

Isolation and characterization of caffeic acid from Cobra Lily (*Arisaema propinquum*) and evaluation of its biological activities

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Abstract: This study investigates the antioxidant and antimicrobial potential of *Arisaema propinquum*, a medicinal herb from the Pir Panjal range of the Kashmir Himalayas. Caffeic acid was isolated and characterized from methanolic extracts of its aerial parts. Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of functional groups indicative of polyphenols, flavonoids, terpenoids, esters, and other metabolites. *In vitro* antioxidant activity was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, superoxide dismutase (SOD) mimetic, and deoxyribose degradation assays. The deoxyribose assay showed dose-dependent inhibition of 63.2%, 69.7%, and 80.3% at concentrations of 100, 150, and 200 µg/mL, respectively, while the methanolic extract exhibited an IC₅₀ of 49.74 µg/mL in the H₂O₂ scavenging assay. Total phenolic and flavonoid contents were quantified as 40.33 ± 1.9 mg GAE/g DW and 32.77 ± 1.35 mg rutin equivalent/g DW, respectively. *In vivo* antioxidant activity was evaluated using *Saccharomyces cerevisiae*, where the extract supported 76.17 ± 0.58% cell survival under oxidative stress. The findings suggest that the antioxidant and antimicrobial properties of *A. propinquum* are attributable to its rich phytochemical profile, supporting its potential in therapeutic applications.

INTRODUCTION

Bioactive compounds have significant therapeutic potential to treat various metabolic disorders including oxidative stress, and pro-inflammatory conditions (Kang & Kim, 2023; Mitropoulou *et al.*, 2023). Some of the bioactive molecules found in plants include antioxidants including phenols, flavonoids, carotenoids, and glucosinolates (Favela-González *et al.*, 2020; Sharma *et al.*, 2021). Comparing the pharmacological effects of plant-derived phytochemicals is a practice

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that is becoming more and more prevalent (Rafiq *et al.*, 2022). In the aftermath of recent systematic advancements across the globe, plant medicinal capabilities have been investigated because of their superior pharmacological action, economic viability, and low toxicity (Abdallah *et al.*, 2023; Chandorkar *et al.*, 2021). As per Epidemiological reports, it has been demonstrated that consuming foods high in bioactive substances with antioxidant activity, such as phenols, flavonoids and carotenoids, has a positive effect on human health and may lower the risk of a number of diseases, including heart diseases, functional disorders pertaining to growing age, cancer, diabetes, Alzheimer's disease, cataract, bone disorders and many others (Bhambhani *et al.*, 2021; Faisal *et al.*, 2023; Olofinisan *et al.*, 2023). A group of substances called ROS is formed as a result of the metabolism of oxygen. These very reactive substances, including the hydroxyl radical (OH•), peroxide (ROO•), and superoxide radicals (O₂•), can seriously harm cells and tissues during a variety of illnesses that are connected to heart disease, cancer, and a number of other health problems (Hassan *et al.*, 2024; Kumar & Pandey, 2015). Large dosages of synthetic antioxidants used to prevent oxidation, such as butylated hydroxyl anisole (BHA), propyl gallate (PG), and butylated hydroxyl toluene (BHT), have been observed to cause internal and external bleeding in rats and guinea pigs (Shahidi & Ambigaipalan, 2018; Ugoeze & Odeku, 2025). In addition to stabilizing membranes and causing stress-induced senescence, terpenes directly detoxify ROS. Alkaloids are capable of scavenging free radicals and preventing oxidation caused by hydrogen peroxide (Khan & Chaudhary, 2024). When used as possible non-enzymatic antioxidants, phenolic substances effectively scavenge free radicals (Varesi *et al.*, 2023). Numerous *in vitro* and *in vivo* systems are looking at these phytochemicals to see whether they may help manage different metabolic and degenerative illnesses (Nisar *et al.*, 2023). A strong *in vitro* and *in vivo* connection (IVIVC) is required for each phytoconstituent pertaining to its pharmacological actions and the outcomes of its structure-activity and relations.

A. propinquum, is an unexplored medicinal and ornamental herb that is known for its massive potential for anticancer, antimicrobial, anti-helminthic, and antioxidant activity, which has been chosen to carry out a study of its chemo-profiling (Van, 2022). This herbaceous plant belongs to the family of Araceae and is one of the well-known medicinal plants among the treasure of medicinal plant wealth in the Northwestern Himalayas (Mubashir & Shah, 2012). The plant usually grows at elevations of 2400 to 3600 m, with most prominence around Pir Panjal Range and flowers from May to July. It is preferably found under shrub canopy around rocks and is exactly a resemblance to a cobra hood (Tabassum *et al.*, 2019). Therefore, the aim of this study was to identify and characterize Caffeic acid from the aerial portions of *A. propinquum* as well as evaluate antioxidant and antimicrobial properties. Also, to the best of our knowledge, no prior studies have been published on the bioactive metabolite chemo-profiling, *in vivo* and *in vitro* antioxidant properties, or isolation of caffeic acid from this particular medicinal herb from the Kashmir Himalayas.

MATERIAL and METHODS

Sample Collection and Authentication

The plant samples of *Arisaema propinquum* were collected from Pir Panjal Mountain ranges of Dobjan, Mughal Road at an altitude of 2694 m asl with geographic location of 33°40'57.56" N 74°39'33.80" E. Plant specimens of *A. propinquum* bearing Voucher No.2681-(KASH) were submitted to KASH herbarium of Centre for Biodiversity and Taxonomy (CBT), University of Kashmir, India in order to get authentication for the plant specimen.

