



Comparative Study Of Antioxidant Activity Of Few Plants From Seraj Valley (H.P) India

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Abstract:

Background Medicinal plants are well-documented for their role as sources of natural antioxidants, comprising a diverse array of bioactive compounds with distinct physicochemical properties. Reactive oxygen species (ROS) are known to contribute to the pathogenesis of several chronic conditions, including cancer and diabetes. Antioxidants mitigate the damaging effects of these reactive molecules, thereby offering protection at the cellular level. Plant-derived phytochemicals with antioxidant potential also exhibit neuroprotective, anti-aging, and anti-inflammatory activities.

Methods In this study, four plant species—*Pteridium aquilinum*, *Vitex negundo*, *Urtica dioica*, and *Bidens pilosa*—belonging to the families Dennstaedtiaceae, Lamiaceae, Urticaceae, and Asteraceae, respectively, were selected based on their ethnopharmacological relevance. The antioxidant activity of their methanolic extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a standard in vitro method for measuring free radical neutralization. Discoloration of the DPPH solution was used as an indicator of scavenging efficiency.

Results The IC₅₀ values for the methanolic extracts of *Pteridium aquilinum*, *Vitex negundo*, *Urtica dioica*, and *Bidens pilosa* were found to be 202.11 µg/mL (R² = 0.9985), 174.91 µg/mL (R² = 0.9889), 201.05 µg/mL (R² = 0.9983), and 161.77 µg/mL (R² = 0.9947), respectively. Ascorbic acid, used as a reference antioxidant, exhibited an IC₅₀ value of 47.51 µg/mL (R² = 0.9783). Among the tested samples, *Bidens pilosa* demonstrated the most potent antioxidant activity.

Conclusion The findings indicate that the methanolic extracts of these four plant species possess considerable antioxidant activity, with *Bidens pilosa* emerging as the most effective. These species may serve as viable sources of natural antioxidants for potential therapeutic and industrial applications.

Index Terms - DPPH-RSA, antioxidant activity, flavonoids, phenolic acids, medicinal plants

I. INTRODUCTION

Naturally derived compounds from higher plants have long been recognized for their antioxidant properties. In recent years, there has been growing interest in the role of oxygen-derived free radicals in biological systems, especially due to their contribution to the development of chronic health conditions. As a result, considerable attention is now being directed toward understanding the protective roles that naturally occurring antioxidants play within living organisms. As part of our ongoing efforts to discover effective antioxidants from natural sources, we examined the extracts of four plant species, each from a distinct botanical family, to evaluate their ability to neutralize free radicals. Medicinal plants serve as crucial natural reservoirs of antioxidants, which help mitigate oxidative stress and lower the risk of various diseases. These beneficial effects are largely attributed to bioactive compounds such as flavonoids, phenolics, alkaloids, tannins, and vitamins. These

compounds combat free radicals—such as reactive oxygen species (ROS) and reactive nitrogen species (RNS)—by neutralizing them and thereby minimizing cellular damage [1].

Phytochemicals also exhibit antioxidant activity through several other mechanisms, including metal ion chelation (binding metals like iron and copper to inhibit oxidative reactions) [2], modulation of antioxidant enzyme activity (enhancing enzymes such as superoxide dismutase, glutathione peroxidase, and catalase) [3], and prevention of lipid peroxidation (protecting cell membranes from oxidative degradation) [4]. Numerous medicinal plants, including turmeric (rich in curcumin) [5], amla (high in vitamin C) [6], garlic (containing sulfur-based antioxidants) [7], ginger (with gingerol) [8], and tulsi or holy basil (with adaptogenic and antioxidant properties) [9], have demonstrated potent antioxidant effects through these mechanisms.

Pteridium aquilinum (Figure 1), commonly referred to as bracken fern, is a widely distributed fern species found in varied ecosystems, from temperate to tropical climates. It thrives under different environmental conditions due to its high adaptability. Traditionally, this plant has been employed in folk remedies for its potential therapeutic benefits, including anti-inflammatory, antimicrobial, and antioxidant activities. Its phytochemical profile includes a variety of bioactive constituents such as flavonoids, alkaloids, phenolic acids, terpenoids, sterols, and saponins. [10].



