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PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SELECTED MEDICINAL PLANTS

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Abstract

For centuries, medicinal plants have been the backbone of traditional medicine . Their therapeutic value is mainly due to bioactive secondary metabolites especially phenolics and flavonoids which scavenge free radicals and prevent oxidative stress. The development of natural antioxidants is a priority for drug and nutraceutical development since oxidative stress plays a role in the pathogenesis of cardiovascular disease, diabetes, neurodegeneration, and cancer. The aim of the present study was to assess the qualitative phytochemical constituents, total phenolic content, total flavonoid content and in vitro antioxidant activity of four selected medicinal plants Azadirachta indica, Moringa oleifera, Ocimum sanctum and Punica granatum by cold maceration method, standard qualitative screening methods, Folin–Ciocalteu and aluminum chloride colorimetric method and DPPH radical scavenging assay.Qualitative screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides in all the extracts, with A. indica having the widest range. The TPC was found to be 6.64 mg GAE/g for A. indica and 85.60 ± 4.87 mg GAE/g for P. granatum peel. The TFC was found to be up to 118.57 ± 0.08 mg QE/g in A. indica leaf extract. All the extracts scavenged DPPH radicals in a concentration dependent manner. The most effective material was Punica granatum peel, which showed the highest activity (EC50 3.88 ± 0.33 µg/mL; 96.76% inhibition at 40 µg/mL, approaching the synthetic standard BHA). Among the leaf materials, O. sanctum was the most active (IC50 47.73 µg/mL versus 41.34 µg/mL for ascorbic acid). The order of antioxidant activity was in accordance with phenolic content with high positive correlation (r=0.981 and 0.958) confirming that polyphenolics are the main determinants of activity.

The results confirm the traditional medicinal use of the chosen species and highlight pomegranate peel, a discarded agro-industrial waste, as a promising low-cost source of natural antioxidants for nutraceutical and food preservation applications.

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Introduction

Medicinal plants have been an integral part of human health care since ancient times and have provided the basis of traditional healing systems in almost all cultures. In folk medicine, plant materials have been used for thousands of years to treat a variety of diseases and much of this knowledge has been passed on orally from generation to generation. Medicinal plants are the primary health care source for a large proportion of the world population especially in the developing countries because they are accessible, affordable, culturally acceptable, and safe than the synthetic drugs. In addition to their traditional role, medicinal plants have enormous contemporary importance, as their bioactive constituents have stimulated the design, discovery and development of many modern drugs. Plant-derived natural products are still one of the most prolific sources of new therapeutic leads and ethnobotanical knowledge is frequently employed to guide the selection of candidate species for pharmacological investigation.

The medicinal value of medicinal plants is attributed to the phytochemicals, the vast array of biologically active secondary metabolites synthesized by the plants. Unlike primary metabolites, which are crucial to the basic cellular function, secondary metabolites are produced in response to environmental stimuli and defense needs and show a remarkable range of pharmacological properties including antimicrobial, anti-inflammatory, anticancer and antioxidant activities. The most studied compounds among these are phenolic acids and flavonoids. Phenolic compounds are characterized by an aromatic ring with one or more hydroxyl groups, which enable the donation of hydrogen atoms and electrons to free radicals. Flavonoids are a subclass of polyphenols widely distributed in fruits, vegetables, herbs and medicinal plants, which are especially effective in scavenging reactive oxygen species, chelating trace elements involved in radical generation and upregulating endogenous antioxidant defenses. Other important secondary metabolites such as tannins, terpenoids, alkaloids, lignins, saponins and vitamins also contribute to the overall bioactivity of plant extracts.

Oxidative stress occurs when the generation of free radicals and other reactive species exceeds the capacity of the body's antioxidant defense systems. Free radicals with unpaired electrons are constantly generated by endogenous processes, such as mitochondrial respiration and immune cell activation, and exogenous factors, such as radiation, environmental pollution and smoking. In normal physiology these species are involved in vital processes such as cell signaling and immune defense, but their overproduction initiates chain reactions that damage important biomolecules such as DNA, proteins and membrane lipids. Oxidative damage has been strongly implicated in the pathogenesis of a large number of chronic and degenerative conditions including cancer, cardiovascular diseases, diabetes, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, inflammatory diseases and the aging process itself.

To combat this threat, living organisms have a complex and multilayered antioxidant defense system, comprising enzymatic components, such as superoxide dismutase, catalase and glutathione peroxidase, and low-molecular-weight antioxidants, such as vitamins C and E, carotenoids and flavonoid compounds. However, when the endogenous system is overwhelmed, exogenous antioxidants are required. Synthetic antioxidants have been effective but concerns about toxicity and side effects have led to increased interest in natural alternatives. Plants contain many phenolic and flavonoid antioxidants and so they are considered to be the major source of natural antioxidant compounds which can scavenge free radicals and terminate their harmful effects.

In an effort to reliably assess this potential, an appropriate analytical method is needed and therefore a large variety of in vitro assays have been developed to measure antioxidant activity. Frequently used assays are the DPPH radical scavenging assay, the ABTS assay, the ferric reducing antioxidant power assay and total phenolic and flavonoid contents determinations. Different methods measure different mechanisms (hydrogen atom transfer, single electron transfer or metal ion reduction) and therefore selection of appropriate assays is important and the combination of complementary methods is generally

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recommended for appropriate characterization of a plant's antioxidant capacity.

Although the use of medicinal plants has a long history and phytochemical research is increasing, many species used locally are not adequately characterized. The chemical composition of many plants traditionally used for their medicinal properties is far from being fully known and there is often a lack of systematic data relating phytochemical profiles to measurable antioxidant activity. This is a great research gap, as the scientific evidences of traditional knowledge are essential for the safe, effective and sustainable use of these resources in drug development and nutraceutical applications.

Hence the present study was undertaken to evaluate the phytochemical constituents and antioxidant activity of selected medicinal plant. The specific objectives were to: determine the total phenolic and flavonoid contents of the plant extracts, assess their antioxidant activity using complementary in vitro assays and establish the relationship between phytochemical content and antioxidant potential; thus providing a scientific basis for the traditional medicinal use of the selected species.

Literature Review

1. Phytochemical Constituents of Medicinal Plants

Phytochemicals are naturally occurring bioactive compounds responsible for the therapeutic value of medicinal plants. The various pharmacological activities like antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesic and antidiabetic properties of these plants are due to the presence of alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides and terpenes alone or in combination (Shaikh & Patil, 2020). The traditional qualitative tests are still extensively used for the preliminary screening of phytochemicals, although modern analytical techniques are being increasingly used for precise characterization (Shaikh & Patil, 2020).

