture (0.6 M sulfuric acid; 28 mM sodium phosphate; 4 mM ammonium molybdate). All samples were incubated in water bath (95 °C) for 90 min. After incubation at 95°C, the samples were cooled to room temperature, and the absorbance at 760 nm of all the samples was measured. Positive control was prepared by using ascorbic acid instead of extracts. The standard curve of ascorbic acid at different concentrations (50-750 µg/ml) was prepared. Overall, the results were expressed as micrograms (µg) of ascorbic acid equivalent (AAE) per milligram (mg) of the dry weight of tested samples (35). furthermore, the antioxidant capacity was calculated using the following formula:

Percentage of total antioxidant capacity =

[(absorbance of control – Absorbance of sample)/ Absorbance of control] × 100.

Statistical analysis

All measurements were taken in triplicate and the results were reported as means ± standard deviation (SD). The results of the analysis of variance analysis (ANOVA) results determined the significant difference between the means and $p < 0.05$ is considered significant. .

Results and Discussion

Plants from five families growing in Mianwali District were selected for the present research work. Ethnobotany findings show that locals/people inhabiting the region from where study plants were selected. The results were organized according to the scientific name, families, vernacular names, portions used, methods of use and medicinal usages (Table 1). These plants belonged to five different botanical families and exhibited both wild and cultivated characteristics. Phytochemical and antioxidant activity were estimated using methanol extracts. According to previous findings, methanol extracts had a significant total phenolic and flavonoid content (36). Based on the investigations methanol is the preferred solvent. However, another study reported that methanol extraction provides strong polarity and high yields (37).

A linear regression equation from the results of the gallic acid (R^2= 0.992) calibrate curve, with ascorbic acid standards (R^2= 0.995) serving as positive controls. The total phenolic content of selected plant extracts ranged from 27.07 to 59.11 mg GAE/g DW (Table 2). Solanaceae had phenolic content values of 32.77, 34.85, 42.79, 43.91 and 44.75 mg GAE/g DW, while the Brassicaceae had phenolic content values of 27.09, 29.05 and 32.78 mg GAE/g DW in methanol extracts. *Rhazya stricta* (59.11 mg GAE/g DW) of the Apocynaceae and *Cicer arietinum* (51.92 mg GAE/g DW) of the Fabaceae had the highest phenolic content, which highlighting the importance of plants (Table 2). A single-factor analysis of variance (ANOVA) was used to test the hypotheses (32). This demonstrated the statistical viability of measures for GAE standard. It is important to remember that the amount of phenolics depends on the environmental stress and exposure of plants to various physicochemical stimuli (38). Apple pomace has been tested with a variety of solvent systems, including ethanol, methanol, ethyl aetate,

Table 1. Indigenous ethnobotanical uses of selected plants of Mianwali, Pakistan

S. No.	Scientific Name	Family	Local name	Status	Part (s) used	Mode of action	Medicinal values (uses)
1	*Solanum nigrum* L.	Solanaceae	Mako	Wild	Leaves, fruits	Extract, Powdered	Stomach, liver diseases
2	*Solanum melongena* L.	Solanaceae	Baingan	Cultivated	Leaves, fruits	Decoction	Hypotension
3	*Solanum surattense* Burm. f.	Solanaceae	Kundiari	Wild	Fruits, leaves	Powdered, decoction, Juice	Piles, blood purification, man debility
4	*Withania coagulans* (Stocks) Dunal	Solanaceae	Paneer	Wild	Fruits, seeds	Infusion, decoction	Cooling effect, blood purification
5	*Withania somnifera (L.)* Dunal	Solanaceae	Ratkan	Wild	Roots, fruits	Powdered	Tonic, rheumatism
6	*Sisymbrium irio* L.	Brassicaceae	Khub Kalan	Wild	Seeds	Powdered	Fever
7	*Raphanus sativus* L.	Brassicaceae	Mooli	Cultivated	Roots, leaves	Infusion, eaten in raw	Digestion, asthma, chest
8	*Brassica rapa* L.	Brassicaceae	Sarsoo	Cultivated	Whole plant	Poultice	Burns
9	*Fagonia indica* Burm.*f.*	Zygophyllaceae	Dhamasa	Wild	Whole plant	Powdered, decoction	Piles, cooling effect, urinary
10	*Rhazya stricta* Decne.	Apocynaceae	Sihar	Wild	Aerial parts	Powdered, decoction	Throat infection, diabetes,
11	*Cicer arietinum* L.	Fabaceae	Chanra	Cultivated	Whole plant	Infusion	Sun stroke, spermatorrhoea

