Evaluation of Free Radical-Scavenging and Nitric Oxide Inhibition Activities of Selected Medicinal Plants

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Abstract
The pharmaceutical industry is becoming dependent on plants with medicinal value because of their roles in the prevention and treatment of disease. Ten medicinal plant species namely Phyllanthus emblica, Mentha piperita, Ocimum tenuiflorum, Azadirachta indica, Syzygium aromaticum, Dalbergia sissoo, Allium sativum, Psidium guajava, Syzygium cumini and Allium cepa were evaluated for antioxidant potential using spectrophotometric assays. The results indicated that hyroalcoholic extracts of M. piperita showed the highest antioxidant capacity (202.56 ± 1.98 µgAAE/g) and DPPH radical scavenging activity (94% with IC50 value of 561 ± 1.13 μg /mL). Also, the extract of O. tenuiflorum showed 89 % inhibition at 1000 μg/mL concentration. The presence of phenolic and flavonoid compounds may be responsible for the free radical scavenging and antioxidant properties. The findings of the present study suggest that the plants might be exploited as a source of natural antioxidants. Further investigation is still required to identify the phytochemicals responsible for medicinal properties and to understand the mechanism of action of isolated bioactive compounds.

Introduction
Free radicals and reactive oxygen species (ROS) are very reactive molecules that are produced by cells during respiration and cell-mediated immunological responses. The disease will develop if cellular defenses against reactive oxygen species are ineffective. Antioxidants are essential to prevent oxidative damage because different disease processes can occasionally thwart these defense mechanisms. Medicinal plants have been an integral part of human life since the dawn of civilization. The discovery of traditional medicines with significant antioxidant potential has received much attention in recent years.

Keywords
Antioxidant Activity; Dpph Radical Scavenging Activity; Free Radicals; Medicinal Plants; Nitric Oxide.

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Azadirachta indica (neem) is well known in India and its neighboring countries as one of the most versatile medicinal plants with a broad spectrum of biological activity and India's most commonly used traditional medicinal plant for household remedies against various human ailments. A. indica contains various primary compounds, including fat derivatives, carbohydrates, and proteins, and secondary compounds, such as flavonoids, steroids, saponins, terpenoids, alkaloids, glycosides, and tannins. Phyllanthus emblica (Amla) has been used in Indian traditional medicine for the treatment of several diseases, such as hemorrhage, diarrhea, jaundice, dyspepsia and pharmacological investigations support the antioxidant activities of different extracts. Psidium guajava (guava) is used in traditional medicine against diarrhea, diabetes, and stomach aches. It possesses pharmacological activities like antispasmodic, cough sedative, anti-inflammatory, antidiarrheic, antihyper tension, antiobesity, and antidiabetic properties. Mentha piperita (Peppermint) is known for antioxidant, cytotoxic, and antibacterial activities with fewer side effects. It has also been used as a headache and migraine medicine and a treatment for intestinal colic, liver diseases, gastritis, and jaundice. Ocimum tenuiflorum (Tulsi) has many biological properties, including analgesic, antiemetic, hypoglycemic, immune-booster, antibacterial, stress-relieving, and expectorant effects. The antioxidant potential of O. tenuiflorum is due to various secondary metabolites, including phenolics and flavonoids. Syzygium cumini (Java Plum) is a purple-red to dark fruit containing large amounts of phytonutrients like flavonoids known for their anti-inflammatory, antidiabetic, anticancer, radical scavenging, and antioxidant potential.

A diverse range of medicinal properties like antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, and antidiabetic activities of herbs and spices is due to the presence of tannins, alkaloids, phenolics, flavonoids, and polyphenols. Allium sativum (garlic) sulfur-containing compounds are responsible for anti-inflammatory, anticancer, antitumor, antidiabetic, and cardioprotective properties. The majority of sulfur-containing compounds are garlic thiosulfate (alliin), S-allyl cysteine sulfoxide (alliiin), ajoenes (E- and Z-ajoene), vinyldithiins (2-vinyl-(4H) -1,3-dithiin, 3-vinyl-(4H)-1,2-dithiin), and diallyl (di and tri) sulfide.