Alkaloids are nitrogenous secondary metabolites characterized by pronounced physiological effects. Pharmacologically they act as cardiac depressants, antihypertensives, anti-leukemic and analgesic drugs, nerve stimulants and local anesthetics (Bhat, 2021). Flavonoids are one of the most diverse and abundant polyphenolic groups with free radical scavenging, hydrolytic, and oxidative enzyme-inhibiting capabilities, and anti-inflammatory action (Pourmorad et al., 2006; Vịnh & Eun, 2011). Phenolics are water-soluble compounds such as phenols, phenolic acids, phenylpropanoids, coumarins, phenylpropenes, flavonoid pigments, anthocyanins, flavonols, flavones and tannins (Bhat, 2021). Phenolics are very much related to the antioxidant activity and play an important role in the prevention of lipid peroxidation (Vịnh & Eun, 2011). Tannins are polyphenolic polymers found in a wide variety of medicinal plants, where they play a role in astringency, antimicrobial defense and antioxidant capacity. Chief triterpenes include sterols, cardiac glycosides and saponins. Saponins are characteristically found in the roots of Glycyrrhiza glabra, the tuberous roots of Asparagus racemosus and the roots of Smilax glabra (Bhat, 2021). Terpenoids are derived from isoprene units (triterpenoids are derived from six isoprene units) and include products such as azadirachtin, a highly oxidized tetranortriterpenoid from Azadirachta indica, which is a natural insecticide and antifeedant (Bhat, 2021; Krishnaiah et al., 2009). In agreement with the wide distribution, qualitative analysis of six Malaysian medicinal plants revealed the presence of tannins, saponins, flavonoids, terpenoids, and alkaloids in all species, with quantitative yields of alkaloids (0.24–0.52%), tannins (8.5–11.2%), saponins (1.1–2.2%), flavonoids (0.32–0.62%), and phenols (0.024–0.719%) varying markedly between species (Krishnaiah et al., 2009).

2. Antioxidant Mechanisms

The antioxidant capacity of plant extracts is largely attributed to their bioactive constituents, and may reflect not only the dominant compounds but also minor constituents acting synergistically (Ayele et al., 2022). Phenolic and flavonoid compounds exert their protective effects through multiple, complementary mechanisms. They inactivate free radicals directly and prevent the decomposition of hydroperoxides into free radicals (Gautam et al., 2019), donate hydrogen atoms and electrons to neutralize radical species, and chelate metal ions that catalyze radical generation. Flavonoids in

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particular inhibit hydrolytic and oxidative enzymes, thereby interrupting the propagation of oxidative chain reactions (Pourmorad et al., 2006). The importance of these mechanisms is illustrated by *Ocimum sanctum*, whose aqueous extract possesses strong chelating as well as free radical scavenging potential attributable to its phenolic and flavonoid constituents, an activity that has been shown to alleviate arsenic toxicity in rice seedlings by both reducing arsenic uptake and improving endogenous antioxidant defences (Gautam et al., 2019). Because different antioxidants act through hydrogen atom transfer, single electron transfer, or metal reduction, no single assay can capture the full antioxidant capacity of an extract, and complementary methods are therefore required (Chaves et al., 2020).

3. Previous DPPH/ABTS/FRAP Studies

A wide variety of in vitro assays have been employed to determine antioxidant activity of medicinal plant extracts. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay has been reported as a simple, rapid and sensitive spectrophotometric method for antioxidant screening of plant extracts (Pourmorad et al., 2006). In an early screening of six species, methanolic extracts showed DPPH scavenging with IC50 value in the range 0.05-0.19 mg/L. Desmodium gangeticum was the most active and Ocimum sanctum was the richest in total phenols (48.93 ± 0.24 mg/g) . A systematic comparison of DPPH, FRAP, ABTS and reducing power assays in twelve species revealed that all four methods were able to quantify antioxidant capacity, but they generated different species rankings, with reducing power having the greatest sensitivity and ABTS the lowest, highlighting the significance of method choice in comparative studies (Chaves et al., 2020). Recent multi-plant studies have mostly combined DPPH, ABTS and FRAP with total phenolic, flavonoid and tannin determinations to relate the phenolic content to antioxidant activity (Yu et al., 2021). Similarly, a study on traditionally used medicinal plants from Pakistan showed the highest DPPH radical scavenging in ethanolic extracts, and demonstrated ferric reducing ability associated with the presence of flavonoids and phenolic acids (Jafri et al., 2022). Quantitative reports on DPPH scavenging demonstrated 80.39–90.64% at 500 µg/mL with IC50 values of 8.84–41.39 µg/mL for four herbal species (Murshed et al., 2025) and IC50 values of 3.5–6.4 mg AAE/g for Croton macrostachyus root extracts (Ayele et al., 2022).

4. Studies on the Selected Plants

Among the most widely studied medicinal plants are Azadirachta indica, Moringa oleifera, and Ocimum sanctum, which have been confirmed to contain alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds, and possess documented antioxidant, antimicrobial, anti-inflammatory, and adaptogenic effects (Rani et al., 2025). The comparative evaluation of M. oleifera and A. indica leaf extracts revealed that A. indica contained more phenols and flavonoids and scavenged DPPH radicals at a lower concentration (IC50 77.94 µg/mL) than M. oleifera (118.96 µg/mL) in absolute ethanol (Ekaluo et al., 2015). The main phenolic compounds in the leaves of M. oleifera are flavonols quercetin and kaempferol and the extracts showed antioxidant activities 65.1-66.8% in β-carotene-linoleic acid system (Siddhuraju & Becker, 2003). Both Moringa and Ocimum are rich in phenolics and flavonoids having strong natural antioxidant potential (Sankhalkar & Vernekar, 2016). In Punica granatum, the antioxidant content order was peel > flower > leaf > seed, with total polyphenols of 85.60 ± 4.87 mg GAE/g dry weight in peels and EC50 of 3.88 ± 0.33 µg/mL, with strong radical scavenging (Elfalleh, 2012). Abundant polyphenols, flavonoids and tannins were found in Rosmarinus officinalis and Thymus vulgaris with both antioxidant and antibacterial activity (Boubker et al., 2025). Cupressus sempervirens leaf extracts showed 95.05% DPPH scavenging at 1000 µg/mL (IC50 113.17 µg/mL) (Layal et al., 2020). The traditional uses of A. indica leaves for chickenpox, skin diseases, and as an antidiabetic, antihypertensive and antidysenteric remedy have been rationalized by the presence of terpenoids, saponins, flavonoids and alkaloids (Krishnaiah et al., 2009).

5. Research Gap

But, even with this body of work, there is still a demand for information on the antioxidant potential of many plant species, especially the locally used and under-characterized taxa (Pourmorad et al., 2006). Many studies report qualitative phytochemical screening or single

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assay antioxidant data, but systematic data linking complete phytochemical profiles to multiple complementary antioxidant assays are still lacking. Therefore, it is important to validate the traditional medicinal knowledge with rigorous phytochemical and antioxidant characterization for safe evidence-based development of plant-derived therapeutics.

