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TARGETING OXIDATIVE STRESS WITH CARROT SEED EXTRACTS: A PHYTOCHEMICAL AND IN VITRO ANTIOXIDANT STUDY

Biswanath Prusty ¹, Santanu Kumar Hotta ², Jochhana Rani Bhuyan ³, Chayakanta Behera ³¹Department of Pharmaceutical Analysis, College of Pharmaceutical Sciences, Berhampur, Ganjam, Odisha, pin- 760002, India²Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Berhampur, Ganjam, Odisha, pin- 760002, India³Department of Pharmaceutics, College of Pharmaceutical Sciences, Berhampur, Ganjam, Odisha, pin- 760002, India

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Abstract

The present study aimed to investigate the in vitro antioxidant potential and phytochemical constituents of *Daucus carota* seed extracts. Various solvent fractions (petroleum ether, diethyl ether, chloroform, ethyl acetate, methanol, and water) were analyzed for radical scavenging activity using DPPH, nitric oxide, hydrogen peroxide, and total antioxidant capacity assays. The ethyl acetate extract exhibited the most potent DPPH scavenging activity ($IC_{50} = 1.38 \pm 0.01 \mu\text{g/mL}$), while the aqueous extract showed the highest nitric oxide scavenging effect ($IC_{50} = 3.20 \pm 0.05 \mu\text{g/mL}$). Phytochemical analysis revealed the presence of alkaloids, flavonoids, phytosterols, proteins, and carbohydrates across different extracts. These findings suggest that *Daucus carota* seeds are a promising source of natural antioxidants.

Keywords: *Daucus carota*, antioxidant, phytochemical screening, IC_{50} , free radicals, DPPH, nitric oxide.

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*Corresponding Author

Biswanath Prusty

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Introduction

Carrot (*Daucus carota* L.), a prominent member of the Apiaceae family, is widely cultivated across the globe for its nutritional and therapeutic value. While its roots are primarily consumed as food and valued for their high carotenoid content—particularly α - and β -carotene, which contribute to vitamin A activity—*Daucus carota* also possesses seeds that have been historically utilized in traditional medicine for their diuretic, antimicrobial, and carminative properties [1]. In addition to carotenoids, carrots contain diverse phytoconstituents including flavonoids, polyphenols, alkaloids, glycosides, and essential oils [2].

Oxidative stress, a condition characterized by an imbalance between the generation of reactive oxygen species (ROS) and the biological antioxidant defense system, plays a

critical role in the pathophysiology of numerous chronic diseases, such as diabetes, cardiovascular disorders, cancer, and neurodegenerative diseases [3]. Natural antioxidants derived from medicinal plants are gaining attention as safer alternatives to synthetic antioxidants, which are associated with potential toxicity and adverse health effects upon prolonged use [4, 5].

Although several studies have evaluated the antioxidant potential of *Daucus carota* roots and essential oils, limited scientific data are available on the antioxidant activity and phytochemical constituents of *Daucus carota* seed extracts using different solvent systems. Most existing studies have either focused on single-solvent extractions or on in vivo models with minimal exploration of specific in vitro antioxidant mechanisms [6,7]. This represents a significant research gap, especially considering the therapeutic potential of seeds as a rich source of bioactive compounds.

Rationale of the Study

In the context of increasing interest in plant-derived antioxidants, evaluating the phytochemical content and free radical scavenging activity of *Daucus carota* seed extracts across various solvent systems is imperative. A multi-solvent extraction approach allows for the selective

isolation of different classes of phytoconstituents based on polarity, which may enhance overall antioxidant efficacy. Understanding these solvent-dependent variations in phytochemical composition and antioxidant behaviour may provide a scientific basis for the development of novel natural antioxidant agents.

Aim of the Study

The present study aims to investigate the in vitro antioxidant activity of *Daucus carota* seed extracts using DPPH, nitric oxide (NO), hydrogen peroxide (H₂O₂), and total antioxidant capacity (TAC) assays, and to perform a comprehensive phytochemical screening using standard qualitative tests. The study evaluates the effect of different solvents—petroleum ether, diethyl ether, chloroform, ethyl acetate, methanol, and water—on the extraction efficiency and antioxidant performance of the seeds.