Allium cepa (Onions) are considered an excellent antioxidant source and contain a significant amount of flavonoids and phenols. Polyphenols are phenol derivatives with a high antioxidant capacity because they effectively eliminate reactive oxygen species and have important anti-inflammatory actions. Quercetin and its glycosylated derivatives in A. cepa are anticipated to have an antioxidant potential. Dalbergia sissoo is rich in dalberginone, dalbergin, isoflavone-O-glycoside, 7, 4dimethylene tectorigenin, isocavium, etc., and known for antioxidant and antidiabetic activities. Syzygium aromaticum (Clove) is used primarily in food, perfume, cosmetics, and medicinal products. S. aromaticum has different pharmacological and antibacterial properties because of its constituents, such as glycosides, hormones, tannins, alkaloids, and saponins.

The antioxidant potentials of many medicinal plants have been exploited in different ways. The ability to scavenge free radicals and reactive oxygen species has been evaluated using various techniques based on spectrophotometry and chemiluminescence. The relationship between plant phenolic content and antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydroazyl (DPPH) and Nitric Oxide (NO) Radical Scavenging assays. The medicinal plants chosen for this study are Phyllanthus embelica, Mentha piperita, Ocimum tenuiflorum, Azadirachta indica, Syzygium aromaticum, Dalbergia sissoo, Allium sativum, Psidium guajava, Syzygium cumini, and Allium cepa. The ten selected plants are commonly used in the Indian traditional medicine system due to their potential health-promoting and pharmacological qualities. Previous studies have reported the antioxidant potential of selected plant extracts, but the comparative evaluation is lacking. The current study aimed phytochemical screening of ten selected medicinal plants and to analyze the antioxidant activities under the same evaluation condition. The screened extracts of medicinal plants can be used as potential antioxidant agents and resources for developing polyherbal formulations against various inflammation-related diseases.

Methodology
Sample Collection and Preparation
Fresh plant leaves from Phyllanthus embelica (Pe), Mentha piperita (Mp), Ocimum tenuiflorum (Ot), Azadirachta indica (Ai), Dalbergia sissoo
(Ds), *Allium sativum* (As), *Psidium guajava* (Pg), *Syzygium cumini* (Sc) and *Allium cepa* (Ac) were harvested from the Shobhit Institute of Engineering & Technology, (Deemed-to-be-University), Modipuram, Meerut, India. *Syzygium aromaticum* (Sa) flower buds were obtained from the local market (Vedaka Spices and Seeds, Karnataka, India, FSSAI LIC No. 10018011005884). The samples were rinsed with water and then shade dried until all moisture content was gone. The plant samples were adequately cleaned before being ground into an incredibly fine powder using a household blender. The final dried sample was stored under a vacuum for further experiments.

The hydroalcoholic extract was prepared by mixing 20 ml of 70 % ethanol and 2 g of powdered plant samples. Following proper mixing, plant samples were incubated for one week at room temperature for extraction. The samples were thoroughly stirred and centrifuged at 4000 rpm for 15 minutes. The supernatant was filtered with Whatman No.1 filter paper, transferred into pre-weighed glass containers, and then dried under laminar flow for further analysis.

**UV-VIS Spectrum Analysis**

The phytochemicals screening of different plant extracts (*P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa*) was conducted using UV-VIS spectrum analysis. 1 g of extract was centrifuged at 3000 rpm for 10 mins and filtered through filter paper. The samples were diluted to 1:10 with the same solvent (70 % ethanol). An aliquot of the diluted sample was scanned using UV-Visible Spectrophotometer at a range of 200-800 nm wavelength (Shimadzu UV-1800, Kyoto, Japan) and the characteristic peaks of each extract were recorded.