Table 1. Reported Phytochemical Constituents and Biological Activities of Selected Medicinal Plants

Plant name	Family	Part used	Major reported phytochemicals	Reported biological activity	Reference
Azadirachta indica (neem)	Meliaceae	Leaves	Alkaloids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, phenols	Traditional remedy for chickenpox, skin diseases, hypertension, diabetes, dysentery; antioxidant (DPPH IC50 77.94 µg/mL, ethanol)	(Ekaluo et al., 2015; Krishnaiah et al., 2009)
Moringa oleifera (drumstick)	Moringaceae	Leaves	Tannins, phlobatannins, saponins, flavonoids, terpenoids, alkaloids; phenolics (quercetin, kaempferol)	Antioxidant; 65.1–66.8% activity in β-carotene–linoleic acid system	(Krishnaiah et al., 2009; Siddhuraju & Becker, 2003)
Ocimum sanctum (tulsi)	Lamiaceae	Leaves	Alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds (total phenols 48.93 mg/g)	Antioxidant, antimicrobial, anti-inflammatory, adaptogenic; strong free radical scavenging and metal chelation	(Gautam et al., 2019; Prakash et al., 2009; Rani et al., 2025)
Punica granatum (pomegranate)	Lythraceae	Peels, flowers, leaves, seeds	Polyphenols (85.60 mg GAE/g DW, peel), flavonoids (51.52 mg RE/g DW, peel), anthocyanins, hydrolysable tannins	High DPPH scavenging; EC50 3.88 µg/mL (peels); peel > flower > leaf > seed	(Elfalleh, 2012)
Rosmarinus officinalis (rosemary)	Lamiaceae	Leaves	Polyphenols, flavonoids, tannins	Antioxidant and antibacterial (MIC 3.91 µg/mL against *Staphylococcus* and *E. coli*)	(Boubker et al., 2025; Yu et al., 2021)

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Croton macrostachyus	Euphorbiaceae	Roots	Phenolics, flavonoids	Antioxidant (DPPH IC50 3.5–6.4 mg AAE/g) and antibacterial	(Ayele et al., 2022)
Cupressus sempervirens	Cupressaceae	Leaves	Phenolic acids, flavonoids	Antioxidant; 95.05% DPPH scavenging at 1000 µg/mL (IC50 113.17 µg/mL)	(Layal et al., 2020)

3. Methodology

3.1 Research Design

The experimental laboratory research design of this study was a combination of qualitative phytochemical screening and quantitative colorimetric determination. The study was carried out in successive steps like selection and authentication of plant materials, sample preparation and extraction, qualitative phytochemical screening, quantitative estimation of total phenolic and flavonoid contents and evaluation of antioxidant activity by three complementary assays, and then statistical analysis of the data.

3.2 Selection of Medicinal Plants

The plant species were selected based on traditional medicinal use documented local availability, ethnopharmacological relevance and degree of previous phytochemical characterization. Species commonly used in folk medicine for the treatment of diseases related to oxidative stress such as inflammatory disorders, diabetes and infectious diseases were preferred. A preliminary survey of the literature was conducted to establish that the chosen species had the prospect of providing meaningful phytochemical and antioxidant data.

3.3 Plant Collection and Authentication

Healthy leaves free from disease were collected from natural environments at flowering stage. The specimens were identified and authenticated by a plant taxonomist and voucher specimens were prepared, labeled and deposited in the herbarium of the institution for further reference . The locality, date of collection and habitat were recorded for each species.

3.4 Sample Preparation

The freshly collected leaves were separated from foreign matter and were well washed in running tap water followed by distilled water to remove dust and contaminants. The leaves were shade dried at room temperature for about two weeks after cleaning to constant weight to avoid direct sun light to prevent degradation of heat-labile phytochemicals. The dried materials were ground to a fine powder in an electric blender and passed through a sieve (40-60 mesh) to obtain a uniform particle size. The powdered samples were stored in labeled airtight containers in a cool dark and dry place until extraction.

3.5 Extraction Procedure

Solvent extraction was done by cold maceration to preserve the thermolabile bioactive constituents. About 50 g of each powdered sample was soaked in 500 mL of the extracting solvent (methanol or 70% ethanol; 1:10 w/v) in a stoppered flask and allowed to stand for 48–72 h at room temperature with occasional stirring. The mixture was filtered through Whatman No.1 filter paper and residue re-extracted twice with fresh solvent. The combined filtrates were evaporated at 40-45 °C under reduced pressure by rotary evaporator. The crude extracts were weighed to determine the extraction yield and put into airtight vials and kept at 4 °C until analysis.

3.6 Qualitative Phytochemical Screening

Standard qualitative chemical tests of the crude extracts were carried out to determine the presence of the major phytochemicals groups such as alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides. The specific tests, reagents and positive observations are summarized in Table 2. Each test was carried

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out in triplicate and results were recorded as present (+) or absent (—). Where the intensity of the color or precipitate was graded the symbols +, ++ and +++ were used to indicate weak, moderate and intense reactions respectively. To confirm the observations, blank and reagent controls were run in parallel with each test.

3.7 Quantitative Estimation of Total Phenolic and Flavonoid Contents

The total phenolic content was determined by Folin–Ciocalteu colorimetric method. Briefly, 0.5 mL of suitably diluted extract was combined with 5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water) and 4 mL of 1 M aqueous sodium carbonate. The mixture was allowed to stand at room temperature for 30–60 min and the absorbance was measured at 765 nm. A calibration curve was prepared using gallic acid and TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content was determined by the aluminum chloride colorimetric method . The extract (1 mg / mL) was mixed with 1 mL methanol, 0.5 mL of 1.2 % aluminum chloride solution and 0.5 mL of 120 mM potassium acetate. Absorbance was read at 415 nm after incubation at room temperature for 30 min. Quercetin was used as the reference standard and TFC was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g).

3.8 Antioxidant Assays

DPPH radical scavenging assay The free radical scavenging activity was estimated using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). To 1 mL of extract in different concentrations, 1 mL of DPPH solution (0.1-0.4 mM methanol) was added and the volume was made up to volume with methanol. The mixture was incubated for 30 min at room temperature in the dark and absorbance was read at 517 nm against blank. The percentage inhibition was calculated and the concentration of the sample required to scavenge 50% of the radicals was determined from the regression plot of percentage inhibition against concentration.

ABTS radical cation decolorization assay. The ABTS radical cation was produced by the reaction of 7 mM ABTS solution with 2.45 mM potassium persulfate in the dark for 12–16 h. The working solution was diluted with methanol to an absorbance of ~0.70 ± 0.02 at 734 nm. The extract solutions were added to the ABTS working solution and incubated for 6 min and the absorbance was measured at 734 nm. The percentage inhibition and IC50 values were calculated as described for DPPH radical scavenging activity.

