

Antioxidant Properties of Common Edible Plants in Preventing Age-Related Diseases

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ABSTRACT

Background: Oxidative stress contributes to biological processes associated with ageing, while edible plants provide phenolic compounds and other constituents with in-vitro antioxidant activity. **Objective:** To compare the total phenolic content and antioxidant capacity of ten commonly consumed edible plants obtained from Lahore and determine their relationships across complementary antioxidant assays. **Methods:** This analytical laboratory study evaluated 30 independently sourced samples representing ten edible plants. Methanolic extracts were assessed for total phenolic content, 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) activity, and ferric-reducing antioxidant power. Plant groups were compared using one-way analysis of variance with Tukey's post-hoc test. Pearson correlations were calculated from ten plant-level mean values. **Results:** Amla had the highest total phenolic content (82.45 ± 2.14 mg GAE/g), DPPH inhibition ($88.60 \pm 1.90\%$), ABTS activity (91.35 ± 2.20 μ mol TE/g), and FRAP value (76.40 ± 1.85 μ mol Fe²⁺/g), followed by pomegranate. Tomato had the lowest values across all measurements. Differences among plants were significant for every outcome (all $p < 0.001$). Total phenolic content was strongly correlated with DPPH ($r = 0.995$), ABTS ($r = 0.995$), and FRAP activity ($r = 0.998$; all $p < 0.001$). **Conclusion:** The selected edible plants differed substantially in in-vitro antioxidant capacity, with amla and pomegranate showing the highest values. These laboratory findings identify locally available sources of antioxidant compounds but do not establish bioavailability or prevention of age-related disease. **Keywords:** Antioxidants; edible plants; total phenolic content; oxidative stress; healthy ageing; Lahore.

INTRODUCTION

Ageing is accompanied by progressive alterations in redox homeostasis that can increase susceptibility to oxidative damage. Reactive oxygen and nitrogen species are generated during normal cellular metabolism and participate in physiological signalling; however, excessive production or inadequate antioxidant defence can damage lipids, proteins, cellular membranes, and DNA. Accumulated oxidative damage has been implicated in endothelial dysfunction, atherosclerosis, neuronal injury, and other biological processes associated with age-related cardiovascular and neurodegenerative disorders (1–3).

Diet represents a potentially modifiable source of exogenous antioxidants. Fruits, vegetables, herbs, legumes, and other edible plants contain vitamin C, vitamin E, carotenoids, phenolic acids, flavonoids, and related phytochemicals that can donate electrons, neutralise reactive species, chelate pro-oxidant metals, and influence inflammatory and cellular-signalling pathways (4). The antioxidant capacity of a plant food is not fixed, however, because it can vary according to species, cultivar, maturity, season,

geographical origin, soil conditions, storage, processing, and the solvent and technique used for laboratory extraction (5–7). Consequently, antioxidant values reported for plants grown or marketed in one geographical setting may not accurately represent comparable foods available in another setting.

Oxidative stress contributes to several mechanisms involved in cardiovascular ageing, including lipid oxidation, vascular inflammation, and impaired endothelial function. Prospective studies and meta-analyses have consistently associated greater consumption of fruits, vegetables, and legumes with lower cardiovascular morbidity and all-cause mortality, although these observational relationships do not independently establish causality or identify the contribution of individual plant foods (8–10). Evidence from Mediterranean dietary interventions has further suggested that dietary patterns rich in plant foods, olive oil, and nuts can contribute to cardiovascular risk reduction (11). These findings support the relevance of plant-rich dietary patterns but do not provide direct information about the comparative antioxidant capacity of individual edible plants commonly available in Pakistan.

Plant-rich dietary patterns have also been investigated in relation to cognitive ageing. Greater adherence to Mediterranean and Mediterranean–Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay dietary patterns has been associated with slower cognitive decline or a lower incidence of Alzheimer's disease (12,13). Long-term intake of flavonoid-rich foods has similarly been associated with a lower risk of Alzheimer's disease and related dementias (14). More recent observational evidence has linked higher fruit and vegetable consumption, particularly greater dietary variety, with better cognitive function or a slower rate of cognitive decline among older adults (15–18). These associations remain subject to residual confounding by education, socioeconomic circumstances, physical activity, smoking, comorbidities, and other lifestyle factors and therefore cannot be interpreted as proof that antioxidant-rich foods directly prevent neurodegenerative disease.