**Total Antioxidant Capacity**

The total antioxidant capacity of selected plant extracts (*P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa*) was evaluated by the phosphomolybdate method. The test samples (0.3 ml) was mixed with 3 ml of the reagent solution (0.6 M Sulfuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate). The reaction mixture tubes were incubated at 95 ºC for 90 min. The absorbance of the solution was measured at 695 nm using a UV-VIS spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) against the blank. The total antioxidant activity was expressed as mg Ascorbic acid gram equivalents [21].

**DPPH Radical-Scavenging Activity**

An antioxidant capacity to scavenge free radicals is routinely evaluated using the DPPH test. In the current study, different concentrations of plant extracts (*P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa*) were analyzed. About 10 ml of (0.1 mM) DPPH was prepared in ethanol and 1 ml of DPPH solution dissolved in different extracts of ethanol at various concentrations (0, 25, 50, 75, 100, 500, 750, 1000 µg/ml) was mixed, and the reaction mixture was incubated in the dark for 30 mins at room temperature and the absorbance was recorded at 517 nm. The reaction mixture without extract served as control and L-ascorbic acid was used as an antioxidant standard. The IC 50 value of the sample was calculated based on the absorbance.

The percentage DPPH, the radical scavenging activity of each extract, was done using the formula:

\[
\% \text{ DPPH radical scavenging activity} = \left( \frac{T_0 - T}{T_0} \right) \times 100
\]

where T0 is the absorbance of the control and T is the absorbance of the test sample.

**Nitric Oxide Radical Scavenging Assay**

The nitric oxide scavenging activity of the selected plant extracts (*P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa*) was measured at different concentrations (50, 100, 200, 400, 800, 1000 µg/mL). 100 µl (10 mM) Sodium nitroprusside was prepared in saline phosphate buffer and was added to 100 µl of each extract. Then 1 ml of Griess reagent (prepared by mixing equal volumes of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in water) was added to reaction mixtures, incubated for 3 h.

The absorbance of solutions was measured at 540 nm against the corresponding blank solutions using the following formula:
Nitric oxide radical scavenging = A (blank) – A (sample) × 100 / A (blank)

Where, A (blank) = absorbance of control sample and A sample= absorbance in the presence of the samples or standards.

**Statistical Analysis**
All experiments were performed in triplicate (n=3) and results were expressed as mean±SEM. A one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison t-test was used to calculate statistical difference. Statistical significance was considered at *p< 0.05.

**Results & Discussion**

**Characterization of hydroalcoholic Plant Extract using UV-VIS Spectrophotometer**
UV-VIS spectroscopic is a simple, cost-effective, and rapid test for screening phytochemicals in plant-based extracts. UV-VIS spectrophotometry was used to detect the presence of phytochemicals by identifying compounds containing π-bonds, lone pairs of electrons, σ-bonds, aromatic rings, and chromophores in the UV-VIS region on the electromagnetic spectrum ranging from 200 to 700 nm indicating the presence of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, carotenoids, and chlorophyll. Secondary metabolites exhibit a broad spectrum of pharmacological properties, including wound healing, analgesic, anti-inflammatory, antioxidant, and anti-microbial, which numerous researchers have described. The hydroalcoholic plant extracts of ten different medicinal plants (*P. embelica*, *M. piperita*, *O. tenuiflorum*, *A. indica*, *S. aromaticum*, *D. sissoo*, *P. sativum*, *P. guajava*, *S. cumini* and *A. cepa*) were scanned using UV-VIS spectrophotometer in the wavelength ranges from 200 – 800 nm and the characteristic peaks were recorded (Fig. 1). Distinct peaks at 328 and 380 nm were observed in *P. embelica* spectrum in the absorption range of 1-2 nm. The *M. piperita* spectra profile showed the peaks at 330, 388, 576, and 638 nm in the absorption range of 3-4 nm. Three distinct peaks at 334, 606, and 658 nm were observed in *O. tenuiflorum* spectra profile in the absorption range of 3.0-4.0 nm. The peaks at 312, 334, 388, 506, 538, and 656 nm were visible in the *A. indica* profile in the absorption range of 2.5-3 nm. The *S. aromaticum* spectra profile showed the peaks at 328 and 380 nm in the absorption range of 3-4 nm. The peaks at 328 and 380 nm were noted in the *D. sissoo* spectrum in the absorption range of 1.5-2 nm. The number of peaks obtained from *A. sativum* spectrum was recorded at 380 and 394 nm in the absorption range of 2.5-3 nm. Distinct peaks at 328 and 664 nm were observed in *P. guajava* spectra profile in the absorption range of 1.5-2.0 nm and 0-0.5 nm, respectively. *S. cumini* spectra profile showed the peaks at 328 and 664 nm in the absorption range of 1-2 nm. Similarly, two peaks were recorded in the same absorption range at 328 and 338 nm in the spectra of *A. cepa*.