Figure 1: *Pteridium aquilinum*

Vitex negundo (Figure 2), known as "Nirgundi" in Indian traditional medicine, is a tall, aromatic shrub that belongs to the Lamiaceae family. Native to parts of Asia, it flourishes in tropical and subtropical environments. This plant is widely used in Ayurveda and other traditional practices to manage conditions such as fevers, joint pain, headaches, and skin disorders. Additionally, it has been used in remedies for insect bites and wounds [11]. The chemical makeup of *Vitex negundo* includes terpenoids, alkaloids, lignans, and steroids, contributing to its medicinal efficacy [12].



Figure 2: *Vitex negundo*

Urtica dioica (Figure 3), more commonly known as stinging nettle, is a herbaceous perennial found across Europe, Asia, North Africa, and North America. Its fine hairs can cause a stinging sensation upon contact, yet it remains highly valued for both nutritional and medicinal purposes. Young leaves are edible when cooked and are rich in vitamins and minerals. Medicinally, the plant has been utilized to address ailments such as joint inflammation, iron deficiency, and seasonal allergies. It is rich in various phytochemicals, including flavonoids, phenolic acids, essential oils, alkaloids, fatty acids, and plant sterols [13].



Figure 3: *Urtica dioica*

Bidens pilosa (Figure 4), commonly referred to as blackjack or Spanish needle, is a branched annual or perennial herb from the Asteraceae family. Typically reaching up to a meter in height, it is recognized for its slender appearance and widespread presence in tropical and subtropical areas. In traditional medicine, it is used for treating inflammation, infections, and wounds. The plant's phytochemical constituents include flavonoids, glycosides, phenolic acids, sterols, saponins, tannins, and terpenoids, which together contribute to its diverse pharmacological properties[14].

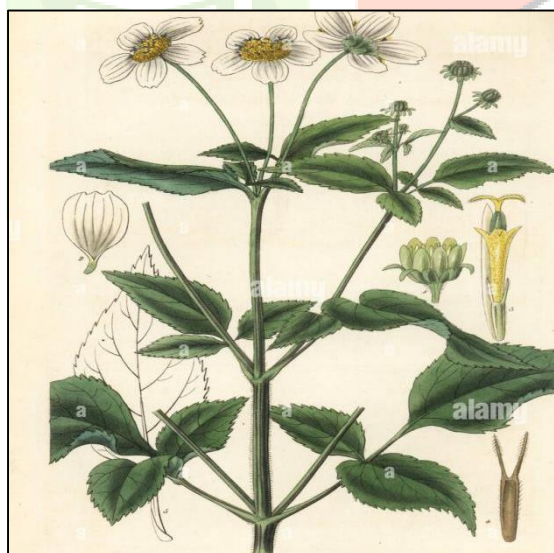


Figure 4: *Bidens pilosa*

Antioxidants are essential in counteracting oxidative stress induced by free radicals, which are implicated in the etiology of numerous chronic diseases, including malignancies, cardiovascular dysfunctions, and neurodegenerative disorders. The present investigation seeks to elucidate the antioxidant efficacy of *Pteridium aquilinum*, *Vitex negundo*, *Urtica dioica* and *Biens pilosa* by conducting a phytochemical assessment and quantifying its free radical scavenging activity via in vitro methods.

II. MATERIAL AND METHODS

Plant Material

Specimens of all four plant species were harvested from distinct ecological zones within the Seraj Valley of Mandi District, Himachal Pradesh, India.

Chemicals and reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl) was procured from Sigma-Aldrich. All solvents and reagents used were of analytical grade and supplied by Merck. UV-Visible absorbance measurements were carried out using a Shimadzu UV-2200 spectrophotometer.

DPPH reagent preparation

DPPH solution was prepared by mixing 4 mg of DPPH powder with 100 ml of methanol to make 0.1 mM concentration.

Extraction and sample preparation

Aerial parts (leaves) of *Pteridium aquilinum* and *Vitex negundo*, along with whole plant material from *Urtica dioica* and *Bidens pilosa*, were air-dried, pulverized, and subjected to methanolic extraction via Soxhlet apparatus. Extracts were then concentrated. 10 mg of extract was accurately weighed and dissolved in 10 ml of methanol to produce the 1000 µg/mL stock solution. Sample solution was prepared with concentration variations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL from stock solution.

Antioxidant activity

The antioxidant activity of the extracts was evaluated by their ability to reduce DPPH radicals. A decrease in absorbance at 517 nm indicated scavenging activity. Ascorbic acid was employed as a positive control. The percentage inhibition of DPPH radicals was calculated using the formula:

$$\text{Inhibition (\%)} = (A_0 - A_1 / A_0) \times 100$$

Where; A_0 is the absorbance of control and A_1 is the absorbance of test [15].