Pakistan produces and consumes a diverse range of fruits, vegetables, herbs, and other edible plants. Previous Pakistani investigations have demonstrated measurable antioxidant activity in locally grown Moringa leaves, medicinal plant extracts, tree-derived plant materials, and edible wild fruits, while also showing that growing conditions, season, location, and extraction procedures can materially influence measured antioxidant values (5,6,19,20). Nevertheless, a standardized comparative evaluation of commonly consumed edible plants obtained from Lahore using several complementary antioxidant assays remains limited. Such local analysis is important because no single antioxidant assay captures all chemical mechanisms through which plant constituents may act.

The 2,2-diphenyl-1-picrylhydrazyl assay evaluates free-radical-scavenging activity, the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay measures radical-cation decolourisation, and the ferric-reducing antioxidant power assay estimates reducing capacity (21–23). Total phenolic content can be estimated using the Folin–Ciocalteu method, although the resulting value represents overall reducing substances rather than individual phenolic compounds (24). The use of several complementary methods therefore provides a broader analytical characterization than reliance on a single assay (7). The present laboratory-based analytical study compared the total phenolic content and in-vitro antioxidant capacity of ten commonly consumed edible plants obtained from markets in Lahore, Pakistan. It also examined the relationship between total phenolic content and antioxidant activity measured using the 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and ferric-reducing antioxidant power assays. It was hypothesised that antioxidant capacity would differ among the selected plants and would be positively associated with their total phenolic content. The study was intended to generate local analytical baseline data relevant to future nutritional and healthy-ageing research rather than to establish clinical prevention of cardiovascular or neurodegenerative disease.

MATERIALS AND METHODS

This analytical laboratory study incorporated a cross-sectional collection of edible plant samples from markets in Lahore, Pakistan. Sample collection and laboratory analysis were completed within a defined

six-month study period. Ten plants commonly consumed in the local population were selected on the basis of their availability, routine dietary use, and previously reported antioxidant constituents. These comprised spinach, mint, coriander, tomato, carrot, beetroot, guava, apple, pomegranate, and Indian gooseberry, commonly known as amla.

Three independently sourced samples of each plant were purchased from three different markets, retail outlets, or supermarkets located in different areas of Lahore, producing an analytical sample of 30 independently collected plant samples. Each purchased sample weighed approximately 500 g. Fresh, intact, and visibly undamaged specimens were included. Samples showing fungal contamination, insect damage, decomposition, abnormal odour, marked discolouration, or other evidence of deterioration were excluded. Imported and highly processed plant products were not included because the study focused on fresh foods available to the local population.

Each sample was placed in a clean, labelled polyethylene bag and transported to the laboratory on the day of purchase while protected from direct sunlight. Labels recorded the common plant name, sample code, collection source, and date of purchase. The identity of each plant was verified by a trained botanist using common and scientific nomenclature. Only the edible portion of each specimen was analysed. Visible soil, dust, and debris were removed by washing the samples with tap water followed by distilled water. Excess surface moisture was removed using clean tissue paper, after which each independently collected sample was cut into smaller pieces and dried in a hot-air oven at 40°C until a constant weight was achieved. The dried material was ground into a fine powder using an electric grinder and stored in labelled airtight containers at 4°C until extraction.

For each independently collected sample, 10 g of dried plant powder was combined with 100 mL of 80% methanol in a covered flask and agitated for 24 hours at room temperature. An aqueous methanol solution was selected because of its ability to extract a broad range of phenolic and antioxidant compounds from plant material (6,7). The mixture was filtered through Whatman No. 1 filter paper, and the residual plant material was extracted a second time using the same procedure. The two filtrates obtained from each sample were combined. The solvent was removed using a rotary evaporator maintained below 40°C, and the resulting concentrated extract was stored in a clean labelled container at 4°C. Before each assay, a measured quantity of the extract was reconstituted in methanol to obtain the required working concentration.

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method (24). An aliquot of each plant extract was mixed with Folin–Ciocalteu reagent, followed by sodium carbonate solution, and the reaction mixture was incubated in the dark to allow colour development. Absorbance was measured at 765 nm using a calibrated ultraviolet-visible spectrophotometer. Gallic acid was used to construct the standard calibration curve, and total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dried plant extract.

