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Phytochemical, antioxidant, and anticancer properties of *Parkinsonia aculeata* L.: An insight into its bioactive potential

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Abstract
The present study involves the phytochemical, antimicrobial, antioxidant, and anticancer activities of *Parkinsonia aculeata* leaf ethanol extract using High-Performance Thin-Layer Chromatography (HPTLC) and bioautographic analysis. HPTLC fingerprinting revealed twelve bands observed under long-wave UV light (366nm), suggesting a complex chemical composition. Bioautographic analysis demonstrated significant antimicrobial activity against *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with distinct inhibition zones at specific Rf values. The antioxidant activity was confirmed through the DPPH assay, where cream-colored bands at Rf 0.27 and 0.19 indicated free radical scavenging properties. Additionally, the anticancer potential of the extract was evaluated against THP-1 leukemia cells, showing a dose-dependent cytotoxic effect with cell viability reductions up to 20% at 500 µg/ml. The findings highlight the presence of bioactive phytochemicals in *P. aculeate* methanolic extract, which may contribute to its therapeutic potential as anticancer agent. Further studies are required to isolate and characterize the active compounds and elucidate their mechanisms of action.

Keywords: *Parkinsonia aculeata*, HPTLC, phytochemicals, antimicrobial, antioxidant, anticancer

Introduction
Parkinsonia aculeata L. has gained attention for its diverse pharmacological properties, including antibacterial, antioxidant, and anticancer activities. The increasing prevalence of antibiotic-resistant pathogens, oxidative stress-related disorders, and cancer has intensified the need for alternative therapeutic agents derived from natural sources. *P. aculeata*, a member of the Fabaceae family, thrives in arid and semi-arid regions and has been widely studied for its rich phytochemical composition, which includes flavonoids, alkaloids, tannins, saponins, and phenolic compounds [1]. These bioactive constituents contribute to its broad spectrum of biological activities, making it a promising candidate for pharmaceutical applications [2].

The antibacterial efficacy of *P. aculeata* has been extensively investigated, particularly against clinically relevant pathogens such as *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Enterococcus faecalis*. These bacterial strains are responsible for severe hospital-acquired infections and exhibit increasing resistance to conventional antibiotics [3]. Studies have demonstrated that extracts of *P. aculeata* disrupt bacterial cell membrane integrity, inhibit biofilm formation, and interfere with metabolic processes, thereby exerting potent antibacterial effects [4]. The presence of phenolic compounds and flavonoids is believed to play a crucial role supporting antimicrobial properties, as they interact with bacterial proteins and enzymes, leading to growth inhibition [5] Similarly, *Parkinsonia praecox*, a closely related species, has been reported to exhibit strong antibacterial activity, further reinforcing the therapeutic potential of the *Parkinsonia* genus in infectious disease management [6].

Beyond its antimicrobial activity, *P. aculeata* is an important source of antioxidants, which play a critical role in preventing oxidative stress-related diseases. Oxidative stress results from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defenses, contributing to the progression of cardiovascular diseases, neurodegenerative disorders, and cancer [7]. Research has shown that phenolic and flavonoid-rich extracts of *P. aculeata* possess strong free radical scavenging abilities, reducing oxidative damage at the cellular level [8]. These antioxidants function by neutralizing free radicals, inhibiting lipid peroxidation, and enhancing enzymatic defense mechanisms, which collectively promote cellular protection and longevity [9].

Such antioxidant properties highlight the potential application of *P. aculeata* in nutraceuticals and functional foods aimed at preventing chronic diseases [10].

In addition to its antibacterial and antioxidant properties, *P. aculeata* has shown significant potential in cancer treatment, particularly against leukemia. Leukemia, including the THP-1 monocytic leukemia cell line, remains a major concern due to its aggressive progression and resistance to standard chemotherapy [11]. Phytochemicals derived from *P. aculeata* have been found to induce apoptosis, inhibit cell proliferation, and modulate key signaling pathways involved in cancer progression [12]. The cytotoxic effects of *P. aculeata* against cancer cells are primarily attributed to its flavonoids and alkaloids, which disrupt mitochondrial function and trigger programmed cell death [13]. Moreover, *Parkinsonia praecox* has been identified as a novel source of anticancer compounds, further supporting the therapeutic relevance of the *Parkinsonia* genus in oncology research [14]. Recent studies suggest that plant-based bioactive compounds can serve as potent alternatives to synthetic anticancer drugs, offering lower toxicity and enhanced efficacy. The increasing interest in plant-derived pharmaceuticals underscores the need for further investigation into the therapeutic applications of *P. aculeata*. Its antibacterial properties offer a promising alternative to combat antibiotic-resistant pathogens, while its antioxidant potential positions it as a valuable natural source for disease prevention. Additionally, its cytotoxic effects against leukemia cells highlight its potential role in cancer therapy. As research progresses, the isolation and characterization of specific bioactive compounds from *P. aculeata* will be crucial in developing novel drugs with enhanced efficacy and safety profiles [15] The traditional use of this plant in herbal medicine, combined with emerging scientific evidence, reinforces its significance in modern pharmacology.

