

Comparative Analysis of *in-vitro* Nitric Oxide Reducing Antioxidant Activity of Arid Region Plants of Rajasthan

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Abstract: Arid-region plants are exposed to diverse environmental stresses that may contribute to the production of bioactive secondary metabolites with antioxidant properties. These free radicals are believed to be a source of cellular and molecular damage leading to numerous chronic ailments. The present study evaluated and compared the nitric oxide (NO) scavenging and antioxidant potential of three arid-region plants, *Leptadenia pyrotechnica*, *Lepidagathis trinervis*, and *Corchorus depressus*. Plant extracts prepared using different solvent systems were assessed for their ability to scavenge nitric oxide radicals, and antioxidant activity was comparatively evaluated based on their half-maximal inhibitory concentration (IC₅₀) values. The results demonstrated differential antioxidant activity among the investigated plant species and extracts. Among the three plants, *L. pyrotechnica* and *L. trinervis* exhibited the strongest nitric oxide scavenging activity, with the 50% ethanol extract showing the most pronounced antioxidant potential and the lowest IC₅₀ value compared with the corresponding extracts of *L. trinervis* (leaf) and *C. depressus*. The comparatively higher activity of *L. pyrotechnica* and *L. trinervis* (stem) suggests a greater abundance or efficacy of antioxidant phytoconstituents in the 50% ethanol extract. Overall, the findings indicate that these arid-region plants, particularly *L. pyrotechnica* and *L. trinervis* represent promising sources of natural antioxidant compounds and warrant further phytochemical characterization and evaluation of their potential therapeutic applications.

Keywords: Antioxidant, Nitric oxide, Plant extract, *Leptadenia pyrotechnica*, *Lepidagathis trinervis*, *Corchorus depressus*

1. Introduction

Adverse plant circumstances, such as harsh temperatures, drought, heavy metals, nutritional shortages, and high salt, produce large levels of reactive oxygen species (ROS), which can lead to oxidative stress (Chaves et al., 2020). To avoid this, cells have a complex antioxidant system with enzymatic and non-enzymatic elements. Plants include a vast variety of bioactive chemicals with strong antioxidant properties (Barua et al., 2014). Natural antioxidants derived from plant extracts are becoming increasingly popular in science due to their role in disease prevention. The majority of research on the possible effects has focused on free radical scavenging, metal chelation, anti-inflammatory, and anticancer properties (Chen et al., 2017). The antioxidant activity of all plant components, including leaves, bark, roots, flowers, fruits, and seeds, has been widely researched. Phenolic molecules are abundant in plants and have been identified as the primary contributors to their antioxidative and varied biological characteristics (Liu, 2004).

Nitric oxide (NO) is an important biomolecule of radical nature that takes part in numerous physiological and biological processes. NO is a vital bioregulatory molecule that plays crucial roles in mammalian cellular physiology such as vascular homeostasis, neurotransmission, intracellular and extracellular signalling, platelet activation, prevention of oxidative stress and cytokine activation, and wound healing acceleration (Can et al., 2022). In studies on NO, sodium nitroprusside (SNP) is generally the preferred radical source. SNP is stable in the solid state, but in aqueous solution it generates NO which interacts with oxygen to

produce nitrite ion as the stable end product. When antioxidants were present in the medium, NO was scavenged and the remaining NO was determined after converting to nitrite. In the spectrofluorometric method, NO remaining after antioxidant scavenging action caused the quenching of resorcinol fluorescence (Can et al., 2022).

Thus, "total" antioxidant activity is assessed to assess total antioxidant capacity using methods such as nitric oxide scavenging antioxidant activity. The NO test is widely used because of its excellent accuracy, sensitivity, and adaptability across testing methods, with a low vulnerability to modest changes in reaction circumstances (Silvestrini et al., 2023). Medicinal plants from arid and semi-arid regions are rich sources of bioactive compounds with potential antioxidant and therapeutic properties. *Leptadenia pyrotechnica*, *Lepidagathis trinervis*, and *Corchorus depressus* are traditionally valued medicinal plants whose phytochemical constituents may contribute to free-radical scavenging activity (Jain et al., 2026; Kumari et al., 2024; Pancholi, 2022). Therefore, evaluation of their antioxidant potential can provide valuable insights into their therapeutic significance. The current investigation aims to gain a comparative understanding of three arid plants *L. pyrotechnica*, *L. trinervis* and *C. depressus* capacity for total antioxidative power *in-vitro* using the NO activity.

2. Material and Method

1) Chemicals

Sodium nitroprusside from Merk, Sodium Phosphate Dibasic and Sodium Phosphate Monobasic from Rankem,

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Greiss reagent (sulphanilamide in 2% phosphoric acid and naphthylethylene diamine dihydrochloride), ascorbic acid, ethanol from Hi Media Laboratories

2) Plant material collection and identification

Plant *L. pyrotechnica* (green stem), *L. trinervis* (Leaf and stem) and *C. depressus* (stem) was collected from the arid region of Jodhpur city in Rajasthan, India, from 2022 to 2023 (Geographical coordinates: 26.277, 73.437). The collected plant leaves were air-dried and submitted to the Herbarium of the Department of Botany, University of Rajasthan, Jaipur (*L. pyrotechnica*: Voucher no. RUBL 211572; *L. trinervis*: Voucher no. RUBL 5724; *C. depressus*: Voucher no. RUBL 20293), where they were identified and confirmed by, Department of Botany, University of Rajasthan, Jaipur.