Chemicals Required and Extract Preparation

All solvents (MeOH, CHCl₃, EtOAc, H₂O) and chemicals (L-ascorbic acid, agarose, hydroxyurea) were procured from Sigma-Aldrich, Merck, or Hi-Media Mumbai Pvt. Ltd. Fresh leaves, stems, and seeds were rinsed, shade-dried (25±2°C, 7–10 days), and powdered (0.4 mm sieve). For extraction, 50 g of powder underwent successive cold maceration (24–26°C) with

500 mL each of CHCl_3 (48 h), EtOAc (48 h), and MeOH (72 h), with intermittent agitation every 6 h. The filtrates (Whatman No. 1) were concentrated via rotary evaporation (40–50°C), and the crude extracts were further purified by silica gel column chromatography (MeOH: CHCl_3 gradient, 0–100%), with fractions monitored by TLC (silica GF₂₅₄, UV 254/366 nm). Residual solvents were verified via GC-MS, and extracts were stored at 4°C in amber vials after yield calculation (% w/w).

Phytochemical screening

Using established procedures, the crude hydroalcoholic extract of *A. propinquum* was put through preliminary qualitative phytochemical screening to determine the presence of numerous bioactive constituents, including primary and secondary metabolites (Khade *et al.*, 2023; Visweswari *et al.*, 2013).

- Alkaloids: Identified using Hager's reagent (saturated picric acid)- a yellow precipitate denotes a positive result.
- Cardiac Glycosides: Keller-Killiani test produces a blue-green coloration at the interface, indicating deoxy sugars.
- Terpenoids: Salkowski reaction yields a reddish-brown interface after addition of concentrated H_2SO_4 , confirming presence.
- Saponins: Froth test revealed persistent foam (>10–15 min) after vigorous shaking with distilled water, indicating the presence.

Estimation of total phenolic content

With certain adjustments, the Folin-Ciocalteu colorimetric technique was used to determine the total phenolic content (Sánchez-Rangel *et al.*, 2013). Leaf and stem extract, later Folin-Ciocalteu phenol reagent in an aliquot of 0.3 mL (2.25 mL) was added. 6.5% sodium carbonate (2.25 mL) was added after five minutes, and the mixture was incubated at room temperature for 90 minutes. At 725 nm, the mixture's absorbance was determined with a UV-Visible spectrophotometer (Cystronics, 19769-118). The same procedure was used to construct a standard calibration curve for gallic acid in the concentration range of 0-200 g/mL. The findings were represented as mg of gallic acid equivalent (GAE) per gram of extract.

Estimation of total flavonoid content

Using quercetin as the reference, the total flavonoid content was calculated using the aluminum colorimetric technique (Shraim *et al.*, 2021). In the range of 0-200 $\mu\text{g/mL}$, a calibration curve for quercetin was created. Briefly, 0.5 mL of each of the extract and standard was put in separate test tubes, and each was then combined with 10 percent Al_2Cl_3 , 1M $\text{CH}_3\text{CO}_2\text{K}$, 1.5mL of MeOH, and 2.8 mL of DW. The identical procedure was followed to make a blank, except that 1/2 mL of DW was used in lieu of the standard and that the Al_2Cl_3 was likewise retrained by the distilled water. Incubation was followed for 30 minutes at 25 °C. A 415 nm absorbance measurement was made using UV-Visible spectrophotometer (Cystronics, 19769-118). Per gram of extract, the amount of flavonoid was represented as mg quercetin equivalent (QE).

Fourier Transform Infrared Spectrometer

Fourier Transform Infrared (FTIR) analysis was performed using an Agilent Cary 630 spectrometer. Samples were prepared as potassium bromide (KBr) pellets by mixing ~2 mg of dry extract with 200 mg of spectroscopic-grade KBr and compressing under vacuum. Spectra were recorded in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. A background scan of pure KBr was subtracted. Spectral data were processed and plotted as % transmittance versus wavenumber using OriginPro 8.5 software, and characteristic peaks were assigned to functional groups.

Column Chromatography for Pure Compound Separation

An extract mixture (1:1 of MeOH: CHCl_3) of about 43 gms was completely dried and mixed with silica gel (mesh size of 60 to 120 mm), then poured into a long cylindrical glass tube for

separation of pure compounds. Both ends of the column were packed with non-absorbent cotton for the collection of different fractions. Solvents were passed through the column at uniform gravity rates from lower percentage of non-polar to an increasing order of polarity. Elutes were collected for each percentage in 50 mL of conical flasks for further analysis of TLC in order to check purity of collected fractions. The spectral techniques used for the pure isolated compound were done via ^1H -NMR and ^{13}C -NMR measurements. These were performed by Bruker Avance spectrometer at an operating frequency of 500 MHz using Dimethyl sulphoxide (DMSO).

Anti-Oxidant Activity

1,1-diphenyl-2-picryl-hydrazyl analysis

The usual approach was used to quantitatively test the extracts' radical scavenging abilities (Soni & Sosa, 2013). Three milliliters of plant extract were mixed with one mL of a 0.1mM solution of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) in methanol (100–300 μg /mL), where reference ascorbic acid antioxidant was used. At 517 nm, the reaction mixture's discoloration was seen after 30 minutes of incubation UV-Visible spectrophotometer (Cystronics, 19769-118).

Superoxide anion radical scavenging activity (SOD Assay)

The technique described by Stankovic (2011) was used to calculate superoxide anion scavenging activity with a few minor modifications. 100 NBT (0.1 mg), 200 μL of EDTA (12mM) and MeOH and 100 μL riboflavin solution of 20 μg were all mixed together, and final volume was raised up to 3 mL with the help of Phosphate buffer (50mM). At 590 nm, absorbance was observed using phosphate buffer as a blank after 5 minutes of illumination. Plant extracts of various concentrations were collected (100, 200 and 300 μg), raised using 150 μL of MeOH, followed by the addition of 100 μL $\text{C}_{17}\text{H}_{20}\text{N}_4\text{O}_6$, 200 μL $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$, 200 μL MeOH, and 100 μL $\text{C}_{40}\text{H}_{30}\text{C}_{12}\text{N}_{10}\text{O}_6$, at last phosphate buffer was added up to 3 mL. Absorbance was recorded at 590 nm on a UV-Visible spectrophotometer (Cystronics, 19769-118). Superoxide anion scavenging activity is boosted as the reaction mixture's absorbance decreases.