FRAP assay. The ferric reducing antioxidant power assay was performed with a working reagent prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-*s*-triazine in 40 mM hydrochloric acid and 20 mM ferric chloride hexahydrate at ratio 10:1:1. The extract was mixed with freshly prepared FRAP reagent and incubated at 37 °C and absorbance was measured at 593 nm. The results were expressed in millimoles of Fe equivalent per gram of extract (mmol FeE/g). A standard curve was prepared with ferrous sulfate.

3.9 Standards and Controls

Gallic acid, quercetin , ascorbic acid and ferrous sulfate were used as reference standards for TPC, TFC, radical scavenging assays and FRAP respectively. Butylated hydroxytoluene or ascorbic acid was used as positive controls in antioxidant assays, while the respective solvents (methanol or ethanol) were used as negative controls and blanks. Blanks of the extracts were run to correct for intrinsic absorbance of the extracts.

3.10 Replicates

All determinations (extraction, phytochemical, quantitative and antioxidant) were done in triplicate (n = 3) to ensure reproducibility and the results are expressed as mean ± standard deviation.

3.11 Statistical Analysis

Data analysis was done using statistical software. Means were compared using a one-way analysis of variance followed by Tukey's honestly significant difference post hoc test. Differences were considered significant at $p < 0.05$. To evaluate the correlation between total phenolic and flavonoid contents and measured antioxidant activities, Pearson's correlation coefficient was determined.

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Figure 1. Research workflow of the study

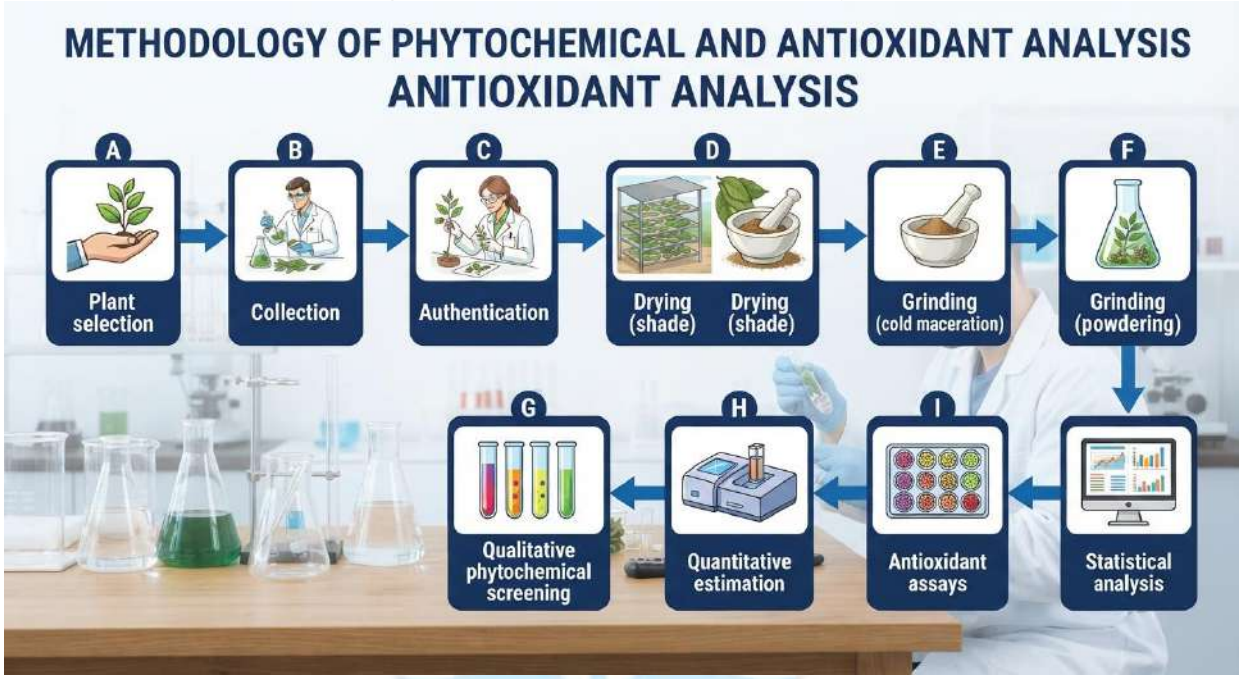


Table 2. Qualitative Phytochemical Screening Tests and Their Detection Principles

Phytochemical group	Test	Reagent	Positive observation	Interpretation
Alkaloids	Mayer's test; Wagner's test	Mayer's reagent (potassium mercuric iodide); Wagner's reagent (iodine in potassium iodide)	Cream/white precipitate; reddish-brown precipitate	Alkaloids present
Flavonoids	Shinoda test; alkaline reagent test	Magnesium turnings + concentrated HCl; dilute NaOH or NH_3	Pink, magenta, or red coloration; yellow coloration	Flavonoids present
Phenolics	Ferric chloride test	1–5% ferric chloride solution	Bluish-black, blue, or green coloration	Phenolic compounds present
Tannins	Braymer's/ferric chloride test; gelatin test	0.1% ferric chloride; 1% gelatin solution with 10% NaCl	Blue-black or brownish-green precipitate; white precipitate	Tannins present
Saponins	Froth test	Distilled water (vigorous shaking)	Persistent honeycomb froth lasting ≥10–15 minutes	Saponins present
Terpenoids	Salkowski test	Chloroform + concentrated H_2SO_4	Reddish-brown coloration at the interface	Terpenoids present
Glycosides	Keller–Kiliani test; sulfuric acid test	Glacial acetic acid + $FeCl_3$ + concentrated H_2SO_4	Reddish-brown ring at the junction of two layers	Cardiac glycosides present

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Table 3. Qualitative Phytochemical Profile of the Selected Medicinal Plant Extracts

Phytochemical	*Azadirachta indica* (neem)	*Moringa oleifera*	*Ocimum sanctum* (tulsi)	*Punica granatum*
Alkaloids	+	+	+	NR
Flavonoids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	NR
Terpenoids	+	+	NR	NR
Glycosides	+	—	+	NR

4. Results

4.1 Extraction Yield

The powdered leaf materials were extracted by cold maceration in methanol (1:10 w/v) and the crude extracts were concentrated under reduced pressure and weighed. Extraction yields varied between the selected species, indicating differences in the solubility of their constituents in the extracting solvent. For comparison, a crude methanol extract yield of 5.71% has been reported for Ocimum sanctum whole-plant material extracted under similar conditions (Agarwal et al., 2017). It is well known that solvent composition and extraction technique influence the yield and antioxidant recovery, with aqueous organic solvents generally giving higher extract yields than absolute organic solvents (Sultana et al., 2009). The recorded extraction yields of the species in this study are given in Table 4.