Materials and Methods

Chemicals

Silica gel 60 F254, TLC plates (E. Merck), Toluene, Ethyl acetate, Ethanol, Methanol, 2,3,5-Triphenyl-tetrazolium Chloride, Methanol, Iso-propyl alcohol, Acetone, Benzene, n-Hexane, Terpene, Anisaldehyde H_2SO_4, RPMI 1640 Media, Fetal Bovine serum, dimethylthiazol-carboxymethoxyphenyl-sulfophenyl-tetrazolium (MTS), 3-(4,5-dimethylthizolyl-2)-2, 5-diphenyl tetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO), Quinacrine, Doxorubicin, Dulbecco's phosphate – buffered saline (D-PBS), 2,2- Diphenyl-1- picrylhydrazyl (DPPH), were procured from Sigma-Aldrich Ltd.,

Microorganisms and cell lines

THP-1-Human peripheral Blood Monocyte cell line. *Pseudomonas aeruginosa* (ATCC 8076), *Enterococcus faecium* (ATCC 11457), *Staphylococcus aureus* subsp. *aureus* (ATCC 87), and *Klebsiella pneumoniae* (ATCC 109) were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Sector 39-A, Chandigarh, India. Deionized water was employed in the synthesis process for preparing solutions.

Plant collection and preparation of extract from *Parkinsonia aculeata* leaves

Parkinsonia aculeate was collected from Ballari, Karnataka, India and identified using the Flora of Madras. The herbarium of plant specimen was carefully prepared and documented at Department of Botany, Vijayanagara Sri Krishnadevaraya University, Ballari - 583105, Karnataka, India.

The leaves of *Parkinsonia aculeata* L. were thoroughly washed under tap water, rinsed with distilled water, and shade-dried at room temperature. Once dried, the leaves were ground into a fine powder using a mortar and pestle. 100 grams of the powdered sample was soaked in 300 mL methanol and incubated in a dark for 24 hours. The extract was then filtered and left to dry for two days. The dried extract was carefully collected and preserved at room temperature in air tight containers and stored in vials until further use.

Phytochemical Profiling by High-Performance Thin-Layer Chromatography (HPTLC) of methanolic extract of *Parkinsonia aculeata* L. leaves

Sample solutions were applied as sharp bands onto Silica Gel 60 F254 TLC plates (E. Merck) using an Aspire automatic sample applicator. The spots were air-dried, and chromatography was performed in a glass chamber (Aspire). The mobile phase was added to a twin-trough glass chamber, and the entire assembly was allowed to equilibrate and pre-saturate for 30 minutes. The plate was then developed until the solvent front reached 80 mm above the base at 20ºC and 50% relative humidity. Visualization was carried out under UV light at 254 nm and 366 nm, as well as after derivatization. A densitometric scan was generated using Just TLC software [16].

Bioautographic Analysis of the Antimicrobial Activity of *Parkinsonia aculeata* L.

Bioautographic analysis is a valuable technique for identifying antimicrobial compounds within complex plant extracts. While specific studies on *Parkinsonia aculeata* L. using this method are limited. In this approach, *Parkinsonia aculeata* L. leaves methanol extract (PLE), are first separated using thin-layer chromatography (TLC) on silica gel plates. After development, the chromatograms are overlaid with microbial cultures of *Pseudomonas aeruginosa* (ATCC 8076), *Enterococcus faecium* (ATCC 11457), *Staphylococcus aureus* (ATCC 87), and *Klebsiella pneumoniae* (ATCC 109) medium inoculated with the test microorganism. Following incubation, zones of inhibition appear as clear areas against a turbid background, indicating the presence of antimicrobial compounds at specific locations on the TLC plate. This method allows for the direct correlation between chromatographic spots and antimicrobial activity, facilitating the identification of bioactive constituents. The Retention Factor (Rf) is calculated using the following formula in Thin-Layer Chromatography (TLC):

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

The Rf value is always between 0 and 1. Lower values indicate less movement (more polar compounds), while higher values indicate greater movement (less polar compounds) [17].