Deoxyribose assay

The hydroxyl radical scavenging activity was assessed via the deoxyribose degradation assay (Ghosh *et al.*, 2013). A 1 mL reaction mixture, containing 200 μL each of monopotassium phosphate (100 mM), 2-deoxy-D-ribose (15 mM), and ferric chloride (500 μM), plus 100 μL each of EDTA (1 mM), ascorbic acid (1 mM), hydrogen peroxide (10 mM), and the sample, was incubated at 37°C for 1 hour. To develop the chromogen, 1 mL of 1% (w/v) thiobarbituric acid and 1 mL of 2.8% (w/v) trichloroacetic acid were added. The mixture was heated at 80°C for 20 minutes to form the pink malondialdehyde-thiobarbituric acid (MDA-TBA) adduct. After cooling, absorbance was measured at 532 nm using a UV-Visible spectrophotometer (Cystronics; 19769-118).

H₂O₂ radical Assay

The standard process is outlined by Gülçin *et al.* (2005). The phosphate buffer solution included 40mM hydrogen peroxide (pH 7.4). The standard $\text{C}_6\text{H}_8\text{O}_6$, along with prepared extracts, was dissolved in distilled water before 1mL of each substance was added to a 0.6 mL solution of 40 mM H_2O_2 . The ascorbic acid concentrations used were 25, 50, 100, 125 g/mL for the test extract. 30 minutes of room temperature incubation were given to the mixture. Utilizing a UV Spectrophotometer (Shimadzu, UV 1601) at 230 nm, the absorbance of H_2O_2 was measured in comparison to a phosphate buffer solution-only blank solution to eliminate background noise. Both Ascorbic acid and hydrogen peroxide absorbance in phosphate buffer were utilized as controls. Every experiment including the spectrometric observations were repeated many times to eliminate the error.

Calculations: The following formula was used to determine the ability to scavenge the radicals:

$$\text{Percentage inhibition} = [(\text{Ac}-\text{As}) / \text{Ac}] \times 100$$

Where A_c =control absorbance for blank and A_s =absorbance of the reaction mixture including a reference material or plant extract.

Spot assay for yeast cells

Cell viability was assessed using a spot assay with *Saccharomyces cerevisiae* strains. Cells were cultivated to mid-exponential phase ($A_{600} \sim 0.6$ – 1.0 , approximately 10^6 cells/mL) in liquid YPDA medium (1% yeast extract, 2% peptone, 2% glucose, 0.003% adenine hemisulfate) at 28°C with shaking at 160 rpm. To evaluate the effect of stress, cells were resuspended in fresh medium containing 2 mM hydrogen peroxide (H_2O_2). Cells were then treated with different concentrations (25, 50, 100, 200, 300, 400, 500, and 750 $\mu\text{g/mL}$) of methanol, ethyl acetate, or chloroform extracts. Controls included untreated cells and cells exposed to H_2O_2 without extract. For the spot assay, saturated cultures were incubated for 1 hour at 28°C with shaking (160 rpm) in YPD medium supplemented with 150 mM hydroxyurea. Subsequently, cultures were serially diluted (10^{-1} to 10^{-4}) in sterile phosphate-buffered saline. Aliquots (3 μL) of each dilution were spotted onto solid YPDA plates (2% agar). Plates were incubated at 28°C, and cell growth was documented after 24, 48, and 72 hours (Pereira *et al.*, 2003).

In vivo Antioxidant Activity

With varied produced doses of 200, 400, and 500 g/mL of the H_2O_2 (4mM), *in vivo* study has been conducted. The extract was applied to cells in the exponential phase for an hour at 28°C and 160 rpm previous to being given stress. The cells were subsequently stressed with H_2O_2 (4mM) by being incubated for 2hr at 28°C and 160 rpm. Each sample was inoculated in triplicate on YPD broth plates following incubation, using the necessary dilution in sterile liquid YPD media. Yeast cells were calculated and assessed to control plates (untreated cells), which were thought to indicate hundred percentage success of the yeast cell after the plates had been incubated for 48hr at 28°C. The capacity of the extracts or reduce the oxidative fatal effects brought on by hydrogen peroxide served as a measure of their antioxidant activity. Tolerance was calculated as a percentage of survival (Soobrattee *et al.*, 2005).

Anti-Bacterial Activity

The antimicrobial efficacy of the plant extracts was evaluated against four bacterial strains: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. All strains were cultured according to standard protocols at 37°C in nutrient broth for 24 hours prior to the assay. A modified agar well diffusion method was employed to assess susceptibility. Briefly, a standardized bacterial inoculum (adjusted to 0.5 McFarland standard) was uniformly swabbed onto the surface of solidified Mueller-Hinton Agar (MHA) plates. Wells (approximately 6 mm in diameter) were aseptically punched into the agar using a sterile cork borer. Subsequently, 25 μL of each extract solution was carefully dispensed into separate wells using a micropipette. Plates were allowed to stand for 10 minutes at room temperature for pre-diffusion before incubation at 37°C for 18 hours. Following incubation, the diameter of the inhibition zones (including the well diameter) was measured in millimeters using a digital caliper. Streptomycin sulfate (1000 $\mu\text{g/mL}$) served as the positive control, while the respective extraction solvents were used as negative controls. All assays were performed in triplicate.

Evaluating Minimal Inhibitory Concentration

The agar dilution technique was modified to evaluate the MIC. To ensure that the extracts dissolved well in the agar, Tween-20 (0.5%) was used as the agar medium. 20 mL of agar in a boiling tube was autoclave sterilized to ensure that the environment was free from microbes. Different concentrations ranging from 0.2-25.6 g/mL were prepared. DMSO solvent was used for extract dilution.

