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DPPH Radical Scavenging of Crude Extracts and Their Polar and Non-Polar Fractions of *Berberis Vulgaris L*

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Abstract

The present study the antioxidant potential of crude methanolic extract and solvent-partitioned fractions of *Berberis vulgaris* L. using the DPPH free radical scavenging assay was assessed. The plant material was extracted with methanol followed by fractionation into *n*-hexane, chloroform, ethyl acetate, butanol and remaining methanol fractions to determine how polarity influences antioxidant capacity. Results showed that a clear concentration-dependent response across all fractions. The butanol fraction showed the strongest antioxidant activity (88.15±1.87% at 100 µg/mL), followed by the methanol fraction (83.00±1.33%) and ethyl acetate fraction (67.00±1.55%). The non-polar n-hexane fraction showed the lowest activity (39.45±1.09%). The standard drug showed consistently higher radical inhibition across all concentrations, validating the assay. This study indicates that *B. vulgaris* contains potent antioxidant constituents predominantly present in its polar fractions. These findings support the traditional medicinal applications of the plant and highlight its potential value for pharmaceutical and nutraceutical development.

KEYWORDS

Berberis vulgaris, Antioxidant activity, DPPH assay, Solvent fractions, Phytochemicals, Free radical scavenging

1.0 INTRODUCTION

Reactive oxygen species (ROS) and free radicals play a critical role in oxidative stress, which is strongly associated with aging and numerous chronic diseases, including cancer, cardiovascular disorders, diabetes, neurological degeneration, and inflammatory conditions[1-3]. While synthetic antioxidants such as BHA and BHT are widely used in food and pharmaceutical industries, concerns over toxicity and adverse biological effects have led to an increased demand for natural antioxidants derived from plants[4-6].

Medicinal plants have also continued to be one of the most rich sources of natural bioactive molecules[7-8]. The well-known ones include the Berberis vulgaris L. (barberry) which has been used in a wide range of therapeutic uses at the traditional and folk medicine

levels[9-10]. Other parts of the plant have been utilized to treat digestive problems, liver problems, fever, infections and inflammation[11-12]. The plant contains a wide spectrum of phytochemicals including isoquinoline alkaloids (notably berberine), flavonoids, tannins, phenolic acids, vitamins, and other secondary metabolites, many of which exhibit biological activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and cardioprotective effects[10, 13].

Since polarity affects the solubility and extraction efficiency of phytochemicals, analyzing solvent fractions provides insight into the distribution of antioxidant constituents within the plant [14-15]. The DPPH free radical scavenging assay is a widely accepted method to evaluate antioxidant capability due to its simplicity, reproducibility, and sensitivity[16].The objective of this study is to determine the DPPH radical scavenging potential of crude extract and different polarity-based fractions of B. vulgaris, enabling the identification of fractions rich in antioxidant molecules.

2.0 MATERIALS AND METHODS

Fresh plant material of *Berberis vulgaris* was collected fromKyberPakhtunkhwa Pakistan. The plant was identified by the plant taxonomist at the University of Swabi. The collected material was washed, shade-dried at room temperature, and ground into fine powder using a laboratory grinder.

2.1 Extraction and Fractionation

Approximately 200 grams of plant powder was subjected to methanolic maceration for several days with occasional stirring. The extract was filtered using Whatman filter paper and concentrated under reduced pressure. The concentrated crude extract was further fractionated sequentially with solvents of increasing polarity: n-hexane, chloroform, ethyl acetate, and butanol. The remaining portion was considered the methanol fraction. All extracts were stored at 4°C until analysis [17].

2.2 DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the DPPH radical scavenging assay. Solutions of each extract and fraction were prepared at concentrations ranging from 10–100 µg/mL. After incubation in the dark, the reduction in absorbance was measured at 517 nm. Radical scavenging percentage (%) was calculated relative to the standard antioxidant [18].

2.3 Statistical Analysis

All tests were performed in triplicate and values were represented as mean ± standard deviation. Results were interpreted based on concentration-dependent activity patterns.

3.0 RESULTS

The antioxidant activity of crude extract and solvent fractions showed notable variation depending on solvent polarity and concentration. All tested samples demonstrated a concentration-dependent increase in DPPH radical scavenging activity. At the highest tested concentration (100 µg/mL), the butanol fraction exhibited the strongest inhibition (88.15±1.87%), followed by the methanol extract (83.00±1.33%) and the ethyl acetate fraction (67.00±1.55%). The chloroform extract showed moderate activity (60.88±1.56%), while the n-hexane extract displayed the lowest scavenging effect (39.45±1.09%). At all concentrations, the standard antioxidant demonstrated significantly higher activity compared to plant extracts. The antioxidant activities are tabulated in Table 1.

Table 1. Antioxidant activity of crude extracts and various fractions of *Berberis vulgaris*

Concentration	n-hexane	Chloroform	Ethyl Acetate	Methanol	Butanol	Standard
10 µg/mL	13.65±1.87	30.66±1.88	36.98±2.00	45.02±1.54	47.65±1.60	92.18±0.54

20 µg/mL	19.44±1.87	35.09±1.40	44.12±1.89	54.10±1.00	55.09±1.70	94.76±0.40
40 µg/mL	26.00±1.43	40.54±1.43	48.09±1.88	60.12±1.03	65.05±1.82	95.87±0.43
60 µg/mL	32.45±1.67	45.29±1.60	55.02±1.92	73.88±1.67	78.88±1.23	92.34±0.01
80 µg/mL	38.02±1.87	53.89±1.60	60.34±1.76	78.89±1.45	84.19±1.17	93.11±0.90
100 µg/mL	39.45±1.09	60.88±1.56	67.00±1.55	83.00±1.33	88.15±1.87	94.98±0.55