III. RESULT AND DISCUSSION

DPPH Radical Scavenging Activity

The antioxidant capacities of the four plant extracts were quantitatively assessed using the DPPH radical scavenging assay, employing concentrations ranging from 50 to 250 µg/mL for plant extracts and 5 to 60 µg/mL for the standard (ascorbic acid). This method evaluates the extract's ability to donate hydrogen atoms to the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl), resulting in a measurable decrease in absorbance at 517 nm due to the formation of the reduced compound, 1,1-diphenyl-2-picrylhydrazine.

The IC_{50} values were determined for each extract which are indicative of the concentration required to achieve 50% radical inhibition. The methanolic extracts of *Pteridium aquilinum*, *Vitex negundo*, *Urtica dioica*, and *Bidens pilosa* exhibited IC_{50} values of 202.11 µg/mL ($R^2 = 0.9985$), 174.91 µg/mL ($R^2 = 0.9889$), 201.05 µg/mL ($R^2 = 0.9983$), and 161.77 µg/mL ($R^2 = 0.9947$), respectively (Figure 5-8). In comparison, ascorbic acid, used as the reference compound, demonstrated a significantly lower IC_{50} value of 47.51 µg/mL ($R^2 = 0.9783$) (Figure 9), indicating a superior radical scavenging potential.

Among the studied extracts, *Bidens pilosa* demonstrated the highest antioxidant activity, as evidenced by its lowest IC_{50} value. The relatively potent free radical neutralization observed in *Bidens pilosa* can be attributed to its rich phytochemical content, particularly flavonoids and phenolic compounds. In contrast, the remaining three extracts showed moderate activity. These findings corroborate existing literature and further substantiate the therapeutic potential of these species as natural sources of antioxidants.

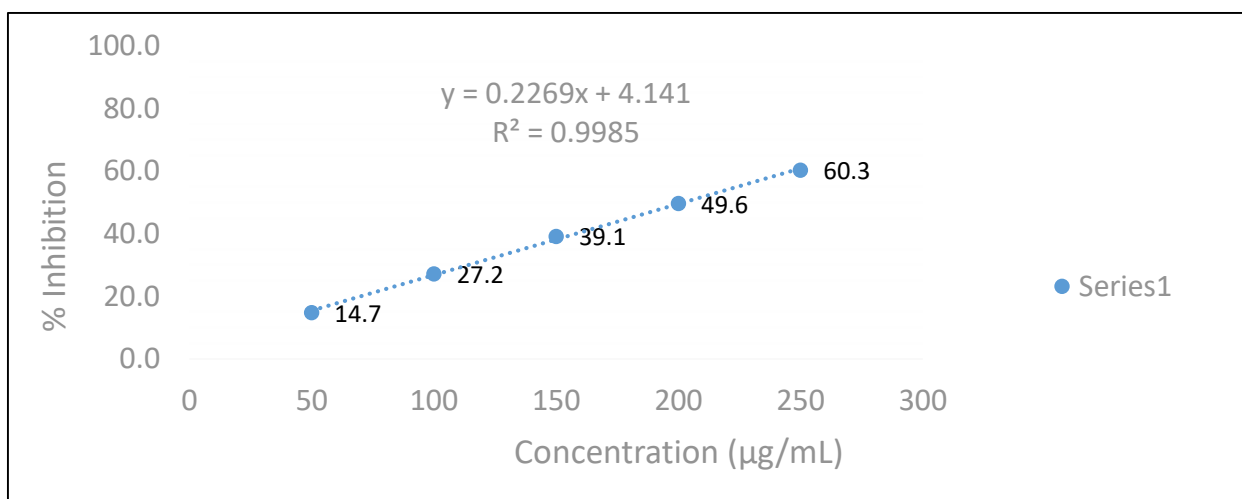


Figure 5: DPPH Radical Scavenging assay *Pteridium aquilinum*.