RESULTS

Phytochemical Screening

Phytoconstituents of methanolic plant extracts revealed the presence of secondary metabolites, including alkaloids, flavonoids, terpenoids and tannins, while primary metabolites like soluble proteins, carbohydrates, and carotenoids were found to be absent. Saponins and alkaloids were found to be absent. This absence is based on the defined detection thresholds of the standard qualitative tests employed (e.g., no precipitate formed in Wagner's test for alkaloids; no persistent froth observed in the froth test for saponins), which were consistently observed across all three experimental replicates. The qualitative estimation is shown in Table 1.

Table 1. Preliminary phytochemical screening of MeOH extracts of *A. propinquum*.

Phytoconstituents	Colorimetric assay	Methanolic Extract
Carbohydrates	Benedicts test	+
	Barfoed's test	
	Molish's test	
Soluble proteins	Bradford's test	+
Anthraquinone glycoside	Borntrager's test	+
Terpenoids	Salkowski test	+
	Liebermann-Burchard test	
Alkaloids	Wagner's test	-
	Dragendorff's test	
	Mayers test	

Flavonoid and Total Phenolic Content

Both of the important secondary metabolites, including the total phenolic content and total flavonoid for the crude methanolic stem/leaf extract were 40.32 ± 1.94 gallic acid equivalents/g and 32.76667 ± 1.35 rutin equivalents/g respectively where R^2 was 0.999 (Table 2). As antioxidants, phenolic substances can function because of their redox characteristics. The total phenolic content may serve as a foundation for a quick assessment of antioxidant activity since the hydroxyl groups on phenols increase their capacity to scavenge free radicals. Plant secondary metabolites including flavonoids (flavones, flavanols), and condensed tannins, rely on the presence of free OH groups, particularly 3-OH, for their antioxidant action. Both *in vivo* and *in vitro*, plant flavonoids exhibit antioxidant action (Parimala & Shoba, 2013). Baba and Malik (2015) evaluated total phenolic content of 45.17 ± 1.70 gallic acid equivalents/g for the methanolic root extract of *Arisaema jacquemontii*, and the total flavonoid content of 35 ± 2.20 rutin equivalents/g.

Table 2. Total phenolics and flavonoids content of the methanolic extract of *A. propinquum*.

Total Phenolic content ^a	40.329 ± 1.94
Total Flavonoid content ^b	32.766 ± 1.35

a= mg gallic acid equivalent (GAE)/g DW; b= mg rutin equivalent/g DW.

Functional Group Analysis of Extracts via FTIR Spectra

About 1mg of crude methanol, chloroform, ethyl acetate and n-hexane extract of the aerial part of the said plant was submitted to FTIR analysis to verify the presence of significant FGs. To confirm the presence of important FGs, FTIR analysis of almost 1mg of a crude aerial portion of the mentioned plant extracts made from methanol, chloroform, ethyl acetate, and n-hexane was performed. The main absorption peaks were due to C-H and N-H stretching of alkane at 3649 cm^{-1} , 2914 cm^{-1} , 3116 cm^{-1} , 2847 cm^{-1} . In this extract, the rest of the low-frequency peaks correspond to the C=O (1707 cm^{-1}) and the C-H stretching (1617 cm^{-1}). Peaks at 1584 cm^{-1} , 1401 cm^{-1} , 1274 cm^{-1} , 1215 cm^{-1} , 1177 cm^{-1} , 1148 cm^{-1} , 1021 cm^{-1} , 1095 cm^{-1} , 995 cm^{-1} ,

and 1661 cm^{-1} make up the fingerprint area in the IR spectrum of this extract. Similar FT-IR was performed for the EA extract of the plant and the major absorption peaks were found to be 3377 cm^{-1} (O-H), 2918 cm^{-1} and 2847 cm^{-1} (C-H) and 1703 cm^{-1} (C=O). Later on, the methanol extract was also subjected to FT-IR analysis and the major absorption frequencies were found to be 3347 cm^{-1} , 2918 cm^{-1} , 1684 cm^{-1} , and 1707 cm^{-1} . All of these peaks indicate to the existence of non-polar and polar phytoconstituents such as terpenoids, amines, carboxylic acids, ketones, and alkenes (Figure 1).

