Research Article

In Vitro Antioxidant Potential of Zn Nanoparticle From Leaf Extract of Hypoxis Aurea Using The DPPH Radical Scavenging Assay

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Abstract:

The present study evaluated the in vitro antioxidant potential of the leaf extract of Hypoxis aurea using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

Fresh leaves of Hypoxis aurea were subjected to ethanolic Soxhlet extraction to obtain the bioactive plant extract. The prepared leaf extract was subsequently employed as a phytogenic reducing and stabilizing agent for the green synthesis of zinc nanoparticles (ZnNPs) using zinc acetate as the precursor salt. The antioxidant activity was assessed at different concentrations of the extract by measuring the reduction in DPPH radical absorbance.The percentage of DPPH radical scavenging activity increased with increasing extract concentration, indicating concentration-dependent antioxidant activity. The findings demonstrate Hypoxis aurea leaf extract (PM-1) demonstrated significant DPPH radical-scavenging efficacy which exhibited a peak inhibition of 63.46% at a concentration of 1000 µg/mL. The antioxidant efficacy of sample was inferior to that of ascorbic acid.

Therefore, H. aurea may represent a promising plant source of natural antioxidants with potential biological applications.

Keywords: DPPH (2,2-diphenyl-1-picrylhydrazyl), Inhibition, Absorbance, Control.

Introduction: Antioxidant activity is an important biological property that helps protect cells from damage caused by free radicals and reactive oxygen species (ROS). Antioxidants neutralize these harmful molecules and help maintain the balance between oxidation and the body's natural antioxidant defense system. Oxidative stress, driven by an imbalance between reactive oxygen species (ROS) production and endogenous antioxidant defenses, is a pivotal mechanism in the pathogenesis of numerous chronic diseases, including diabetes, cardiovascular disorders and neurodegenerative conditions Daniela Vilasboas-Campos etal. (2021), Musa Oyewole Salawu etal. (2023). Medicinal plants serve as significant reservoirs of natural antioxidants, such as polyphenols, flavonoids, and terpenoids, which mitigate oxidative damage through free radical scavenging mechanisms. Chuying Chen etal.(2020). Saravanan Shanmugam etal.(2019).

Hypoxis aurea is a small, hairy perennial herb, 10–30 cm tall, growing from a rounded to cylindrical tuberous rootstock (corm) about 1–2.5 cm across. Leaves are basal, linear and grass-like (10–30 cm × 2–6 mm) with scattered long hairs. Flowers are golden-yellow, star-shaped, and borne singly or in pairs on slender hairy scapes shorter than the leaves. The six perianth lobes are elliptic-lanceolate (6–12 mm). The fruit is a clavate capsule containing black, tuberculate seeds. In the Western Ghats the plant grows in lateritic grasslands and on plateaus, flowering with the south-west monsoon (June–September).Hypoxis aurea, a member of the Hypoxidaceae family, has been traditionally utilized in ethnomedicine, yet its specific in vitro antioxidant capacity remains under-characterized in contemporary pharmacological literature. The primary objective of this study was to evaluate the free radical scavenging potential of the leaf extract of Hypoxis aurea using the DPPH assay, a widely accepted method for assessing hydrogen-donating antioxidant activity BN Ramesh etal.(2017). Raghavendra H L etal.(2017).

Therefore, the present study was undertaken to synthesize Hypoxis aurea leaf extract and evaluate its antioxidant potential.

Material and method:

Synthesis of Hypoxis aurea leaf extract by ethanolic Soxhlet method:

Collect fresh leaves and allow it to remove surface contaminants, followed shade-drying to prevent the thermal degradation of heat-sensitive phytoconstituents Jenson jacob etal.(2020).. The dried material is then

mechanically pulverized into a coarse powder to increase the surface area for solvent interaction Jenson jacob etal.(2020). Now the coarse powder allows to Soxhlet ethanolic extraction. Soxhlet extraction allows for continuous cycling of the solvent through the plant material, ensuring thorough extraction of target analytes over a prolonged period, typically ranging from 6 to 24 hours depending the plant matrix density Jenson jacob etal.(2020). Małgorzata Dzięcioł etal.(2024). Once extraction finished, the solvent is evaporated under reduced pressure using a rotary evaporator at controlled temperatures (typically below 40-50°C) to obtain a concentrated crude extract, which is then stored at low temperatures prior to analysis Jenson jacob etal.(2020). Khushboo Bhardwaj etal. (2016).

Synthesis of ZnO nanoparticles using H. aurea leaf extract (HA-ZnONPs)

1. Prepare 90 mL of 0.1 M zinc acetate dihydrate in double-distilled water under magnetic stirring.
2. Add 10 mL of 1% HAEE dropwise (extract: salt = 1: 9 v/v) while stirring at 60–70 °C.
3. Adjust the pH to 12 with 2 M NaOH added dropwise. A pale-white/cream precipitate forms, indicating Zn $(OH)_2$/ZnO formation.
4. Continue stirring at 70 °C for 2 h, then age the mixture overnight at room temperature.
5. Centrifuge at 10,000 rpm for 15 min. Wash the pellet three times with distilled water and once with ethanol to remove unbound phytochemicals.
6. Dry at 80 °C in a hot-air oven, then calcine at 400 °C for 2 h in a muffle furnace. Grind to a fine powder and store in an air-tight vial.