Free-radical-scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl method (21). Plant extracts were combined with freshly prepared 2,2-diphenyl-1-picrylhydrazyl solution, while a control containing the radical solution without plant extract was analysed under the same conditions. The reaction mixtures were incubated in the dark for 30 minutes at room temperature, and absorbance was recorded at 517 nm. Reduced absorbance represented greater radical-scavenging activity. For the comparative analysis reported in this study, activity was expressed as percentage inhibition relative to the control using the following calculation:

$$\text{Percentage inhibition} = [(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100.$$

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical-cation decolourisation assay was performed according to the method described by Re et al. (22). The radical solution was generated by combining 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) with potassium persulfate and incubating the mixture in the dark for 12–16 hours. The solution was diluted before analysis, combined

with the plant extract, and measured at 734 nm after six minutes. Trolox was used as the reference standard, and activity was expressed as micromoles of Trolox equivalents per gram of dried plant extract.

Ferric-reducing antioxidant power was assessed using the method described by Benzie and Strain (23). Fresh ferric-reducing antioxidant power reagent was prepared using acetate buffer, 2,4,6-tripyridyl-s-triazine solution, and ferric chloride solution. Plant extract was added to the freshly prepared reagent and incubated at 37°C, after which absorbance was recorded at 593 nm. Greater absorbance indicated stronger reducing capacity. Results were expressed as micromoles of ferrous-ion equivalents per gram of dried plant extract.

Blank, control, and reference-standard solutions were included with each analytical run. Fresh reagents were prepared according to the relevant assay procedures, and the spectrophotometer was calibrated before measurements. The three independently purchased market samples for each plant were treated as source-level replicates. Each determination for an individual extract was performed in technical triplicate, and the mean of the three technical readings was used as the value for that independently collected sample. Technical replicate readings were therefore not treated as separate independent observations during inferential analysis. Uniform sample preparation, extraction, storage, incubation, and spectrophotometric procedures were applied to all plant samples to minimise measurement variability.

Data were entered and analysed using IBM SPSS Statistics version 25.0. Continuous assay results were summarized as mean and standard deviation using the independently collected samples as the analytical units. The distributions of model residuals and the homogeneity of variances were assessed before inferential testing. One-way analysis of variance was used to compare mean antioxidant measurements among the ten plant groups, followed by Tukey's post-hoc procedure for adjusted pairwise comparisons. For the comparative correlation analysis corresponding to the plant-level summary data, one mean value for each plant was used, giving ten plant-level observations. Pearson correlation analysis was applied to assess the relationships between total phenolic content and the 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and ferric-reducing antioxidant power measurements. All tests were two-sided, and a p-value below 0.05 was considered statistically significant. The study involved the laboratory analysis of commercially available edible plant materials and did not involve human participants or experimental animals. Informed consent was therefore not applicable.

RESULTS

A total of 30 independently collected samples representing ten edible plants were analysed. Antioxidant measurements differed substantially among the plant groups. Amla had the highest total phenolic content at 82.45 ± 2.14 mg GAE/g extract, followed by pomegranate at 79.80 ± 2.35 mg GAE/g extract. Tomato had the lowest total phenolic content at 25.90 ± 1.05 mg GAE/g extract. The absolute difference between amla and tomato was 56.55 mg GAE/g extract.

Amla also produced the highest values in all three antioxidant assays, with DPPH inhibition of $88.60 \pm 1.90\%$, ABTS activity of 91.35 ± 2.20 $\mu\text{mol TE/g}$ extract, and a FRAP value of 76.40 ± 1.85 $\mu\text{mol Fe}^{2+}/\text{g}$ extract. Pomegranate had the second-highest corresponding values of $86.75 \pm 2.10\%$, 89.20 ± 2.45 $\mu\text{mol TE/g}$ extract, and 73.65 ± 2.05 $\mu\text{mol Fe}^{2+}/\text{g}$ extract. Tomato had the lowest values in all three assays. Relative to tomato, amla showed a 52.85-percentage-point higher DPPH inhibition, 52.95 $\mu\text{mol TE/g}$ extract higher ABTS activity, and 46.55 $\mu\text{mol Fe}^{2+}/\text{g}$ extract higher FRAP activity.

One-way analysis of variance demonstrated differences among plant groups for total phenolic content, DPPH inhibition, ABTS activity, and FRAP activity, with all overall p-values below 0.001. The corresponding omega-squared values ranged from 0.989 to 0.992, indicating that most of the observed variation in the assay values occurred between plant groups. Tukey-adjusted comparisons showed no statistically significant difference between amla and pomegranate in any of the four measurements.

Both plants had higher values than apple and tomato for all measurements, with adjusted p-values below 0.001.