Materials and Methods

Chemicals and Reagents

Ascorbic acid, butylated hydroxytoluene (BHT), and 2,2-diphenyl-1-picrylhydrazyl (DPPH; Chemical Code: D2342) were procured from Otto Chemie Pvt. Ltd. (Mumbai, India). All other chemicals and solvents used in the study were of analytical grade and obtained from standard commercial suppliers.

Plant Material and Authentication

The seeds of *Daucus carota* L. (Apiaceae) were purchased from Pahuja Seeds Pvt. Ltd., Azadpur, New Delhi, India. The plant material was taxonomically identified and authenticated by a qualified botanist from the College of Pharmaceutical Sciences, Odisha, where a voucher specimen was deposited for future reference.

Botanical Description of the Plant

Daucus carota is a biennial herbaceous plant characterized by a rosette of finely divided leaves and a fleshy, orange-colored taproot during its first year of growth. In the second year, the plant produces flowering stalks bearing compound umbels with white to pale violet flowers. The seeds are small, oval, ridged, and aromatic, often used for their essential oil and phytochemical content (Ismail et al., 2024). A representative image of the roots and seeds used in this study is shown in **Figure 1**.



Figure 1. Dried seeds of *Daucus carota* L. used for extraction and phytochemical studies.

Preparation of Seed Extracts

The dried seeds were coarsely powdered using a

mechanical grinder. Approximately 100 g of seed powder was subjected to successive extraction using a Soxhlet apparatus with solvents of increasing polarity: petroleum ether, diethyl ether, chloroform, ethyl acetate, and methanol. Each extraction was carried out for 6–8 hours. An aqueous extract was prepared separately by decoction—boiling 10 g of seed powder in 100 mL of distilled water for 30 minutes, followed by filtration. All extracts were concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use.

In Vitro Antioxidant Assays

DPPH Radical Scavenging Activity

The free radical scavenging activity was determined using the DPPH assay as described by McCune and Johns (2002) [8]. A methanolic solution of DPPH (0.1 mM) was mixed with different concentrations of the extracts, and the absorbance was measured at 517 nm after 30 minutes of incubation in the dark.

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was evaluated following the method reported by Fiorentino et al. (2008) [10]. Sodium nitroprusside in phosphate-buffered saline (PBS) was used to generate nitric oxide, which reacted with Griess reagent to produce a pink chromophore measured at 546 nm.

Hydrogen Peroxide (H₂O₂) Scavenging Activity

Hydrogen peroxide scavenging was determined using the method of Ruch et al. (1989) [11]. A solution of H₂O₂ (40 mM) was prepared in PBS (pH 7.4) and mixed with extract samples. The absorbance was measured at 230 nm after 10 minutes.

Total Antioxidant Capacity (TAC)

The phosphomolybdenum assay was employed to assess the total antioxidant capacity as per Prieto et al. (1999) [9]. Extracts were incubated with a reagent containing sulfuric acid, sodium phosphate, and ammonium molybdate. The resulting green phosphate/Mo(V) complex was measured at 695 nm after 90 minutes at 95 °C.

Solubility and Morphological Observations

The solubility of seed powder was qualitatively observed in various solvents. Good solubility was noted in non-polar solvents such as petroleum ether, diethyl ether, ethyl acetate, and benzene. Moderate or limited solubility was observed in methanol, water, and chloroform.



Figure 2. Solubility behaviour of *Daucus carota* seed powder in various solvents.

Morphologically, the powdered seeds appeared light brown, ovoid, and ridged under astereomicroscope, consistent with standard botanical descriptions.

Phytochemical Screening

Preliminary qualitative phytochemical analysis of all extracts was carried out using standard procedures:

Carbohydrates: Molisch's, Fehling's, Benedict's, and Iodine tests

Steroids and Triterpenoids: Liebermann–Burchard and Salkowski tests

Glycosides: Keller–Killiani, Borntrager's, Legal's, and Baljet tests

Alkaloids: Mayer's, Hager's, and Dragendorff's reagents

Flavonoids: Shinoda test and Lead acetate test

Tannins: Ferric chloride and Gelatin test

Proteins: Millon's, Xanthoproteic, Biuret, and Ninhydrin tests

Saponins: Foam test

All observations were recorded based on the colour or precipitate formation to confirm the presence or absence of phytoconstituents.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). A p-value of less than 0.05 was considered statistically significant.