Antioxidant assay by DPPH Method (96-Well Plate)

Principle:

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical with a deep violet colour. Antioxidants reduce DPPH, causing a colour change from violet to yellow, which can be measured spectrophotometrically at 517 nm.

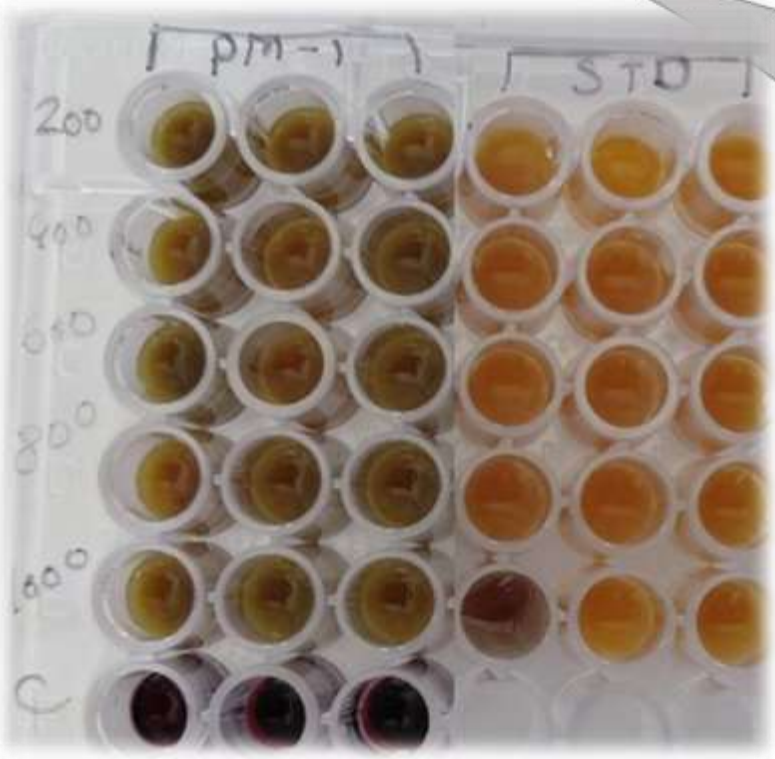
Reagent Preparation:

1. DPPH Solution (0.1 mM): Dissolve 3.94 mg of DPPH in 100 mL methanol. Store in dark bottle and use freshly.
2. Sample and Standard Preparation: Dissolve samples and standard in methanol to prepare a series of concentrations (e.g., 200, 400, 600, 800, 1000 µg/mL).

   Procedure:

   1. Add 100 µL of each test sample or standard into wells in triplicates.
   2. Add 100 µL of freshly prepared 0.1 mM DPPH solution to each well (except blank).
   3. Add 100 µL methanol to blank wells (no DPPH).
   4. Cover plate with aluminum foil and incubate in the dark for 30 minutes at room temperature.
   5. Read absorbance at 517 nm using a microplate reader.

Observation:



Antioxidant Activity of Sample by DPPH Method (96-Well Plate)
Observation table:

Sr. no	Sample code	Conc. µg/ml	OD			Mean	Percent inhibition
1	**Control**	-	1.456	1.564	1.485	1.502	
2	**Standard (Ascorbic Acid)**	200	0.369	0.345	0.355	0.356	76.27
		400	0.345	0.341	0.336	0.341	77.31
		600	0.326	0.342	0.343	0.337	77.56
		800	0.256	0.245	0.255	0.252	83.22
		1000	0.123	0.122	0.121	**0.122**	**91.88**
3	**Sample- PM-1 Hypoxis aurea**	200	0.798	0.799	0.799	0.799	46.81
		400	0.689	0.688	0.687	0.688	54.18
		600	0.675	0.677	0.678	0.677	54.94
		800	0.589	0.588	0.587	0.588	60.84
		1000	0.546	0.544	0.556	**0.549**	**63.46**

Antioxidant Activity of Sample by DPPH Method (96-Well Plate)

Result- Present study reveals, Sample-PM-1 (Hypoxis aurea) exhibited a concentration-dependent enhancement in DPPH radical scavenging efficacy, rising from 46.81% at 200 µg/mL to 63.46% at 1000 µg/mL. The inhibition percentages at concentrations of 400, 600, and 800 µg/mL were 54.18%, 54.94%, and 60.84%, correspondingly. Ascorbic acid exhibited notably elevated DPPH scavenging activity among the evaluated doses, with 91.88% inhibition at 1000 µg/mL.

Discussion – The DPPH radical scavenging experiment was conducted to assess the antioxidant capacity of SamplePM-1 (Hypoxis aurea), with ascorbic acid as the benchmark. The antioxidant efficacy was assessed through the decrease in DPPH absorbance, with an elevated percentage of inhibition signifying enhanced radical-scavenging capability. This gradual rise signifies an improved radical-scavenging capability as focus escalates.
Conclusion – Sample-PM-1 (Hypoxis aurea) demonstrated significant DPPH radical-scavenging efficacy. Sample-PM-1 exhibited a peak inhibition of 63.46% at a concentration of 1000 µg/mL. The antioxidant efficacy of Sample-PM-1 (Hypoxis aurea) was inferior to that of ascorbic acid.

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