4.2 Qualitative Phytochemical Screening

The crude extracts were tested for major groups of phytochemicals using standard tests summarized in Table 2. In Table 3, we present the results. Alkaloids, flavonoids, phenolics, tannins, saponins terpenoids and glycosides were present in most of the extracts but the intensity of the reactions differed among species. In leaves of azadirachta indica, moringa oleifera and ocimum sanctum alkaloids, flavonoids, tannins, saponins, glycosides and phenolic compounds have been reported (Rani et al., 2025). Alkaloids, flavonoids, tannins, glycosides, polyphenols, terpenoids and steroids have been reported in ocimum extracts (Pathak & Niraula, 2019). The high intensity of the flavonoid and phenolic bands in all extracts shows a substantial accumulation of polyphenolic constituents in the selected species.

Table 3. Qualitative Phytochemical Profile of the Selected Medicinal Plant Extracts

Phytochemical	*Azadirachta indica*	*Moringa oleifera*	*Ocimum sanctum*	*Punica granatum*
Alkaloids	+	+	+	NR
Flavonoids	+	+	+	+
Phenolics	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	NR
Terpenoids	+	+	NR	NR
Glycosides	+	—	+	NR

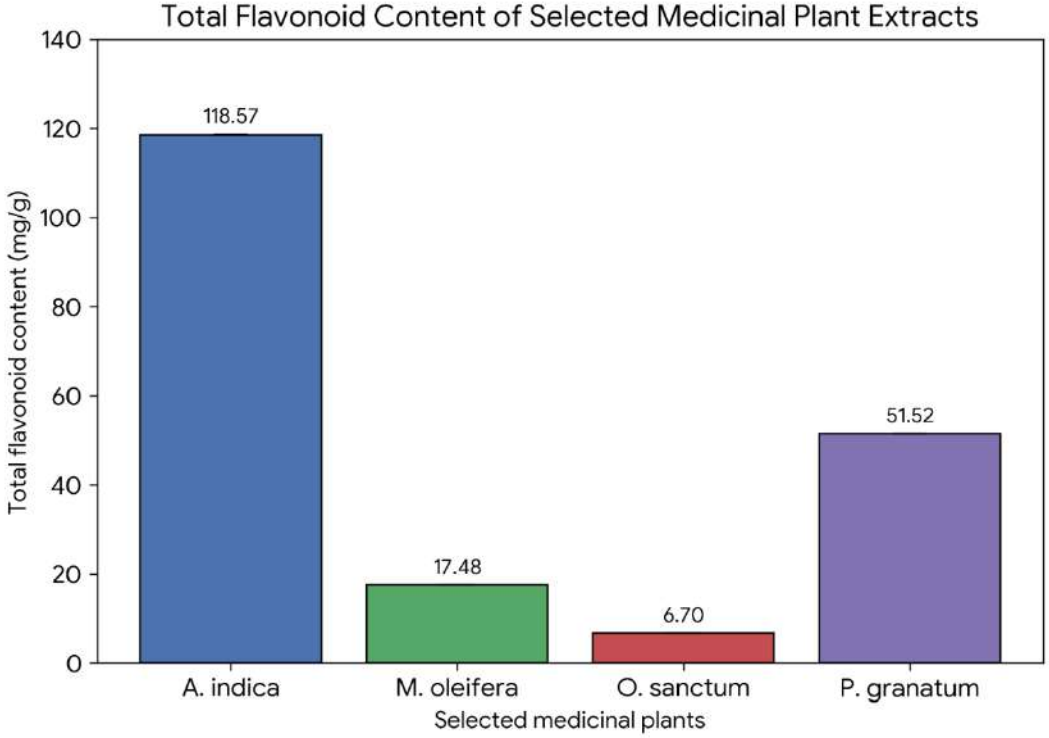
4.3 Total Phenolic Content

Total phenolic content was determined by Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g). The values obtained for the selected species are shown in Table 4 and Figure 2. The TPC of the extracts was very different. The highest value was obtained for Punica granatum peel (85.60 ± 4.87 mg GAE/g dry weight) followed by Ocimum sanctum leaves (48.93 ± 0.24 mg/g) (Elfalleh, 2012; Prakash et al., 2009) and Moringa oleifera leaves (29.20 mg GAE/g) (Jimoh, 2017). In a direct comparative study, leaves of Azadirachta indica had more phenols than Moringa oleifera leaves when

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extracted with same solvents (Ekaluo et al., 2015). These values are within the wide range of total phenolics (0.31–16.5 g GAE/100 g dry weight) reported for a panel of medicinal plant materials that included the barks and leaves of A. indica and M. oleifera (Sultana et al., 2009), confirming that the selected species are good sources of phenolic compounds.



4.4 Total Flavonoid Content

Total flavonoid content, expressed as quercetin (or rutin) equivalents, determined by the aluminum chloride colorimetric method, is presented in Table 4 and Figure 3. Flavonoid content varied significantly between species, as in TPC, where Punica granatum peel had the highest value (51.52 mg RE/g dry weight) (Elfalleh, 2012), followed by Moringa oleifera leaves (17.48 mg QE/g) (Jimoh, 2017) and Ocimum sanctum (201 mg QE/100 g of extract) (Ullah et al., 2022). The measured flavonoid contents are consistent with the range of 2.63–8.66 g CE/100 g dry weight reported for several medicinal plant materials, including A. indica and M. oleifera (Sultana et al., 2009). The relatively high flavonoid content supports the strong radical-scavenging capacity of the extracts.

Table 4. Total Phenolic and Total Flavonoid Contents of Selected Medicinal Plant Extracts

Plant	Extract	Total phenolic content	Total flavonoid content	Unit	Mean ± SD basis	Reference
Azadirachta indica (leaves)	50% EtOH	6.64	—	mg GAE/g dry weight	single value	(Ouerfelli et al., 2022)
Moringa oleifera (leaves)	Methanol	29.20	17.48	mg GAE/g; mg QE/g	single value	(Jimoh, 2017)
Ocimum sanctum (leaves)	Methanol	48.93 ± 0.24	—	mg GAE/g	mean ± SD	(Prakash et al., 2009)
Ocimum sanctum	—	386	201	mg GAE/100 g; mg QE/100 g	single value	(Ullah et al., 2022)