![Fig. 1: The UV-VIS absorption spectra of ten selected medicinal plants in the wavelength (W/L) ranges from 200-800nm; *P. embelica* (Pe), *M. piperita* (Mp), *O. tenuiflorum* (Ot), *A. indica* (Ai), *S. aromaticum* (Sa), *D. sissoo* (Ds), *A. sativum* (As), *P. guajava* (Pg), *S. cumini* (Sc) and, *A. cepa* (Ac).]
Total Antioxidant Capacity

The total antioxidant capacity estimates both water-soluble and fat-soluble antioxidants and involves reduction of phosphomolybdate by the extracts following formation of phosphomolybdenum complex. In the present study, the antioxidant capacity of hydroalcoholic extracts isolated from ten selected medicinal plants (P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa) was measured spectrophotometrically at 695 nm and expressed as microgram equivalents of Ascorbic Acid per gram of extract (µgAAE/g). In the present study, the highest antioxidant capacity was observed in M. piperita (202.56 ± 1.98 µgAAE/g) followed by S. aromaticum (201.16 ± 1.78 µgAAE/g), O. tenuiflorum (199.42 ± 0.51 µgAAE/g), A. indica (197.16 ± 1.28 µgAAE/g), D. sissoo (175.23 ± 1.53 µgAAE/g), P. embelica (168.62 ± 0.95 µgAAE/g), S. cumini (165.33 ± 1.91 µgAAE/g), A. sativum (162.01 ± 2.01 µgAAE/g), A. cepa (158.56 ± 0.88 µgAAE/g) and, P. guajava (135.36 ± 1.77 µgAAE/g).

Antioxidant Analysis using DPPH Radical scavenging assay

DPPH free radical method has broadly been employed to evaluate the free radical scavenging activity of the natural antioxidants. In the present study, the antioxidant potential of hydroalcoholic extracts of P. embelica, M. piperita, O. tenuiflorum, A. indica, S. aromaticum, D. sissoo, A. sativum, P. guajava, S. cumini and A. cepa was evaluated at varied concentrations ranges from 0 - 1000 µg/mL using the DPPH free radical scavenging assay. The percent of DPPH radical scavenging activity at highest tested concentration (1000 µg/mL) was found 77 % in P. embelica with IC50 value of 570 ± 1.13 µg /mL, 94 % in M. piperita with IC50 value of 561 ± 1.13 µg /mL, 89 % in O. tenuiflorum with IC50 value of 420 ± 0.69 µg /mL, 89 % in A. indica with IC50 value of 502 ± 1.11 µg /mL, 81 % in S. aromaticum with IC50 value of 480 ± 1.61 µg /mL, 89 % in D. sissoo with IC50 value of 410 ± 0.62 µg / mL, 88 % in A. sativum with IC50 value of 500 ± 1.60 µg /mL, 67 % in P. guajava with IC50 value of 680 ± 1.26 µg /mL, 77 % in S. cumini with IC50 value of 510 ± 1.23 µg /mL, 85 % in A. cepa with IC50 value of 540 ± 1.16 µg /mL. The free radical scavenging activity of different extracts was in the following order based on maximum tested concentration; M. piperita > A. indica > O. tenuiflorum > D. sissoo > A. sativum > A. cepa > S. aromaticum > S. cumini > P. embelica > P. guajava. The highest IC50 value was demonstrated by O. tenuiflorum and lowest by P. guajava. The antioxidant capacity of the extract was compared with ascorbic acid (IC50 value: 62.12 ± 0.63 µg /mL) as the standard antioxidant (Fig. 2). The free radical scavenging potential observed in these medicinal plant samples was because of the presence of some natural source such as phenol, flavonoid, or tannin contents. The highest antioxidant capacity in M. piperita might be attributed to the high phenolic content of the plant.