acetone and chloroform. Previously, 61 plants from Mianwali District, Pakistan were examined and revealed identical patterns, but with the expected variations due to uncontrollable variables (39). Phenolic experiments were conducted on a large number of plants (n=112). They found methanolic extracts of herbs ranged from 0.22 to 50.3 g of gallic acid equivalent/100 g DW (40). Increased levels of phenolics have been found in *Arabidopsis* when grown in an ultra-violet radiation environment (41). Previous studies indicated that methanolic extract of *Rhazya stricta* contain phenolic content 189.9 µg GAE/mg (42). Phenolic content in dried seeds of *Cicer arietinum* was 29.75 mg/gm (43). Flavonoids are a kind of phenolic compounds that has numerous hydroxyl groups, which provide these phytoconstituents with significant antioxidant properties (44). Our results found that higher total flavonoid content was found in Apocynaceae family by *Rhazya stricta* (124 mg QE/g DW) and *Cicer arietinum* (87 QE/g DW) of Fabaceae family (Table 2). Moreover, Solanaceae had flavonoid contents of 57.97, 62.76, 65.34, 73.45, 85.73 mg QE/g dw (Table 2). Previously, flavonoid content was observed with extract of *Solanum nigrum* 16.42 mg quercetin equivalents (QE)/g) dry extract wt (45). *Solanum melongena* total flavonoid content in different parts of plant during vegetative and flowering stage ranged from 50.56 - 336.39 mg QE/100 (46). Brassicaceae members exhibited total flavonoids 42.54, 54.25, 58.89 mg QE/g DW (Table 2). Khalil and his colleagues have reported n-Hexane, chloroform, ethyl acetate, butanol and aqueous fractions of *Sisymbrium irio* total flavonoid content were 0.09, 10, 30, 24 and 16 mg QE/g DW respectively (47). *Raphanus sativus* L. leaves was previously reported as flavonoid content (44.5 mg QE/g) (48). Total flavonoid content of *Fagonia indica* Burm. f. in methanolic extracts was 24.16 mg of QE/g of dried extract (26). The positive benefits of phenolic and flavonoids may be linked to a variety of activities, the most prominent of which is an antioxidant effect. Therefore, we next examined the antioxidant properties of selected plants of Mianwali.

Since several plant species are used to produce phototherapeutic drugs, there has been research into their antioxidant activity in recent years (49, 50). Plants contain novel compounds with therapeutic properties that require further research. As a result of oxygen intake during cell

Table 2. Total phenolic and flavonoid content of selected plants

Extract	Total Phenolic Content mg GAE/g dw	Total flavonoid Content mg QE/g dw
Solanum nigrum	42.79 ± 0.35	65.34 ± 0.98
Solanum melongena	44.75 ± 038	85.73 ± 1.8
Solanum surratense	43.91 ± 0.28	73.45 ± 0.47
Withania coagulans	32.77 ± 0.49	57.97 ± 0.32
Withania somnifera	34.85 ± 0.24	62.76 ± 0.77
Sisymbrium irio	32.78 ± 0.14	58.89 ± 0.67
Raphanus sativus	29.05 ± 0.19	54.25 ± 0.78
Brassica rapa	27.09 ± 0.35	42.54 ± 1.13
Fagonia indica	27.07 ± 0.49	38.37 ± 0.81
Rhazya stricta	59.11 ± 0.35	124.23± 0.24
Cicer arietinum	51.92 ± 0.42	87.97 ± 0.24

Results are expressed as mean ± standard deviation of triplicates

development, aerobic cells produce free radicals (13). Free radicals reduce membrane fluidity, inhibit enzyme receptor activity and destroy membrane proteins, eventually resulting in death. Antioxidant assists in the treatment of these conditions by allowing the redistribution of elements such as hydrogen atoms and electrons from their hydroxyl groups (51). Various plants exhibited different antioxidant

Table 3. Antioxidant potential of selected plants from Mianwali

Extract	Total antioxidant mg AAE/g dw
Solanum nigrum	125.25 ± 0.50
Solanum melongena	76.82 ± 0.29
Solanum surratense	70.88 ± 0.32
Withania coagulans	118.74 ± 0.83
Withania somnifera	71.54 ± 0.41
Sisymbrium irio	55.82 ± 0.35
Raphanus sativus	82.01 ± 0.49
Brassica rapa	59.02 ± 0.52
Fagonia indica	69.21 ± 0.39
Rhazya stricta	113.15 ± 0.60
Cicer arietinum	129.06 ± 0.31