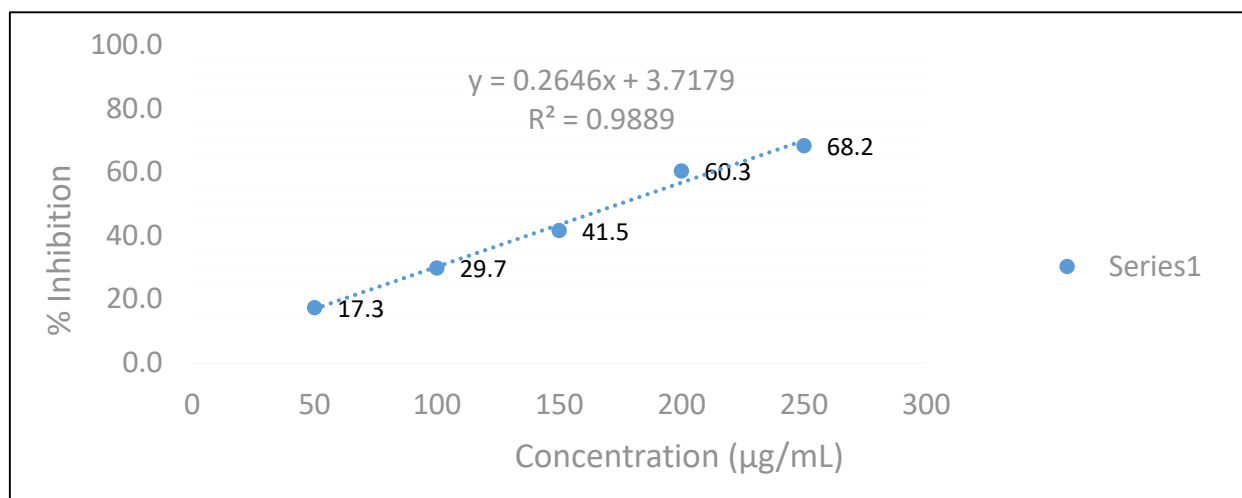


Figure 6: DPPH Radical Scavenging assay *Vitex negundo*.

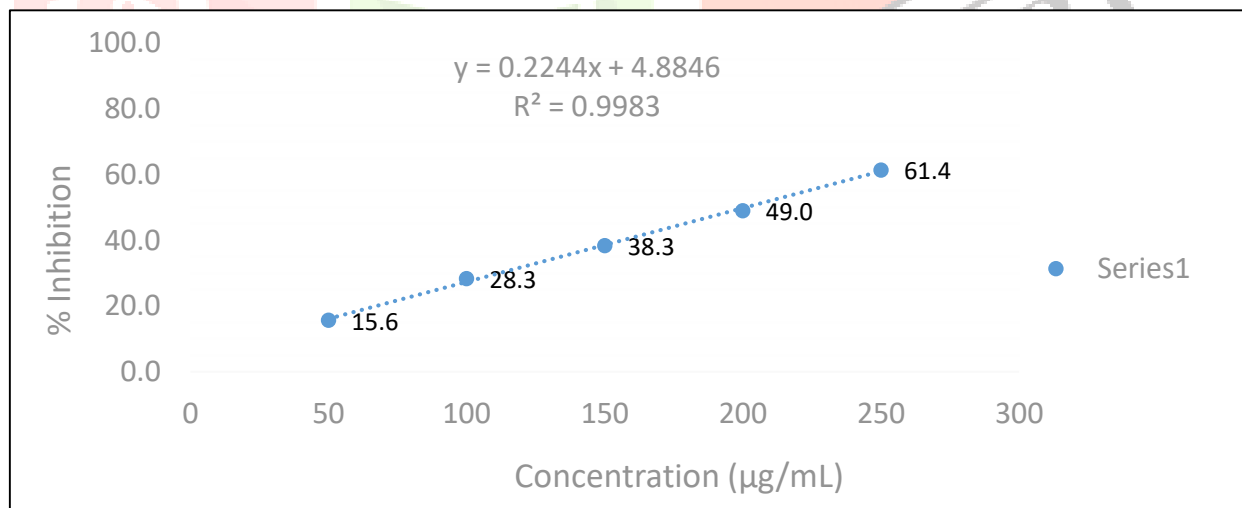


Figure 7: DPPH Radical Scavenging assay *Urtica dioica*.