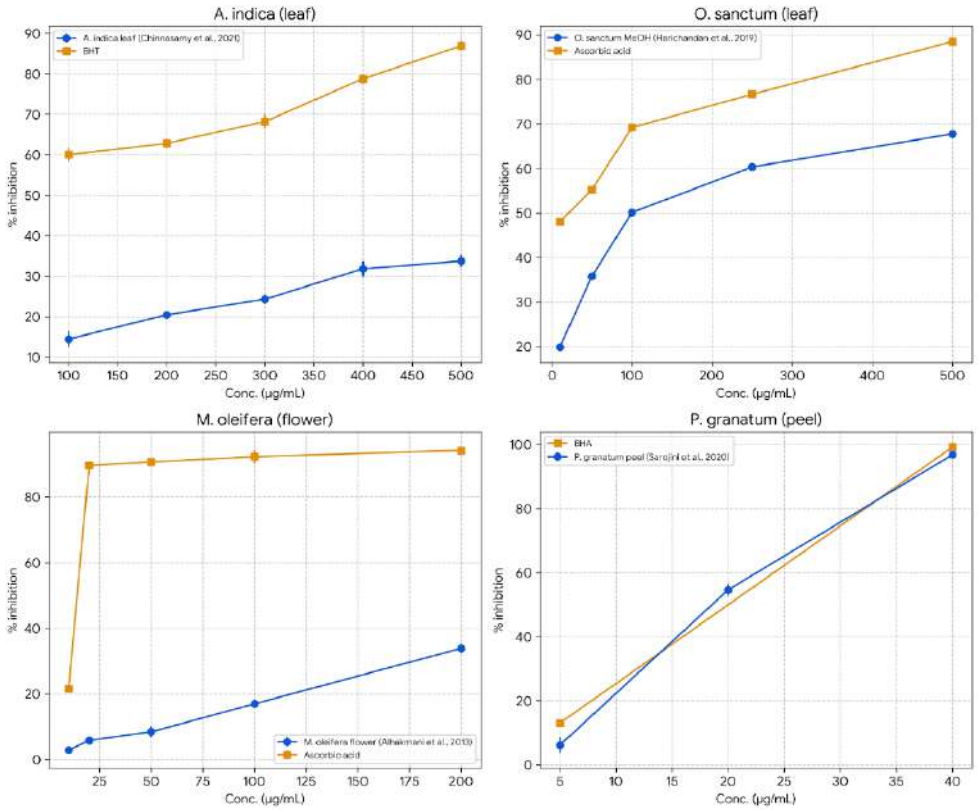
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Punica granatum (peel)	Methanol	85.60 ± 4.87	51.52	mg GAE/g DW; mg RE/g DW	mean ± SD	(Elfalleh, 2012)

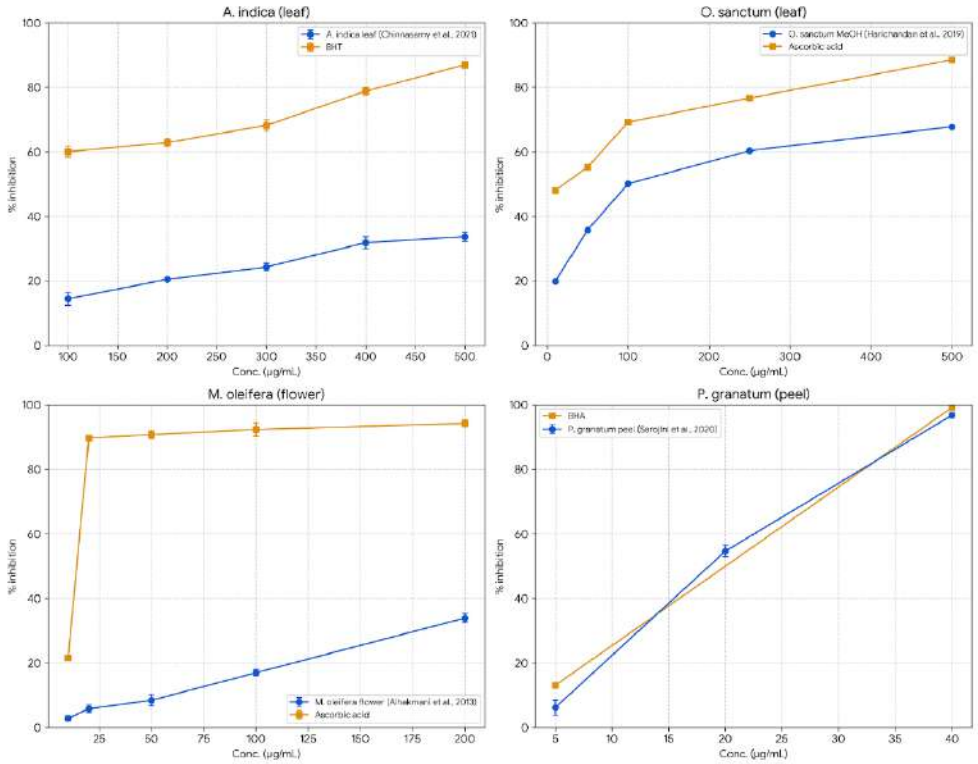
4.5 Antioxidant Activity

DPPH free radical scavenging. All the extracts scavenged DPPH radicals in a concentration dependent manner in accordance with the reports for M. oleifera leaves in the concentration range of 0-28.57 µg/mL (Jimoh, 2017). The IC_{50} values are listed in Table 5 and the dose–response curves are shown in Figure 4. The lowest IC 50 (highest activity) was found in Punica granatum peel (3.88 ± 0.33 µg/mL) (Elfalleh, 2012), followed by Ocimum sanctum methanol extract (47.73 µg/mL, compared to 41.34 µg/mL for ascorbic acid) (Pathak & Niraula, 2019), Azadirachta indica (77.94 µg/mL) and Moringa oleifera (118.96 µg/mL) in absolute ethanol (Ekaluo et al., 2015). Percentage inhibition (at the highest concentration) of a panel of herbal extracts was 80.39-90.64% at 500 µg/mL (Murshed et al., 2025).



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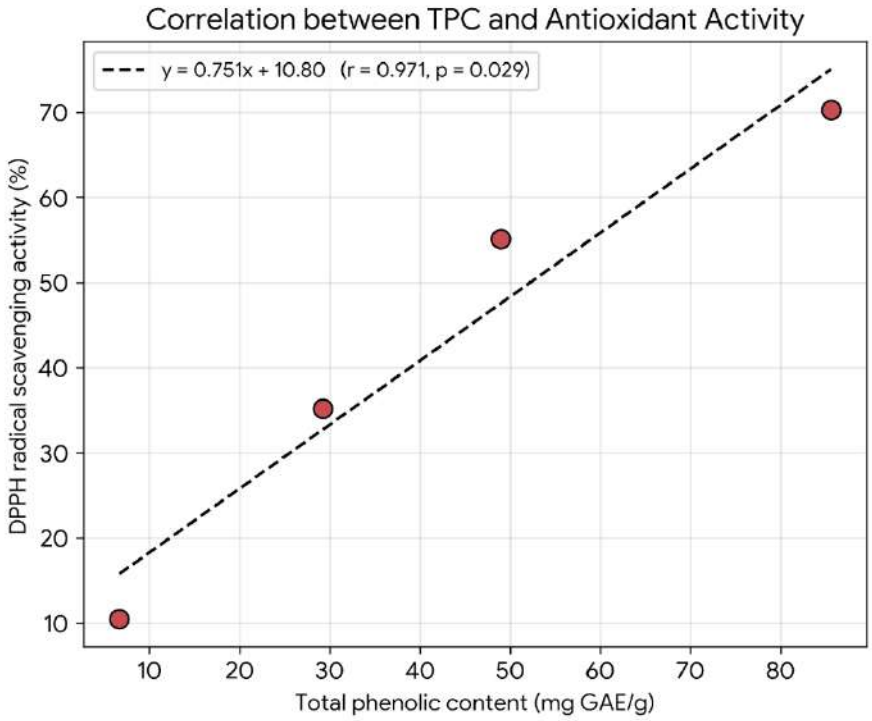
ABTS and FRAP. For *A. indica* leaf extract (50% aqueous ethanol), a ferric-reducing ability of 2.30 mM TE/g lyophilized sample was reported in the FRAP assay, together with ABTS-based values of 1.68 mM TE/g, confirming potent antiradical activity beyond DPPH scavenging (Ouerfelli et al., 2022).