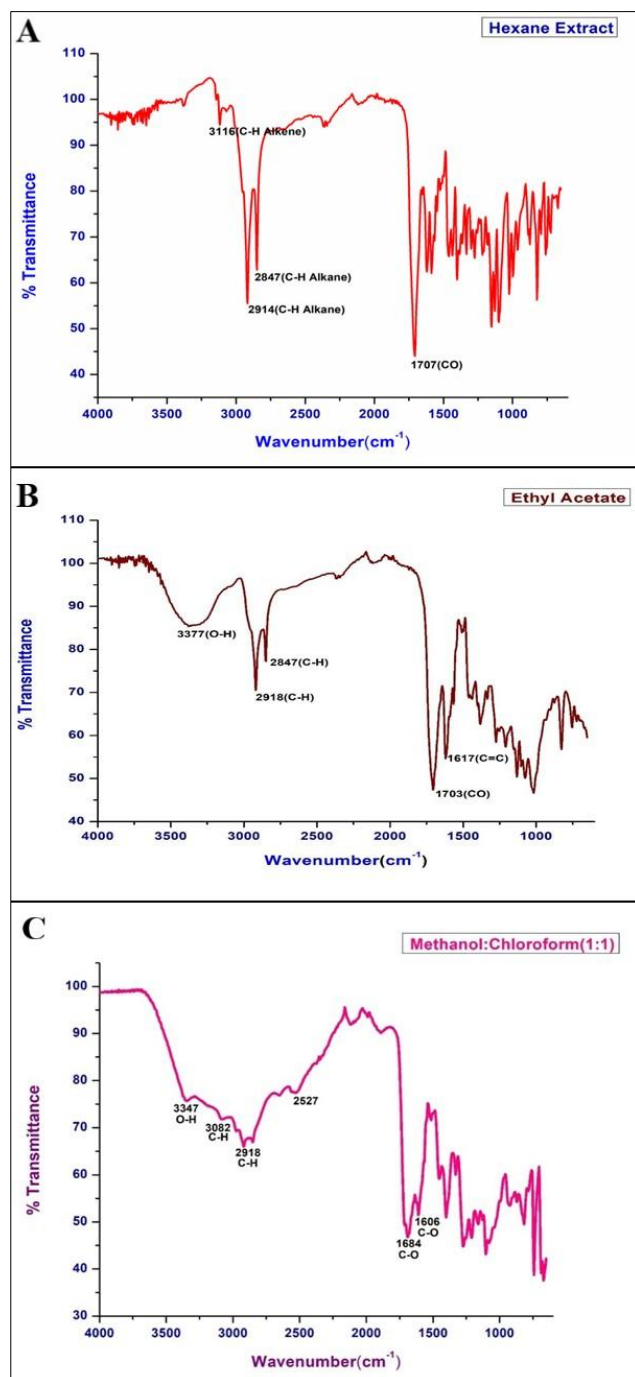


Figure 1. (A) FTIR spectrum of hexane extract of *A. propinquum*; (B) FTIR spectrum of ethyl acetate extract of *A. propinquum*; (C) FTIR spectrum of MeOH: CHCl_3 aerial extract of *A. propinquum*.

Isolated Compound

The obtained isolated compound was the caffeic acid with a molecular formula of $\text{C}_9\text{H}_8\text{O}_4$ with solvent separation system 70:30 (EtOAc: MeOH). The melting point of the CA is $223\text{--}225^\circ\text{C}$. Belongs to hydroxycinnamic acid, which is a cinnamic acid in which the hydroxy groups at

positions 3 and 4 have been replaced for the phenyl ring existing in both cis and transforms, with the latter being more prevalent. The molecular structure of this compound is drawn with ChemDraw version 12.0 (Figure 2). The basic structural identification of target compound was carried out by ^1H -NMR and ^{13}C -NMR at 500 MHz in DMSO with specifications given as:

^1H NMR spectra as follows (500 MHz, DMSO d_6): δ 7.79 (d, $J=15.0$, 1H), 7.00 (dd, $J = 7.5$, 1.4 Hz, 1H), 6.93 (d, $J = 1.4$ Hz, 1H), 6.75 (d, $J = 7.5$ Hz, 1H), 6.51 (d, $J = 15.2$ Hz, 1H), 3.96 (s, 1H), 3.77 (s, 1H).

^{13}C NMR spectra as follows (500 MHz, DMSO d_6): δ 168.94 (s), 148.34 (s), 146.09 (s), 145.41 (s), 128.15 (s), 121.84 (s), 116.23 (s), 115.92 (s), 114.63 (s).

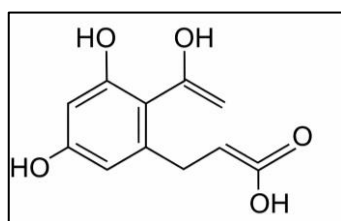


Figure 2. Molecular structure of Caffeic acid.

Anti-Oxidant Activities

DPPH, SOD assay and deoxyribose assay analysis

The methanolic extracts of the aerial parts and seeds of the plant were analyzed for free radical scavenging activities. The extracts of aerial parts showed more activity than seed extracts. The aerial extracts in case of DPPH showed percentage inhibition of 58.9 %, 64.8 % and 77.9 % at 100 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ respectively. In case of Deoxyribose assay the aerial extracts showed % inhibition of 63.2%, 69.7%, and 80.3 % at 100 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$, respectively (Figure 3). For Superoxide anion radical scavenging activity (SOD assay), where the aerial extracts showed percentage inhibition of 61.5%, 67.5%, and 79.8% at 100 $\mu\text{g/mL}$, 150 $\mu\text{g/mL}$, and 200 $\mu\text{g/mL}$, respectively. Among the three performed antioxidant tests for the methanolic leaf and stem extracts we observed IC_{50} value of 26.84($\mu\text{g/mL}$) is quite significant for the Deoxyribose assay than the SOD assay which was having IC_{50} value of 42.89 ($\mu\text{g/mL}$) and with least achieved results for DPPH having an IC_{50} value of 59.47 ($\mu\text{g/mL}$) (Table 3). However, for the methanolic seed extracts of *A. propinquum* the Inhibition concentration values for these three tests showed a similar pattern as IC_{50} value of 108.85 ($\mu\text{g/mL}$) for SOD activity, IC_{50} value of 120.12 ($\mu\text{g/mL}$) for Deoxy ribose assay and for DPPH, we obtained IC_{50} value of 133.89 ($\mu\text{g/mL}$). Rajasree *et al.* (2021) examined the capacity of the MeOH extract of the F1 Hybrid strain of *C. melo* to scavenge nitric oxide, using gallic acid as a reference. The considerable ability of MECM to lessen the creation of NO in the reaction system was revealed in the *in vitro* investigation. Moreover, EA extracts of *Arnebia benthamii* were found to be effective at 78% inhibiting enzyme activity. Lighting up a solution containing riboflavin led to the production of superoxide radicals. According to Lu *et al.* (2004), NBT, a dye, is reduced by superoxide radicals to diformazan, and its presence causes a change in hue. The reaction mixture's decreased absorbance indicates augmented superoxide anion scavenging activity.

Table 3. IC_{50} values of various MeOH aerial extracts against different antioxidant assays.