Results

In Vitro Antioxidant Activity

Table 1- In Vitro Antioxidant activity of seed extract

Extra ct	DPPH ($\mu\text{g/mL}$)	Nitric Oxide ($\mu\text{g/mL}$)	TAC ($\mu\text{g/mL}$)	H ₂ O ₂ ($\mu\text{g/mL}$)
Petroleum Ether	>22.96	20.72	2.45 $\pm$ 0.59	1.41 $\pm$ 0.01
Diethyl Ether	>21.71	19.25	1.31 $\pm$ 0.08	1.94 $\pm$ 0.04
Chloroform	>22.43	15.05	1.26 $\pm$ 0.05	1.79 $\pm$ 0.02
Methanol	1.85 $\pm$ 0.05	9.56 $\pm$ 0.05	1.42 $\pm$ 0.01	—
Ethyl Acetate	1.38 $\pm$ 0.01	5.67 $\pm$ 0.01	2.07 $\pm$ 0.60	—
Water	1.57 $\pm$ 0.05	3.20 $\pm$ 0.05	1.17 $\pm$ 0.05	—
Ascorbic Acid	—	—	—	54.00 $\pm$ 1.53
BHT	—	—	—	76.00 $\pm$ 6.03

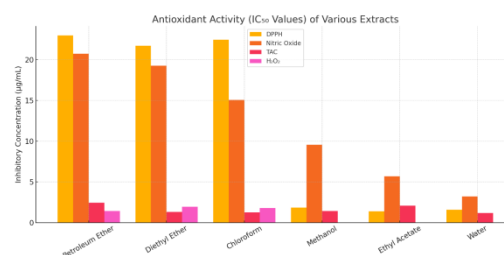


Figure 3- inhibitory concentration (IC₅₀) values for different antioxidant assays (DPPH, Nitric Oxide, TAC, and H₂O₂) across various extracts

The in vitro antioxidant evaluation of *Daucus carota* seed extracts demonstrated significant variation across different solvent systems. The ethyl acetate extract exhibited the most potent DPPH scavenging activity (IC₅₀ = 1.38 $\pm$ 0.01 $\mu\text{g/mL}$), followed closely by the aqueous (1.57 $\pm$ 0.05 $\mu\text{g/mL}$) and methanol (1.85 $\pm$ 0.05 $\mu\text{g/mL}$) extracts. In the nitric oxide scavenging assay, the aqueous extract showed the highest activity (IC₅₀ = 3.20 $\pm$ 0.05 $\mu\text{g/mL}$), followed by ethyl acetate and methanol.

For Total antioxidant capacity (TAC), the water extract again displayed the strongest effect (IC₅₀ = 1.17 $\pm$ 0.05 $\mu\text{g/mL}$), outperforming other solvent extracts. In the hydrogen peroxide (H₂O₂) scavenging assay, petroleum ether extract was the most effective (IC₅₀ = 1.41 $\pm$ 0.01 $\mu\text{g/mL}$), significantly stronger than the standard antioxidants butylated hydroxytoluene (76.00 $\pm$ 6.03 $\mu\text{g/mL}$) and ascorbic acid (54.00 $\pm$ 1.53 $\mu\text{g/mL}$).

Overall, the results suggest that both polar (water, methanol, ethyl acetate) and non-polar (petroleum ether) extracts possess strong antioxidant potential, likely due to the presence of flavonoids, alkaloids, and phenolic compounds.

Preliminary phytochemical screening of *Daucus carota* seeds extracts.

Table 2- Phytochemical Analysis of seed extract

S. No.	Phytochemical Test	Test Procedure	Observation	Inference
1	Alkaloids	Mayer's reagent	Yellow-coloured precipitate	Positive
2	Carbohydrates	Molisch's, Fehling's, Benedict's, Iodine	Strong positive reactions	Positive
3	Flavonoids	Lead acetate test	Yellow-coloured precipitate	Positive
4	Phytosterols (Steroids)	Liebermann–Burchard test	Brown ring at the junction	Positive
5	Proteins	Millon's, Xanthoproteic, Biuret, Ninhydrin tests	Color changes observed	Positive

Note: Observations are based on colorimetric and precipitation reactions confirming the presence of the corresponding class of compounds.