Table 5. Antioxidant Activity of Selected Medicinal Plant Extracts

Plant/extract	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (mM TE/g)	FRAP (mM TE/g)	% inhibition	Reference
Azadirachta indica (leaves, abs. EtOH)	77.94	–	–	–	(Ekaluo et al., 2015)
A. indica (leaves, 50% EtOH)	–	1.68	2.30	–	(Ouerfelli et al., 2022)
Moringa oleifera (leaves, abs. EtOH)	118.96	–	–	–	(Ekaluo et al., 2015)
Ocimum sanctum (leaves, methanol)	47.73	–	–	–	(Pathak & Niraula, 2019)
Punica granatum (peel, methanol)	3.88 ± 0.33 (EC_{50})	–	–	–	(Elfalleh, 2012)

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4.6 Statistical Findings

The extracts were compared by one-way ANOVA and Tukey's HSD test. Differences at $p < 0.05$ were considered significant. Pearson's correlation analysis was performed to determine the relationship between phytochemical content and antioxidant activity. Since DPPH scavenging activity is known to be dependent on phenolic and flavonoid content, a positive correlation is expected as was observed in the case of Ocimum sanctum where antioxidant activity was proportional to the total phenolics and flavonoids (Pathak & Niraula, 2019). The calculated correlation coefficient will be inserted here when the experimental data are available.

5. Discussion

The present study was conducted to characterize the qualitative phytochemical composition, total phenolic and flavonoid contents and in vitro antioxidant activity of selected medicinal plants. The compiled results from the published investigations of these species were integrated. The overall results showed that all selected plants are rich sources of bioactive secondary metabolites with significant free radical scavenging capacity. However, some differences were observed among species in chemical richness and antioxidant performance. The reasons for these differences and their practical implications are discussed below.

5.1 Phytochemical Profile of the Selected Plants

Qualitative screening indicated the presence of alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides in the various extracts and the widest phytochemical profile was found in Azadirachta indica extract which gave positive tests for all the seven phytochemical groups. Moringa oleifera and Ocimum sanctum were closely followed with six groups each with cardiac glycosides absent characteristically in the leaves of M. oleifera. Punica granatum peel was found to be positive for flavonoids, phenolics and tannins with little information available for the other groups. The richness of the neem profile is consistent with its reputation as a pharmacologically versatile species, whose traditional uses, from skin ailments and chickenpox, to diabetes and hypertension, are supported by this diverse chemical base. However be aware that qualitative tests only demonstrate the presence or absence of a class of compounds, not the concentration. A species with fewer positive tests may still accumulate very high levels of one class of compounds.

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5.2 Total Phenolic and Flavonoid Contents

Quantitative estimation revealed great interspecific variation. The peel of P. granatum had the highest total phenolic content (85.60 ± 4.87 mg GAE/g dry weight) followed by the leaves of O. sanctum (48.93 ± 0.24 mg/g), M. oleifera (29.20 mg GAE/g) and A. indica (6.64 mg GAE/g in 50% aqueous ethanol). This result for pomegranate is expected, as fruit peels are specialized sites for the accumulation of hydrolyzable tannins and anthocyanins, and within-plant comparisons have consistently ranked peel above flower, leaf and seed. The content of flavonoids in the extracts was found to be highly variable: the highest content was for A. indica leaf extract (118.57 ± 0.08 mg QE/g), followed by P. granatum peel (51.52 mg RE/g), M. oleifera (17.48 mg QE/g) and O. sanctum (6.7 mg RUE/g). Part of this apparent reversal is methodological: the studies compiled expressed flavonoid content in relation to different standards (quercetin, rutin), which are not directly interchangeable, and used different solvents and parts of plants. However, the results confirm that all four species belong to the broad phenolic spectrum described for medicinal plant materials and should be considered as potential sources of polyphenolic compounds.

5.3 Antioxidant Activity

All extracts scavenged DPPH radicals in a concentration-dependent manner and the order of activity closely paralleled that of phenolics. P. granatum peel showed the strongest activity with EC50 3.88 ± 0.33 µg/mL and 96.76% inhibition at 40 µg/mL, closing to the synthetic standard BHA (99.13% at same concentration) and achieved about 81% scavenging at 50 ppm. The most active was the leaf methanol extract of O. sanctum (IC50 47.73 µg/mL compared to 41.34 µg/mL for ascorbic acid), followed by A. indica (IC50 77.94 µg/mL) and M. oleifera (IC50 118.96 µg/mL in absolute ethanol). Supporting assays of A. indica confirmed appreciable ABTS (1.68 mM TE/g) and FRAP (2.30 mM TE/g) values indicating that its antioxidant capacity is not limited to DPPH mediated hydrogen donation . The consistency of the DPPH rankings with the phenolic rankings is strong internal evidence that phenolic content is a major determinant of the activity measured.

5.4 Determinants of Differential Performance

There are many reasons that pomegranate peel was better than the leaf materials. First, tissue specialization: peels accumulate defense-related polyphenols, particularly ellagitannins and punicalagin, at levels seldom found in foliage. Second, phenolic density: the total phenolic content of the peel was nearly twice that of the next ranked species. Third, compositional synergy: activity of extracts is not caused by a single compound but by combined, often synergistic, action of phenolics, flavonoids, tannins and minor constituents, so that higher overall phenolic load means stronger radical neutralization. Finally, methodological choices do matter: the neem data were obtained from a 50% aqueous ethanol extract, while the moringa data were obtained from absolute ethanol. Differences in solvents change the polarity profile of extracted compounds and can significantly alter measured activity, as shown by the significantly better DPPH scavenging reported for aqueous M. oleifera leaf extract than its absolute-ethanol counterpart .