Sample	IC_{50} DPPH assay ($\mu\text{g/mL}$)	IC_{50} Deoxy ribose assay ($\mu\text{g/mL}$)	IC_{50} SOD ($\mu\text{g/mL}$)
C	70.9 ^a	59.47 ^a	60.38 ^a
S	133.89 ^c	108.85 ^c	120.12 ^c
L	59.47 ^b	26.84 ^b	42.89 ^b

C= Control (Ascorbic acid); L =Stem/Leaves; S =Seeds. Values followed by different superscript letters within a column are significantly different (one-way ANOVA, Tukey's test, $p < 0.05$).

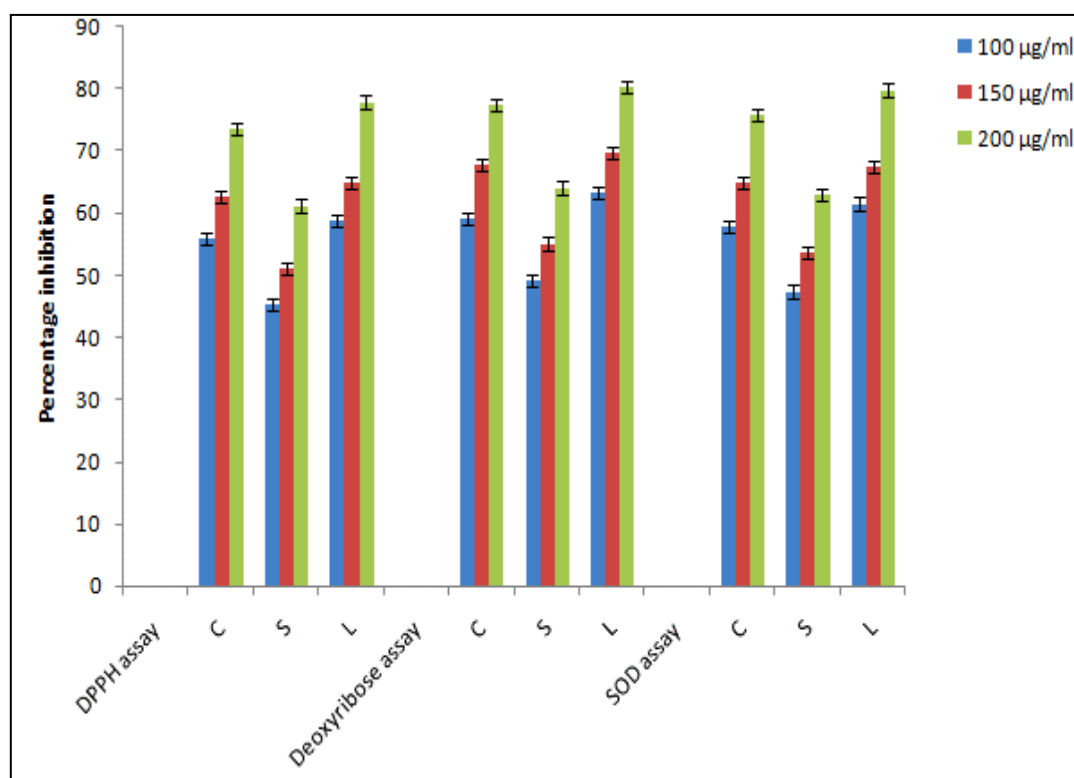


Figure 3. Free radical scavenging activity of *Arisaema propinquum*; Ascorbic acid was included as a positive control. Values are mean $\pm$ SD (n=3); C= Control, S = Methanolic extract of seed, L= Methanolic extract of Stem/Leaves.

Hydrogen peroxide radical assay

Few enzymes are immediately rendered inactive by hydrogen peroxide, a weak oxidizing agent that often does this via oxidizing crucial thiol (-SH) groups. It has a quick ability to permeate cell membranes. Once within the cell, it may interact with Fe^{2+} and Cu^{2+} ions to produce OH, which may be the cause of many of its harmful effects (Lu *et al.*, 2004). In order to safeguard food systems, it is crucial to remove hydrogen peroxide as well as O_2 . The methanolic extract of *A. propinquum* has been evaluated at various concentrations of 25, 50, 75, 100, and 125 $\mu\text{g/mL}$ in order to determine % Inhibition in comparison to the standard ($\text{C}_6\text{H}_8\text{O}_6$) (Figure 4). The determined IC_{50} value for the sample extract and standard were 49.742 and 46.191 $\mu\text{g/mL}$ respectively. In comparison with conventional ascorbic acid, the MeOH extract had considerable dose-dependent H_2O_2 radical scavenging action.

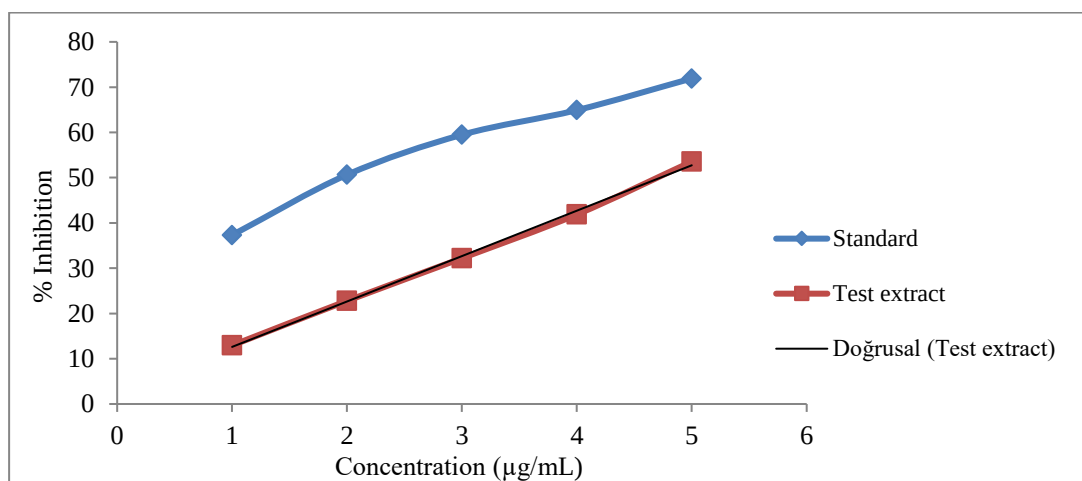


Figure 4. Inhibition of different MeOH extract concentrations of *A. propinquum*.