4. DISCUSSION

The current results prove that B. vulgaris has strong antioxidant compounds with diverse solubility characteristics based on solvent polarity. The most active polar butanol and methanol fraction indicated the high concentrations of flavonoids, phenolic acids, and glycosylated compounds that are considered to be the most effective to neutralize free radicals. The moderately active compounds were ethyl acetate and chloroform fractions which are compound with intermediate polarity. The least active fraction was the non-polar n-hexane fraction which implies little availability of lipophilic antioxidants. The apparent presence of this polarity-dependent pattern can be attributed to the behavior of phytochemicals in other allied medicinal plants reported to react in similar ways to a particular concentration.The concentration dependent rise in the extracts of all extracts is indicative of the presence of active constituents that can neutralize radicals. Standard activity is high, hence validating assays and offering a comparative standard. These findings are in line with the already established literature stating a strong antioxidant property of B. vulgaris which confirms its traditional use in medicine and pharmacological value.

5.0 CONCLUSION

This study demonstrates that *Berberis vulgaris* possesses significant antioxidant potential, particularly in its polar solvent fractions. The butanol and methanol extracts exhibited the highest radical scavenging ability, confirming the presence of bioactive phytochemicals with strong antioxidative properties. These findings highlight the potential of *B. vulgaris* for the development of natural antioxidant formulations and justify further phytochemical and pharmacological investigations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

6.0 REFERENCES

1. Ozcan, A. and M. Ogun, *Biochemistry of reactive oxygen and nitrogen species.* Basic principles and clinical significance of oxidative stress, 2015. **3**: p. 37-58.
2. Rauf, A., et al., *Reactive oxygen species in biological systems: Pathways, associated diseases, and potential inhibitors—A review.* Food science & nutrition, 2024. **12**(2): p. 675-693.
3. Manful, C.F., et al., *Antioxidants and reactive oxygen species: shaping human health and disease outcomes.* International Journal of Molecular Sciences, 2025. **26**(15): p. 7520.
4. Esazadeh, K., et al., *Cytotoxic and genotoxic effects of tert-butylhydroquinone, butylated hydroxyanisole and propyl gallate as synthetic food antioxidants.* Food Science & Nutrition, 2024. **12**(10): p. 7004-7016.
5. Tauchen, J., et al., *Synthetic and semi-synthetic antioxidants in medicine and food industry: A review.* Frontiers in Pharmacology, 2025. **16**: p. 1599816.
6. Ayaz, A., et al., *Harmony in motion: unraveling the nexus of sports, plant-based nutrition, and antioxidants for peak performance.* Antioxidants, 2024. **13**(4): p. 437.
7. Chaachouay, N. and L. Zidane, *Plant-derived natural products: a source for drug discovery and development.* Drugs and Drug Candidates, 2024. **3**(1): p. 184-207.
8. Kumari, N., et al., *Medicinal and aromatic plants as potential sources of bioactives along with health-promoting activities.* Current Food Science and Technology Reports, 2024. **2**(4): p. 359-376.
9. Shakeri, F., et al., *Anti-inflammatory, antioxidant, and immunomodulatory effects of Berberis vulgaris and its*

constituent berberine, experimental and clinical, a review. Phytotherapy Research, 2024. **38**(4): p. 1882-1902.
10. Fatima Hashmi, S., et al., *Genus Berberis: A comprehensive and updated review on ethnobotanical uses, phytochemistry and pharmacological activities.* Chemistry & Biodiversity, 2024. **21**(10): p. e202400911.
11. Rezaei, M. and A. Sarkhosh, *Barberry (Berberis spp.) Domestication and Breeding*, in *Biodiversity and Genetic Improvement of Medicinal and Aromatic Plants I*. 2025, Springer. p. 23-67.
12. Sadiq, A., et al., *3-(((1 S, 3 S)-3-((R)-Hydroxy (4-(trifluoromethyl) phenyl) methyl)-4-oxocyclohexyl) methyl) pentane-2, 4-dione: Design and Synthesis of New Stereopure Multi-Target Antidiabetic Agent.* Molecules, 2022. **27**(10): p. 3265.
13. Wani, U., et al., *Phytochemical and pharmacological studies of berberis vulgaris and its active constituent-berberine: a review.* Int J Res Anal Rev, 2024. **11**: p. 36-49.
14. Ghaffar, N. and A. Perveen, *Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study*. New Zealand Journal of Botany, 2025. **63**(4): p. 627-637.
15. Hussain, F., et al., *Synthesis, in-vitro α-glucosidase inhibition, antioxidant, in-vivo antidiabetic and molecular docking studies of pyrrolidine-2, 5-dione and thiazolidine-2, 4-dione derivatives.* Bioorganic Chemistry, 2019. **91**: p. 103128.
16. Shahidi, F. and A. Samarasinghe, *How to assess antioxidant activity? Advances, limitations, and applications of in vitro, in vivo, and ex vivo approaches.* Food Production, Processing and Nutrition, 2025. **7**(1): p. 1-107.
17. Khan, A.A., et al., *Phytochemical Profiling and Dual In Vitro In Vivo Antidiabetic Assessment of Ethyl Acetate Fraction and Essential Oil From Allium ascalonicum.* Biomedical Chromatography, 2025. **39**(10): p. e70193.
18. Javed, M.A., et al., *Evaluation of pyrimidine/pyrrolidine-sertraline based hybrids as multitarget anti-Alzheimer agents: In-vitro, in-vivo, and computational studies.* Biomedicine & Pharmacotherapy, 2023. **159**: p. 114239.