levels, antioxidant activity of studied plants are mentioned in Table 3. The antioxidant activities of *Cicer arietinum*, *Solanum nigrum*, *Withania coagulans* and *Rhazya stricta* were 129.06, 125.25, 118.74 and 113.15 mg AAE/g DW respectively. We also found that the antioxidant activity of Solanaceae were 70.88, 71.54, 76.82 and 118.74 mg AAE/g DW of plants extract, while *Solanum nigrum* exhibited significant antioxidant activity at 125.25 mg AAE/g DW. Similarly, members of the Brassicaceae demonstrated antioxidant activity values of 55.82, 59.02 and 82.01 mg AAE/g DW of plants extract. However, *Cicer arietinum* (129.06 mg AAE/g DW) demonstrated the highest antioxidant activity. A single factor analysis of variance (ANOVA) was used to evaluate the hypotheses. This demonstrated the statistical validity of measures for the AAE standard. Plant phenolics have been found to have high antioxidant activity. As a result, the ingested antioxidant activity may be attributed to the presence of phenolic content in the plant's extracts (52). Many flavonoid and associated polyphenols have been shown in recent studies to contribute significantly to plants scavenging activity (53, 54).

The total phenolics and flavonoids and antioxidant activity of selected plants were estimated as percentage of their total phenolics and flavonoids and antioxidant activity (Table 4). *Rhazya stricta* had the highest phenolic content and *Cicer arietinum* had the highest antioxidant activity. The plant families with the highest antioxidant activity are *Fabaceae* > *Solanaceae* > *Apocynaceae* > *Brassicaceae* > *Zygophyllaceae* (Table 4). The amount of phenolics and antioxidant potential in two onion varieties was found to be determined by the cultivation process. Those grown organically had higher values when compared to those grown artificially (55). The current work highlights the importance of phenolics in the studied plants, which can be used as an alternative source of antibiotics. Current antibiotics are associated with problems including resistance issues and high toxicity levels (56). Polyphenols–based anti-diabetic therapies with enzyme inhibitory properties may be investigated for the treatment of various hyperglycemic disorders. Plant phenolics have been identified as UV protective molecules in plants and natural phenolics have also been shown to protect against skin cancer (57). The significance of these plants was assessed based on ethnic group cultivation.

Conclusion

Based on the findings of the present study, it is possible to conclude that selected plants have potential total phenolic and flavonoid contents and antioxidant activity. These results suggested that *Rhazya stricta* and *Cicer arietinum* extracts possessed significant phenolic and flavonoid and antioxidant properties. Plant-derived antioxidants with free radical scavenging activity have a broad variety of uses in the treatment and prevention of free radicals related health problems. Nonetheless, further research is needed to qualify as a potential drug against toxicity, identify the active compounds and investigate its mode of action at a safe dosage.

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Authors contributions

AS, and AMC, SZUA, carried out the experiential studies; RFS, and RK participated in the design of the study and performed the statistical analysis, AM, MS, and WKK carried out the written and drafted the manuscript; SZUA participated in the plant identification and collection of materials; MZB, EIA, AY, and GESB wrote and finalized the manuscript. All authors read and approved the final manuscript.

Table 4. The percentage ranking of phenolic and flavonoid contents and antioxidant potential of selected plants

Total Phenolic Content		Total Flavonoid Content		Antioxidant activity	
Scientific Name	Rank	Scientific Name	Rank	Scientific Name	Rank
Rhazya stricta	1	*Rhazya stricta*	1	*Cicer arietinum*	1
Cicer arietinum	2	*Cicer arietinum*	2	*Solanum nigrum*	2
Solanum melongena	3	*Solanum melongena*	3	*Withania coagulans*	3
Solanum surratense	4	*Solanum surratense*	4	*Rhazya stricta*	4
Solanum nigrum	5	*Solanum nigrum*	5	*Raphanus sativus*	5

Withania somnifera	6	*Withania somnifera*	6	*Solanum melongena*	6
Sisymbrium irio	7	*Sisymbrium irio*	7	*Withania somnifera*	7
Withania coagulans	8	*Withania coagulans*	8	*Solanum surratense*	8
Raphanus sativus	9	*Raphanus sativus*	9	*Fagonia indica*	9
Fagonia indica	10	*Fagonia indica*	10	*Brassica napus*	10
Brassica napus	11	*Brassica napus*	11	*Sisymbrium irio*	11

Pearson's Correlation Value between TPC-AOP: 0.70; Values are mean ± SD (n=3)

Compliance with ethical standards

Conflict of interest: All the authors declared that they have no competing interest related to this article.

Ethical issues: None.

Supplementary data

Table S1 Data for Folin-Ciocalteu and phosphomolybdenum assay.

Table S2a ANOVA for GAE Standard Readings.

Table S2b. ANOVA: Single Factor Antioxidant potential.

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