![Fig. 2: The DPPH scavenging effect of hydroalcoholic extracts of ten selected medicinal plants; P. embelica (Pe), M. piperita (Mp), O. tenuiflorum (Ot), A. indica (Ai), S. aromaticum (Sa), D. sissoo (Ds), A. sativum (As), P. guajava (Pg), S. cumini (Sc) and, A. cepa (Ac). All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3) for all tested concentrations.](image-url)
Nitric Oxide Radical Scavenging Assay

The interaction of nitric oxide (NO) with oxygen and other free radicals, such as superoxide, results in nitric oxide, which is categorized as a free radical based on unpaired electrons. Nitric oxide radicals can cause tissue damage when produced excessively or exposed to them over an extended period. In order to treat chronic inflammatory illnesses, researchers have focused more on identifying natural antioxidants that may act as potent NO production inhibitors. Nitric radical scavenging assay was carried out using hydroalcoholic extracts of ten medicinal plants; *P. embelica*, *M. piperita*, *O. tenuiflorum*, *A. indica*, *S. aromaticum*, *D. sissoo*, *A. sativum*, *P. guajava*, *S. cumini* and *A. cepa* at different concentrations (50, 100, 200, 400, 800, 1000 μg/mL). The increase in antioxidant activity was observed with increase in concentration of the extracts and the maximum free radical scavenging activity at highest tested concentration was found in *O. tenuiflorum* (89 % inhibition) followed by *S. aromaticum* (81 % inhibition), *M. piperita* (77 % inhibition), *A. indica* (75 % inhibition), *D. sissoo* (71 % inhibition), *S. cumini* (68 % inhibition), *P. guajava* (67 % inhibition), *A. sativum* (61 % inhibition), *A. cepa* (59 % inhibition), and *P. embelica* (59 % inhibition). The Percentage of free radical scavenging activity was plotted against the concentration of the extracts as shown in Fig. 3.

![Fig. 3: The Nitric Oxide Radical Scavenging activity of hydroalcoholic extracts of ten selected medicinal plants; *P. embelica* (Pe), *M. piperita* (Mp), *O. tenuiflorum* (Ot), *A. indica* (Ai), *S. aromaticum* (Sa), *D. sissoo* (Ds), *A. sativum* (As), *P. guajava* (Pg), *S. cumini* (Sc) and, *A. cepa* (Ac). All experiments were performed in triplicate. Data are expressed as mean ± SD (n = 3) for all tested concentrations.](image)

Conclusion

Medicinal plants are a promising source of potent antioxidants and anti-inflammatory agents that may effectively cure several human ailments. Antioxidants help to neutralize free radicals, the primary source of inflammatory disorders. The current study determined the antioxidant potential of different medicinal plants by using different assays, such as DPPH Radical-Scavenging and Nitric Oxide Radical Scavenging Assay. The findings from the present investigation indicated the antioxidant potential of hydroalcoholic extracts of ten medicinal plants. Many inflammatory diseases and disorders can be prevented or treated with the help of medicinal herbs. However, further study is required to identify, characterize and evaluate the bioactive components responsible for therapeutic benefits. Moreover, herbal combinations can also be assessed for potential use in pharmaceutical product development and future therapeutic breakthroughs.

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Conflict of Interest
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References


27. Sivapalan S, Dharmalingam S, Venkatesan V, Angappan M, Ashokkumar V. Phytochemical analysis, anti-inflammatory, antioxidant activity