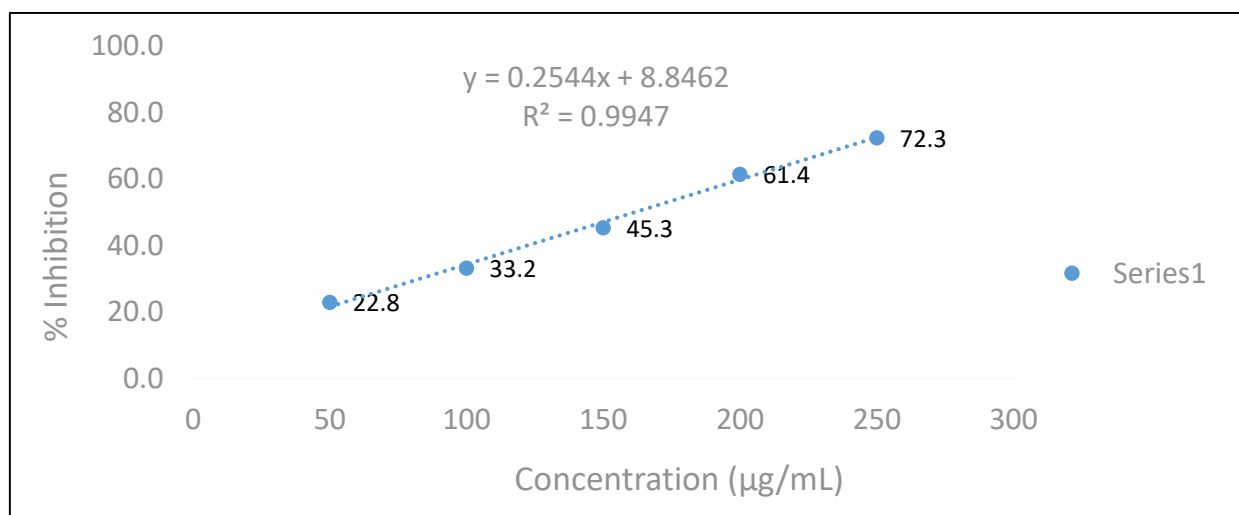


Figure 8: DPPH Radical Scavenging assay *Bidens pilosa*.

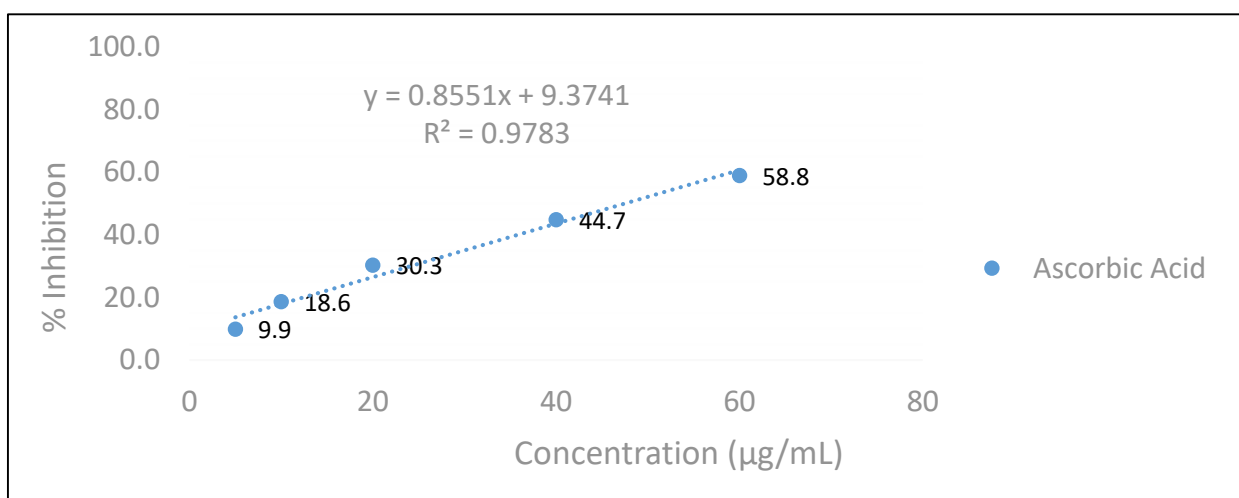


Figure 9: DPPH Radical Scavenging assay Ascorbic acid.

IV. CONCLUSION

The current study highlights the antioxidant efficacy of methanolic extracts derived from *Pteridium aquilinum*, *Vitex negundo*, *Urtica dioica*, and *Bidens pilosa*. Among these, *Bidens pilosa* exhibited the most prominent free radical scavenging activity, as evidenced by its comparatively low IC₅₀ value. These findings suggest that the aforementioned plant species, particularly *B. pilosa*, could serve as accessible and potent natural source of antioxidant compounds. Further investigations, including in vivo and clinical studies, are warranted to fully elucidate their therapeutic potential and facilitate integration into pharmaceutical, nutraceutical, and cosmetic formulations.

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