Table 1. Total Phenolic Content and Antioxidant Activity of Selected Edible Plants

Plant sample	Total phenolic content, mg GAE/g extract	DPPH inhibition, %	ABTS activity, $\mu\text{mol TE/g}$ extract	FRAP value, $\mu\text{mol Fe}^{2+}/\text{g}$ extract
Amla	82.45 $\pm$ 2.14	88.60 $\pm$ 1.90	91.35 $\pm$ 2.20	76.40 $\pm$ 1.85
Pomegranate	79.80 $\pm$ 2.35	86.75 $\pm$ 2.10	89.20 $\pm$ 2.45	73.65 $\pm$ 2.05
Beetroot	62.30 $\pm$ 1.80	72.40 $\pm$ 1.65	75.10 $\pm$ 1.90	60.25 $\pm$ 1.70
Spinach	58.90 $\pm$ 1.95	69.85 $\pm$ 1.80	72.60 $\pm$ 2.15	57.80 $\pm$ 1.65
Mint	55.70 $\pm$ 1.65	67.20 $\pm$ 1.55	70.40 $\pm$ 1.80	54.90 $\pm$ 1.60
Coriander	49.85 $\pm$ 1.50	61.75 $\pm$ 1.40	64.30 $\pm$ 1.65	50.70 $\pm$ 1.45
Guava	45.60 $\pm$ 1.75	59.40 $\pm$ 1.60	62.25 $\pm$ 1.55	48.90 $\pm$ 1.35
Carrot	36.40 $\pm$ 1.30	48.60 $\pm$ 1.25	50.70 $\pm$ 1.40	39.85 $\pm$ 1.20
Apple	28.75 $\pm$ 1.10	39.20 $\pm$ 1.15	42.35 $\pm$ 1.25	31.60 $\pm$ 1.10
Tomato	25.90 $\pm$ 1.05	35.75 $\pm$ 1.00	38.40 $\pm$ 1.15	29.85 $\pm$ 0.95

Values are presented as mean $\pm$ standard deviation for three independently collected samples per plant. The mean of the technical replicate readings was used for each independently collected sample. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric-reducing antioxidant power; GAE: gallic acid equivalent; TE: Trolox equivalent.

Table 2. One-Way Analysis of Variance for Antioxidant Measurements Among Plant Groups

Outcome	F statistic	Numerator df	Denominator df	p-value	Omega squared
Total phenolic content	385.51	9	20	<0.001	0.991
DPPH inhibition	390.37	9	20	<0.001	0.992
ABTS activity	299.17	9	20	<0.001	0.989
FRAP value	320.57	9	20	<0.001	0.990

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric-reducing antioxidant power.

Table 3. Correlations Between Total Phenolic Content and Antioxidant Measurements

Relationship	n	Pearson r	95% CI lower limit	95% CI upper limit	p-value
Total phenolic content and DPPH inhibition	10	0.995	0.978	0.999	<0.001
Total phenolic content and ABTS activity	10	0.995	0.977	0.999	<0.001
Total phenolic content and FRAP value	10	0.998	0.991	1.000	<0.001

CI: confidence interval. Correlations were calculated using the ten plant-level mean values.

The pattern of plant rankings was consistent across the complementary antioxidant assays. Beetroot had the third-highest total phenolic content and antioxidant values, followed by spinach and mint. Coriander and guava occupied intermediate positions, while carrot, apple, and tomato had the lowest measured values. Total phenolic content was strongly and positively correlated with each antioxidant measurement. The correlation was $r=0.995$ for DPPH inhibition, $r=0.995$ for ABTS activity, and $r=0.998$ for FRAP activity, with all p-values below 0.001. These estimates were calculated from the ten plant-level means and indicate that plants with greater measured phenolic content also tended to have greater radical-scavenging and ferric-reducing capacity under the assay conditions.

DISCUSSION

The present study demonstrated substantial variation in total phenolic content and in-vitro antioxidant capacity among ten commonly consumed edible plants obtained from Lahore. Amla and pomegranate consistently produced the highest values across total phenolic content, DPPH, ABTS, and FRAP measurements, whereas apple and tomato produced the lowest values under the selected extraction and assay conditions. The consistency of plant rankings across the three antioxidant assays and the strong correlations with total phenolic content support the study hypothesis that antioxidant capacity differs among plant types and is positively associated with their measured phenolic content.

The high values observed for amla and pomegranate should be interpreted as measurements of their methanolic extracts rather than direct estimates of the antioxidant effects of normally consumed

portions. Extraction solvent, sample preparation, temperature, storage, geographical origin, and maturity can influence the recovery and measured activity of plant constituents (5–7). The results therefore characterize the analysed samples under standardized laboratory conditions and should not be assumed to represent every cultivar, growing season, market source, or household preparation of the same foods.