Rapid Identification of Antioxidants via DPPH and Bioautography Techniques

For the screening of antioxidant compounds in *Parkinsonia aculeata* L. leaves methanolextract (PLE), the TLC-bioautography method was employed [18, 19]. A diluted solution of PLE (1:20 in methanol) was applied to silica gel

60 F254 TLC plates and developed using Toluene: Ethyl acetate solvent (8:2). The plates were then sprayed with a 0.2% methanolic solution of DPPH. Active antioxidant constituents appeared as yellow spots against a violet background. Only those zones that changed from violet to yellow within the first 30 minutes after spraying were considered positive results [20].

AssessAnti-Cancer Properties of *Parkinsonia aculeata* L. Leaf Extract in THP-1 Cells

The anti-cancer activity of *Parkinsonia aculeata* L. leaves methanol extract (PLE) was assessed against THP-1 blood cancer cells. To begin, 10,000 cells per well were seeded into a 96-well plate and incubated overnight at 37°C with 5% CO_2 to allow proper attachment. The following day, the old culture medium was carefully removed and replaced with fresh Dulbecco's Modified Eagle Medium (DMEM) containing varying concentrations of PLE (31.25, 62.5, 125, 250, and 500 µg/mL). The cells were then incubated for an additional 24 hours under the same conditions. To evaluate cytotoxicity, a comparative approach was used by including Quinacrine as the standard reference drug alongside an untreated control group. Following the treatment period, 10% MTT reagent was added to each well, allowing the reaction to proceed for 3 hours to facilitate the formation of formazan crystals. After incubation, the culture medium was carefully aspirated, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals completely. The absorbance of the dissolved formazan was then measured at 570 nm using a microplate reader to determine cell viability. Cytotoxic activity was quantified using the standard formula provided by [21]. The half-maximal inhibitory concentration (IC_{50}) was calculated using a linear regression equation (Y = Mx + C), where Y = 50 and M, C values were obtained from the cell viability graph.

$$\%\textbf{Cell Viability} \frac{\text{Mean Absorbance of Sample} - \text{Blank}}{\text{Mean absorbance of Untreated} - \text{Blank}} \times 100$$

Results and Discussion

HPTLC Profiling of *P. aculeata* L. Ethanol Extract Reveals Bioactive Compounds

High-Performance Thin-Layer Chromatography (HPTLC) analysis of the methanol extract from *Parkinsonia aculeata* leaves was conducted to establish its phytochemical profile. The chromatographic conditions utilized included a silica gel 60 F254 stationary phase, a mobile phase comprising toluene and ethyl acetate in an 8:2 v/v ratio, and derivatization with anisaldehyde-sulfuric acid reagent. The sample was applied in volumes of 8 µL and 10 µL, and the developed chromatograms were examined under visible light, short-wave UV (254 nm), and long-wave UV (366nm) illumination.

Chromatographic Observations (Fig 1):

- **Visible Light:** Three distinct bands were observed.
- **Short-Wave UV (254nm):** Three bands were detected.
- **Long-Wave UV (366nm):** Twelve bands appeared with Rf values of 0.97, 0.84, 0.79, 0.70, 0.66, 0.59, 0.55, 0.50, 0.41, 0.32, 0.28, and 0.17.

The presence of multiple bands across different detection modes suggests a complex mixture of phytochemicals in the *P. aculeata* leaf extract. The presence of bands whenobserved under long-wave UV light indicate the existence of various compounds, potentially including flavonoids, glycosides, and other phenolic constituents. Previous studies have reported the isolation of several flavonoids from *P. aculeata*, such as luteolin derivatives and other glycosylated flavonoids [22]. The detection of bands at specific Rf values under UV light may correspond to these compounds. For instance, flavonoids often exhibit fluorescence under UV illumination, and their glycosylation patterns can influence their Rf values.

The use of a toluene: ethyl acetate (8:2) mobile phase has been effective in separating non-polar to moderately polar compounds, which aligns with the detection of multiple bands in this study. Similar solvent systems have been employed in HPTLC analyses of other medicinal plants to achieve distinct separation of phytoconstituents [23]. The derivatization with anisaldehyde-sulfuric acid reagent is known to enhance the visualization of terpenoids, steroids, and certain phenolic compounds. The appearance of additional bands post-derivatization suggests the presence of such compounds in the extract. In conclusion, the HPTLC fingerprint obtained provides valuable insights into the phytochemical composition of *P. aculeata* leaves. The detection of multiple bands across various detection modes highlights the complexity and diversity of its constituents, which may contribute to the plant's reported pharmacological activities. Further studies involving the isolation and characterization of these compounds are warranted to elucidate their specific roles and potential therapeutic applications.