In vivo anti-oxidant analysis

MeOH extracts have shown significant activity compared to EtOAc and CHCl₃, escalating the percentage survival of *S. cerevisiae* against hydrogen peroxide. Table 4 clearly indicates the significant differences in the survival of *S. cerevisiae*. The present investigation for MeOH extract of *A. propinquum* of *in vitro* and *in vivo* have shown a potent protective effect against stress-causing agents due to the presence of free radical neutralizing phytochemicals including phenols, flavonoids, polyphenolic compounds, which were stated to counterbalance various ROS, including OH•, H₂O₂• ROO• by chelating and providing donors to neutralize reactive ones (Parray et al., 2015).

Table 4. Antioxidant activity of plant extracts on the survival of *S. cerevisiae*.

Treatment	Survival percentage
Control (yeast cells)	100 ± 1.20
H ₂ O ₂ (4mM)	28.73 ^a ± 2.08
H ₂ O ₂ +250µg/ mL	37.64 ^b ± 1.91
H ₂ O ₂ +500µg / mL	64.72 ^c ± 1.15
H ₂ O ₂ +750µg /mL	76.17 ^d ± 0.58

Means ± SD (n = 3) Using SPSS Version 17 (SPSS Inc., Chicago, USA) and means were compared using Tukey's tests at the 5% level of significance, it was shown that letters that are followed by the same letters are not substantially different at $p=0.05$.

Henceforth, significant quantitative growth differences have been achieved in *S. cerevisiae* on Hydroxyurea (150 mM) loaded YPDA media cultures, along with and without MeOH, CHCl₃, and EtOAc extracts of *A. propinquum* (Figure 5). Hydroxyurea (HU) is an eminent chemotherapeutic drug that is extensively utilized in the treatment of solid tumors and myeloproliferative diseases (MPD). As of carcinogenic and mutagenesis potential for HU's, it is often employed to investigate both prokaryotes and eukaryotes. Site-specific DNA damage is caused by hydroxyurea by the production of H₂O₂• and NO• (Sakano et al., 2001). According to the aforementioned data, aerial extracts on HU-loaded YPDA plates protected yeast cells, which points to the extract's potential involvement in scavenging hydrogen peroxide radicals. This supports the extract's antioxidant action and demonstrates the function it plays in defending cells against chemicals that damage DNA.

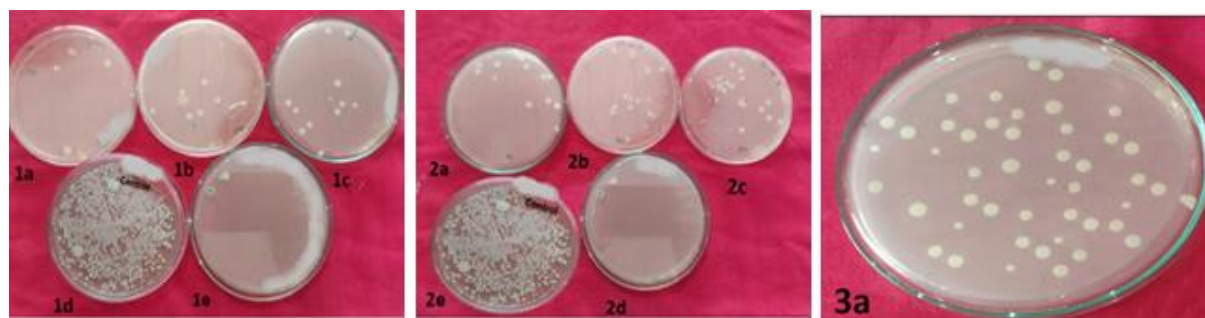


Figure 5. Spot assay analysis for yeast colonies count for different concentrations of extracts of *A. propinquum* where 1a-c= EtOAc treatments (250,500 and 750 µg/mL); 1e = stress (H₂O₂) and 1d= Control; 2a-c = MeOH treatments and 3a, shows growth of Yeasts cells spotted on YPDA + 150 mM HU.

Antibacterial Activity

Few strains were treated with prepared extracts of *A. propinquum* as maximum and minimum zone of inhibition is given in Table 5.

Table 5. Antibacterial activity of plant extracts against selected bacterial strains.

Bacterial strains	Zone of Inhibition (mm)				MIC
	HA ^a	EA ^b	MeOH ^c	5µg Ciprofloxacin	
<i>Salmonella typhi</i> ATCC 6539	8 ± 0.4	10 ± 0.4	17 ± 0.2	25 ± 0.2	>23.2
<i>Pseudomonas aeruginosa</i> ATCC 27853	20 ± 0.28	24 ± 0.18	22 ± 0.8	25 ± 0.8	>1.6
<i>E. coli</i> ATCC 25922	25 ± 0.1	14 ± 0.6	21 ± 0.27	28 ± 0.4	>1.6
<i>Staphylococcus aureus</i> ATCC 25923	15 ± 0.5	23 ± 0.45	17 ± 0.6	25 ± 0.45	>3.2

^aHA: Hexane extract, ^bEA: Ethyl acetate and ^cMeOH: Methanol extracts. 5 µg ciprofloxacin was used as a standard antibiotic for the reference of positive control; however, for the negative control 5 percent DMSO were used without any inhibition zone.