Figure 2. Carbohydrate analysis

Preliminary phytochemical analysis of *Daucus carota* seed extracts revealed the presence of several bioactive constituents. **Alkaloids** were detected by the formation of a yellow precipitate upon treatment with Mayer's reagent. **Carbohydrates** were strongly positive across multiple tests, including Molisch's, Fehling's, Benedict's, and iodine reactions, indicating their abundant presence in the aqueous extract.

The **flavonoid content** was confirmed by the appearance of a yellow precipitate with lead acetate. **Phytosterols (steroids)** were identified in the non-polar extracts using the Liebermann–Burchard test, as evidenced by the formation of a brown ring at the junction. In addition, the extracts tested **positive for proteins**, as indicated by results from Millon's, Xanthoproteic, Biuret, and Ninhydrin tests.

These findings confirm the presence of key phytochemical classes—**alkaloids, carbohydrates, flavonoids, steroids, and proteins**—which are known contributors to antioxidant and therapeutic activity.

Discussion

The present study demonstrates that *Daucus carota* seed extracts possess significant in vitro antioxidant potential, with variations observed across different solvent systems. Among the tested extracts, ethyl acetate, methanol, and aqueous fractions exhibited the most potent free radical scavenging activity, as reflected by their low IC₅₀ values in DPPH, nitric oxide (NO), and total antioxidant capacity (TAC) assays.

The ethyl acetate extract showed the highest DPPH radical scavenging ability (IC₅₀ = 1.38 ± 0.01 µg/mL), followed closely by aqueous and methanolic extracts. Similarly, the aqueous extract demonstrated superior nitric oxide scavenging (IC₅₀ = 3.20 ± 0.05 µg/mL) and overall antioxidant activity, indicating the presence of highly active polar phytoconstituents.

In contrast, non-polar extracts such as petroleum ether and chloroform exhibited noteworthy hydrogen peroxide scavenging activity, suggesting the presence of lipophilic antioxidants. Notably, these extracts performed better than the standard antioxidants butylated hydroxytoluene (BHT) and ascorbic acid in the H₂O₂ assay, indicating strong

oxidative defense potential.

Phytochemical screening supported these findings by confirming the presence of alkaloids, flavonoids, carbohydrates, proteins, and phytosterols. These compounds are well-documented for their antioxidant properties, particularly flavonoids and phenolic compounds, which act as radical scavengers, metal chelators, and enzyme modulators [4].

The antioxidant potential observed in this study aligns with prior reports on *Daucus carota* seeds and roots, further reinforcing the role of plant-based bioactive in oxidative stress management [6]. This study adds new insight by comparing multiple solvents, highlighting the importance of solvent polarity in phytochemical extraction efficiency and antioxidant activity.

Conclusion

This study confirms that *Daucus carota* seed extracts possess potent in vitro antioxidant activity, particularly in ethyl acetate, methanol, and aqueous extracts. These extracts effectively scavenged DPPH, nitric oxide, and hydrogen peroxide radicals and showed strong total antioxidant capacity.

The presence of phytochemicals such as flavonoids, alkaloids, and phytosterols likely contributes to the antioxidant activity. These findings support the traditional use of carrot seeds and highlight their potential as natural antioxidant agents for managing oxidative stress-related disorders.

Future Scope

Further studies are needed to isolate and characterize the active phytoconstituents, evaluate their in vivo efficacy, and explore their potential in developing natural antioxidant-based formulations for therapeutic use (Acne and vulgarise).

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Conflict of Interest

No Conflict of Interest

Informed Consent

We have participated sufficiently in the work to take public responsibility for the content. We give our full and informed consent for the manuscript to be submitted to IJIHD for consideration of publication.

Ethical Statement

Increasing the credibility and validity of research work

Author Contribution

Biswanath Prusty developed methodology, data analysis, and write-up throughout my research work

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