Total phenolic content was closely related to DPPH, ABTS, and FRAP measurements. Phenolic compounds can contribute to antioxidant activity by donating electrons or hydrogen atoms and stabilising reactive species, although the Folin–Ciocalteu assay is not specific to individual phenolic compounds and may respond to other reducing substances (7,24). The almost identical ranking across the three assays suggests that the measured extracts contained constituents capable of acting through both radical-scavenging and reducing mechanisms. Nevertheless, the correlations do not demonstrate that phenolic compounds were the sole cause of the observed activity. Vitamin C, carotenoids, flavonoids, organic acids, and other reducing compounds may also have contributed, but these constituents were not measured separately.

The moderate-to-high values observed for beetroot, spinach, mint, coriander, and guava are relevant because these foods and herbs are locally available and commonly incorporated into meals. Previous studies of plants grown or collected in Pakistan have also identified appreciable antioxidant activity while demonstrating that season, production location, extraction technique, and plant source influence measured values (5,6,19,20). Direct numerical comparison with those studies remains difficult because plant species, edible portions, extraction solvents, assay concentrations, and reporting units differ.

The comparatively lower assay values of apple and tomato should not be interpreted as evidence that these foods have little nutritional value. Individual foods contain different mixtures of fibres, vitamins, minerals, carotenoids, and phytochemicals, and their nutritional importance cannot be ranked solely through a single extract-based antioxidant comparison (4). Tomato, for example, may provide carotenoid compounds that are not fully represented by total phenolic content, while the biological relevance of an apple or tomato also depends on the quantity consumed, the food matrix, processing, digestion, and metabolism. A diverse dietary pattern is therefore more biologically meaningful than selecting foods solely according to laboratory antioxidant rankings.

The findings provide biochemical context for epidemiological research associating greater consumption of fruits, vegetables, and legumes with lower cardiovascular morbidity and mortality (8–10). Plant-rich Mediterranean dietary patterns have also been associated with cardiovascular benefit (11), while Mediterranean and MIND dietary patterns and greater fruit and vegetable variety have been associated with favourable cognitive outcomes in observational and interventional research (12–18). The current study does not test these clinical relationships. It provides only laboratory evidence that selected locally available plant foods contain extractable compounds capable of radical scavenging and ferric reduction under controlled conditions. The study has several strengths. Three independently sourced samples of each plant were included, a uniform extraction procedure was used, and antioxidant activity was evaluated through three complementary assays rather than a single analytical method. Total phenolic content was also examined in relation to each antioxidant measurement, allowing evaluation of the consistency of the observed pattern.

Several limitations should be considered. The samples were obtained from one city during a six-month period, and differences related to season, cultivar, agricultural practices, ripeness, storage, and market handling were not analysed separately. The number of independent samples per plant was limited. Methanolic extraction improves laboratory recovery of several phytochemicals but does not represent normal digestion or household preparation. Results expressed per gram of dried extract cannot be directly converted into antioxidant exposure per usual serving without extraction yields and edible-portion data. The study did not assess bioaccessibility, intestinal absorption, metabolism, circulating biomarkers, tissue effects, cardiovascular outcomes, cognitive function, or disease incidence. Individual

phenolic compounds and other antioxidant constituents were not identified. Finally, the plant-level correlations were based on ten mean values and should be interpreted as ecological analytical associations rather than participant-level or causal relationships. These findings establish comparative in-vitro antioxidant profiles for selected edible plants available in Lahore. They support further work using larger seasonal samples, standardized edible portions, extraction-yield reporting, compound-specific analysis, simulated gastrointestinal digestion, and human biomarker or dietary studies. Such research would be necessary to determine whether the laboratory differences observed among the plants translate into meaningful differences in biological exposure or health outcomes.

CONCLUSION

The selected edible plants differed substantially in total phenolic content and in-vitro antioxidant capacity, with amla and pomegranate producing the highest DPPH, ABTS, and FRAP values and tomato producing the lowest values under the specified laboratory conditions. Total phenolic content was strongly associated with all three antioxidant measurements, indicating a consistent relationship between phenolic content and chemical antioxidant capacity across the analysed plants. These findings identify locally available plant foods with comparatively high concentrations of extractable antioxidant compounds but do not establish their bioavailability, clinical effectiveness, or ability to prevent cardiovascular, neurodegenerative, or other age-related diseases.

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