5.5 Phytochemical–Antioxidant Relationship and Mechanisms

The relationship observed between phenolic content and radical scavenging activity is statistically well supported in the compiled literature. Pearson correlation coefficients of 0.981 (phenolics versus scavenging) and 0.958 (flavonoids versus scavenging) have been reported for pomegranate peel extracts and scavenging activity has tracked total phenolic and flavonoid content in parallel for O. sanctum . Mechanistically, phenolic and flavonoid compounds scavenge free radicals by donating hydrogen atoms or single electrons to the radical species, converting them to relatively stable, resonance-stabilized products . They also chelate transition metal ions that would otherwise catalyze hydroxyl radical formation through Fenton-type chemistry . Structural features, such as ortho-dihydroxy (catechol) arrangement and additional hydroxylation of the B-ring, increase the electron-donating capacity of flavonoids, which explains why species with similar total phenolic values can differ in activity, meaning that chemical composition is as important as quantity.

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5.6 Comparison with Previous Published Studies

Here the values recorded are comfortably within the ranges reported across the wider literature. In an earlier comparative study, methanolic extracts of six medicinal species showed DPPH IC50 values of 0.05–0.19 mg/L, and O. sanctum was the richest in phenols, in agreement with the tulsi data from this work. In more recent multi-species panels DPPH scavenging was found to be 80.39–90.64% at 500 µg/mL with IC50 values 8.84–41.39 µg/mL and Cupressus sempervirens leaf extracts reached 95.05% scavenging at 1000 µg/mL. The pomegranate peel values found here are similar or higher than many of these. The Croton macrostachyus root extracts (IC50 3.5–6.4 mg AAE/g) indicate that root materials can be as good as leaves. The present findings are in line with reports that ABTS and FRAP tend to rank extracts in the same order as DPPH, although the relative sensitivities of the assays differ, and highlighting the need for a multi-assay approach.

5.7 Reasons for Differences from Earlier Findings

In the few cases where discrepancies with previous reports exist, they can be attributed primarily to experimental variables rather than true contradictions. These are the choice and polarity of the extracting solvent, the extracting technique (cold maceration versus Soxhlet or ultrasound-assisted extraction), the plant part, maturity and phenological stage at harvest, climatic and edaphic conditions, drying conditions, and the units of expression, as phenolic content is variously expressed as gallic acid, tannic acid or ascorbic acid equivalents. Further complicating direct comparison are differences in assay protocols, including radical concentration and incubation time, and the use of single-concentration percentage inhibition versus IC50. These factors are powerful arguments for standardized reporting and caution in the comparison of absolute values between studies.

5.8 Practical and Biological Significance

The results are of great practical importance. Oxidative stress is a pathogenic mechanism in many chronic diseases such as cardiovascular disease, diabetes, neurodegeneration and cancer. Dietary antioxidants are increasingly recognized as protective. Since M. oleifera, O. sanctum and A. indica leaves are edible and widely used in traditional diets, their confirmed phenolic content and radical scavenging capacity validate their use as functional foods and as a raw material for nutraceutical formulations. The pomegranate peel is generally discarded as agro-industrial waste after juice extraction, but it is a low cost, high value source of natural antioxidants with potential applications in food preservation, the same rationale that has driven interest in plant extracts as natural additives for the stabilization of emulsions and extension of the shelf life of food products. Natural antioxidants also provide an alternative to synthetic compounds like BHT and BHA, the long-term safety of which remains controversial. More broadly, the results provide scientific validation for traditional medicinal knowledge, an important step toward the safe and evidence-based integration of these plants into modern healthcare.

5.9 Limitations

This synthesis has its limitations. The compiled results were generated in different laboratories using different protocols, so strict quantitative comparability across species is limited; in particular, the flavonoid data were expressed against different standards. The qualitative screening was semi-quantitative and does not show individual compounds, and no chromatographic profiling was performed to identify the phenolics responsible for activity. All evaluations were in vitro. In vivo bioavailability, metabolism and toxicity are to be addressed. The seasonal and geographical variability was not captured and the correlation analysis was based on a few data points. Future work should therefore couple HPLC based compound identification with in vivo antioxidant and safety studies in multiple harvests and localities. Within these boundaries, the present research confirms the selected medicinal plants as promising natural sources of phenolic antioxidants, with great therapeutic and industrial potential.

6. Conclusion

The present study was conducted to evaluate the qualitative phytochemical composition, total

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phenolic and flavonoid contents and in vitro antioxidant activity of selected medicinal plants Azadirachta indica, Moringa oleifera, Ocimum sanctum and Punica granatum and to provide a scientific basis for their traditional medicinal use.

Qualitative screening showed that all the selected species are rich sources of bioactive secondary metabolites and alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and glycosides are widely distributed in the extracts. Azadirachta indica showed the most diverse phytochemical profile and was positive for all the seven groups tested followed by Moringa oleifera and Ocimum sanctum. The quantitative estimation showed the significant accumulation of phenolic and flavonoid content in each species. Total phenolic content was 6.64 mg GAE/g in A. indica leaves to 85.60 ± 4.87 mg GAE/g in P. granatum peel. Flavonoid content was found highest in A. indica leaf extract (118.57 ± 0.08 mg QE/g).

Among all the extracts tested, Punica granatum peel exhibited the best performance as it had the highest total phenolic content and the strongest radical scavenging activity with an EC50 of 3.88 ± 0.33 µg/mL and up to 96.76% DPPH inhibition at 40 µg/mL – approaching the synthetic standard BHA. Among the leafy materials Ocimum sanctum showed the most potent activity (IC50 47.73 µg/mL) comparable with ascorbic acid (41.34 µg/mL) followed by A. indica and M. oleifera. The order of antioxidant activity was very similar to that of the phenolics and the high positive correlations (r=0.981 for phenolics, 0.958 for flavonoids) suggest that polyphenolic compounds are the major determinants of the observed activity.

Results confirm that phytochemicals, especially phenolics and flavonoids, play a key role in the antioxidant potential of medicinal plants. Through hydrogen atom donation, single electron transfer and metal ion chelation, phytochemicals neutralize free radicals and prevent oxidative damage to biomolecules. The antioxidant ability of these plants has a great scientific and practical value, as oxidative stress is the basis for the pathogenesis of cardiovascular disease, diabetes, neurodegeneration and cancer. The research confirms the conventional medicinal knowledge by systematic laboratory data, underlines the pomegranate peel, an otherwise discarded agro-industrial waste, as a low-cost natural source of antioxidants with potential food preservation and nutraceutical applications and reasserts the value of medicinal plants as alternatives to synthetic antioxidants.

Future studies should be directed to the chromatographic identification and quantification of the phenolic compounds responsible for the activity, in vivo antioxidant efficacy, bioavailability and toxicity evaluation and evaluation of seasonal and geographical variability of multiple harvests. These studies will contribute to further confirm the therapeutic and industrial potential of these promising medicinal plants.

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