3) Preliminary plant extraction for screening

The plant leaves were washed with deionised water, shade-dried (15 to 25 days) (Kasiramar & K, 2019) and powdered using an electrical grinder. Soxhlet extraction was performed using 20 g of plant powder in a high-purity cellulose extraction thimble (INSIL Scientific, India of 33X100mm) using solvent 50% ethanol-aqueous, followed by air drying of the extract at room temperature and stored at 4 °C for future experimentations (Borges et al., 2020). Four extracts were prepared from plants that is leaf extract of *Leptadenia pyrotechnica*, leaf and stem extract of *Lepidagathis trinervis* and stem extract of *Corchorus depressus*

4) Nitric oxide scavenging activity

The nitric oxide scavenging activity of plant extracts was determined by method of Singh et al., (2009). In this experiment Sodium nitroprusside (5µm) was dissolved in standard phosphate buffer (0.025M, pH 7.4). 1 ml of sodium nitroprusside was added in 0.05 ml of plant extract (100 µg/ml) and this reaction mixture incubated at 25°C for 5 hrs, after 5 hrs 0.5 ml of incubated solution and 0.5 ml of Greiss reagent (Diluted equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water) were taken and shake welled then absorbance of reaction mixture was determined or recorded at 546 nm on spectrophotometer. For control same procedure was followed (without extract), for standard (50 µg/ml) ascorbic acid was used, the percent scavenging effect (Singh et al., 2008). Radical scavenging activity (RSA) was calculated for Nitric oxide as inhibition percentage using the given formula:

$$\text{RSA (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

5) Statistical analysis

The statistical significance was estimated according to one way ANOVA at $p < 0.01$, $p < 0.001$ and $p < 0.0001$ for antioxidant (GraphPad Prism version 10.6.1)

3. Result and Discussion

The Nitric oxide radical scavenging activity increases as the concentration of the extract increases in all the plant extracts. It was observed that maximum activity was shown by *L. pyrotechnica* and *L. trinervis* (stem extract) plant extract in comparison followed by *L. trinervis* (leaf extract) and lowest

by *C. depressus* (Table 1 and Fig. 1). This demonstrated activity by reacting with lipid peroxy radicals to halt chain propagation processes, resulting in non-radical species such as nitro compounds that inhibit lipid peroxidation. Nitric oxide is spontaneously produced in aqueous solutions when sodium nitroprusside interacts with oxygen (O) to form nitrite (NO), which may be measured using Griess reagent. Scavengers of NO compete with O, resulting in decreased synthesis of NO (N et al., 2009). The 50% ethanol extract of plants *L. pyrotechnica* and *L. trinervis* showed the highest activity and had an IC₅₀ value indicating a good nitric oxide scavenging potential (Barua et al., 2014)

S. No	Plant extract	IC ₅₀ for Nitric oxide (µg/mL)
1	<i>L. pyrotechnica</i> (Leaf)	85.696± 2.499
2	<i>L. trinervis</i> (Leaf)	111.055± 4.910
3	<i>L. trinervis</i> (Stem)	86.792± 0.479
4	<i>C. depressus</i> (Stem)	162.538± 2.493
5	Ascorbic acid	17.291±0.279

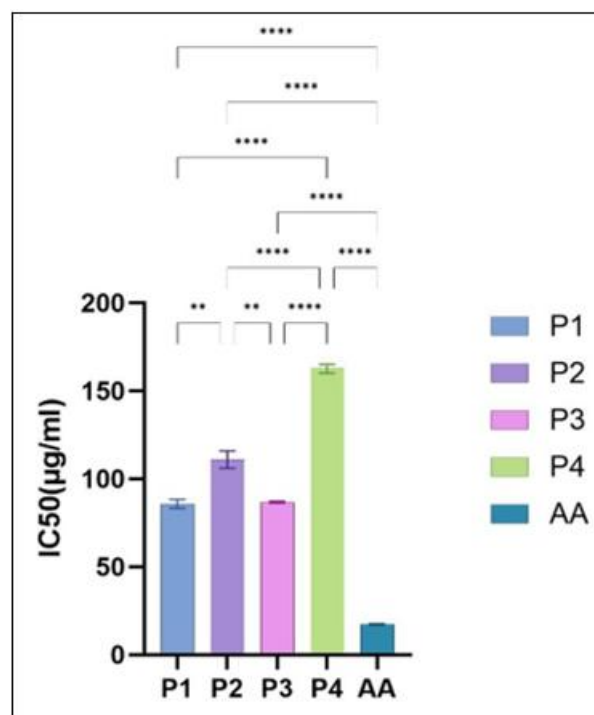


Figure 1: Comparative Antioxidant IC₅₀ graph of 50% ethanol-aqueous extracts of all the plants (P1-*L. pyrotechnica* (leaf); P2-*L. trinervis* (leaf); P3-*L. trinervis* (stem); P4-*C. depressus* (stem); AA- Ascorbic acid). Statistical comparison with one way ANOVA at ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$

4. Conclusion

The study demonstrated variable nitric oxide scavenging and antioxidant activity among the three arid-region plants. *Leptadenia pyrotechnica* and *Lepidagathis trinervis* showed the highest antioxidant potential, particularly in the 50% ethanol extract, as indicated by its comparatively lower IC₅₀ value. These findings suggest that these plants are a promising natural source of antioxidant compounds and warrants further phytochemical investigation.

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