DISCUSSION and CONCLUSION

This study successfully isolated and characterized caffeic acid from the Cobra Lily (*Arisaema propinquum*), further elucidating the phytochemical composition and biological properties of this relatively unexplored medicinal plant native to the Kashmir Himalayas. The findings confirm that *A. propinquum* is a rich source of bioactive phenolic compounds that exhibit potent antioxidant and antibacterial activities, thereby reinforcing the pharmacological relevance of the *Arisaema* genus and expanding its chemotaxonomic profile.

The phytochemical analysis revealed high total phenolic (40.32 ± 1.94 mg GAE/g DW) and flavonoid contents (32.77 ± 1.35 mg RE/g DW) within the methanolic extract of aerial parts, consistent with results reported in related species like *Arisaema jacquemontii* and *A. erubescens* (Tabassum et al., 2019; Rahman et al., 2019). These bioactive secondary metabolites are well known for their health-promoting effects, including free radical scavenging, metal ion chelation, and modulation of cellular signaling pathways (Baba & Malik, 2015). The identification of functional groups such as hydroxyl, amine, and carbonyl via FTIR spectroscopy further highlights the complex phytochemical matrix, which likely synergizes to produce the broad spectrum of observed biological activities. Such diverse chemical profiles in *Arisaema* species suggest these plants represent a valuable reservoir for natural product discovery and drug development.

A key novel contribution of this study is the first reported isolation of caffeic acid from *A. propinquum*. Caffeic acid, a hydroxycinnamic acid derivative, has been extensively investigated for its multifaceted pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Maity et al., 2022; Mirzaei et al., 2021). Previous phytochemical studies on *Arisaema* species have documented various bioactives, such as cytotoxic fatty acid esters in *A. flavum* (Nisa et al., 2022) and lectins in *A. tortuosum* (Dhuna et al., 2005), but this is the first definitive report of caffeic acid in *A. propinquum*. The isolation methodology employed could serve as a foundation for future work targeting the purification and quantification of this compound to standardize extract-based formulations.

The antioxidant potential of *A. propinquum* was demonstrated convincingly across multiple assays. The deoxyribose assay results, with an IC₅₀ value of 26.84 µg/mL, illustrate potent hydroxyl radical scavenging activity, a particularly relevant measure given the hydroxyl radical's role as one of the most highly reactive and DNA-damaging species generated *in vivo*. This assay, supported by the *in vivo* yeast cellular protection model, which recorded a 76.17% survival rate under oxidative stress at 750 µg/mL extract concentration, exemplifies the translational importance of the antioxidant effects observed *in vitro*. Compared with other *Arisaema* species like *A. tortuosum*, which demonstrated relatively modest antioxidant capacity (Azam et al., 2016; Sharma et al., 2023), *A. propinquum* shows superior activity, evidencing interspecies variation and highlighting its potential as a more effective natural antioxidant source.

The antibacterial activity displayed by the methanolic extracts of *A. propinquum* is similarly noteworthy. Zones of inhibition ranged up to 25 mm against *Escherichia coli*, with minimum inhibitory concentrations (MICs) against various pathogens generally exceeding 1.6 µg/mL, indicating potent broad-spectrum activity. This complements previously reported antimicrobial activities in *Arisaema* congeners, such as *A. jacquemontii* (Tabassum et al., 2019) and *A. tortuosum* (Azam et al., 2016), as well as endophytic microbial associations in *A. erubescens* (Akanksha et al., 2021). The effectiveness against Gram-negative bacteria, which often exhibit multidrug resistance due to complex outer membrane barriers, underscores the therapeutic significance and potential for discovering novel antibacterial agents within *A. propinquum*.

Beyond confirming traditional uses and demonstrating bioactivity, the present findings highlight the need for comprehensive characterization of the phytochemical constituents responsible for antioxidant and antibacterial effects. While FTIR analysis indicated the presence of functional groups associated with phenolic and related bioactive compounds, further clarification of compound–activity relationships requires advanced analytical techniques such as NMR, LC–MS/MS, and GC–MS for precise structural elucidation and metabolomic profiling (Mirzaei et al., 2021). Additionally, translation of the observed in vitro activities into therapeutic relevance necessitates in vivo validation using mammalian models to assess antioxidant protection, antibacterial efficacy, safety, and pharmacokinetics, thereby strengthening the mechanistic understanding and potential biomedical applications of the extract (Vázquez et al., 2018).

The genus *Arisaema* is known to harbor an extensive diversity of bioactive compounds (>260 reported), encompassing anticancer, analgesic, anti-inflammatory, antioxidant, and antimicrobial agents (Tabassum et al., 2019). The unique chemical and bioactive profile presented here for *A. propinquum*, particularly its caffeic acid content and potent antioxidant and antibacterial activities, contributes meaningfully to this repository of natural therapeutic resources. Isolation and standardization of active principles, in conjunction with mechanistic studies exploring molecular targets and pathways, remain necessary to harness the full clinical potential of these plants.

This study positions *Arisaema propinquum* as a pharmacologically valuable species, warranting further detailed research. Its potent phenolic profile, first isolation of caffeic acid, and efficacy in multiple antioxidant and antibacterial assays highlight its promise as a source for the development of new natural antioxidant and antimicrobial agents. Future research should prioritize advanced phytochemical characterization, in vivo pharmacological testing, toxicity evaluation, and exploration of synergistic phytochemical interactions to guide drug discovery efforts dedicated to managing oxidative stress, microbial infections, and related pathologies. Such endeavors will strengthen the scientific foundation for the development of standardized plant-based therapeutics derived from *A. propinquum* and related species in the *Arisaema* genus.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJATE belongs to the authors.

Authorship Contribution Statement

S.H.: Investigation, Methodology, Resources, Visualization, Software, Formal Analysis, and Writing-original draft. **A.N.:** Supervision, and Validation, Investigation. **M.R.:** Writing-original draft, Revising, Reviewing and editing. **M.A.Z.:** Resources, Visualization, Software. **A.N.:** Resources, Visualization, Software.

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