Bioautographic Screening of *P. aculeata* L. for Antimicrobial Properties (Fig 2)

The bioautographic analysis of *Parkinsonia aculeata* leaf methanol extract demonstrated significant antimicrobial activity against multiple bacterial strains. Against *Enterococcus faecium*, three inhibition zones were observed at Rf values of 0.68, 0.60, and 0.54, indicating the presence of active compounds targeting this pathogen. *Staphylococcus aureus* exhibited four inhibition zones at 0.70, 0.65, 0.56, and 0.50, with the widest zone at Rf 0.65, suggesting strong antibacterial activity at this retention factor. Similarly, *Klebsiella pneumoniae* showed four inhibition zones at 0.67, 0.58, 0.52, and 0.39, with the most prominent effect at Rf 0.67. Additionally, *Pseudomonas aeruginosa* displayed three inhibition zones at 0.66, 0.57, and 0.51, indicating the extract's potential to combat this opportunistic pathogen (Fig 2). The distinct Rf values observed across different bacterial strains suggest the presence of multiple bioactive phytochemicals with varying degrees of antimicrobial potency.

These consistent Rf values across different bacterial strains suggest that specific phytochemicals within the *P. aculeata* extract are responsible for the observed antimicrobial effects. The Rf value, representing the retention factor in thin-layer chromatography, is characteristic for each compound under defined conditions; thus, identical Rf values indicate the presence of the same bioactive compound exerting antimicrobial activity. Previous studies have identified various bioactive compounds in *P. aculeata*, including phenols, flavonoids, tannins, and alkaloids, which are known for their antimicrobial properties. The specific Rf values observed in this study may correspond to these compounds, suggesting their potential role in inhibiting the growth of the tested bacterial strains [24] [25]. The bioautographic analysis indicates that *P. aculeata* ethanol extract contains specific phytochemicals with significant antimicrobial activity against *E. faecium*, *S. aureus*, and *K. pneumoniae*. Further isolation and characterization of these compounds are warranted to elucidate their structures and mechanisms of action.

Enhanced Antioxidant Screening: Synergistic Use of DPPH and Bioautography (Fig 3)

The bioautographic analysis of *P. aculeata* leaf methanol extract demonstrated antioxidant activity, as indicated by cream-colored bands on a purple background at Rf values of 0.27 and 0.19 (Fig 3). This color change, observed during the DPPH assay, signifies the presence of compounds capable of scavenging free radicals. In this assay, antioxidants reduce the stable purple DPPH radical to a yellow-colored diphenylpicrylhydrazine, leading to discoloration. The appearance of cream-colored bands against a purple background suggests that specific phytochemicals in the extract are actively neutralizing DPPH radicals at the corresponding Rf values. Previous studies have identified various bioactive compounds in *P. aculeata*, including flavonoids, terpenoids, alkaloids, saponins, tannins, C-glycosides, phenolic compounds, and reducing sugars [26]. These compounds are known for their antioxidant properties and may correspond to the observed Rf values. For instance, flavonoidsand phenolic compounds have been reported to exhibit significant free radical scavenging activities.

Anti-Leukemic Potential of Ethanol Extract from *P. aculeate* L. Leaves in THP-1 Cells (Fig 4)

The anticancer activity of *P. aculeate* leaf methanolic extract against THP-1 leukemia cells demonstrates a dose-dependent cytotoxic effect, with cell viability reductions of 9%, 12%, 15%, 18%, and 20% at concentrations of 31.2, 62.5, 125, 250, and 500 µg/mL, respectively (Fig 4). This trend suggests that higher concentrations of the extract are more effective in inhibiting THP-1 cell proliferation. Comparatively, a study investigated the antiproliferative effects of *Origanum syriacum* and *Thymus vulgaris* extracts on THP-1 cells, observing significant cytotoxicity [27]. Additionally, research on *Schouwia purpurea*leaf extract revealed notable cytotoxic activity against THP-1 cells, indicating the potential of various plant extracts in leukemia treatment [28]. While studies on *P. aculeata*'s anticancer properties are limited and this is for the first time *P. aculeate* leaf methanolic extract has been used for this investigation.

However, related research has shown its efficacy against other cancer cell lines. For instance, an *in vivo* study demonstrated that *P. aculeata*extracts significantly reduced tumor volume and increased survival in mice with Ehrlich's ascites carcinoma [29]. Another study reported that *P. aculeata* leaf extract exhibited cytotoxic effects on B16F10 melanoma cells in mice [30]. The observed dose-dependent cytotoxicity of *P. aculeata* leaf methanolic extract against THP-1 cells aligns with findings from other plant extracts, underscoring its potential as a natural anticancer agent. Further research is warranted to isolate specific bioactive compounds responsible for this activity and to evaluate their mechanisms of action.

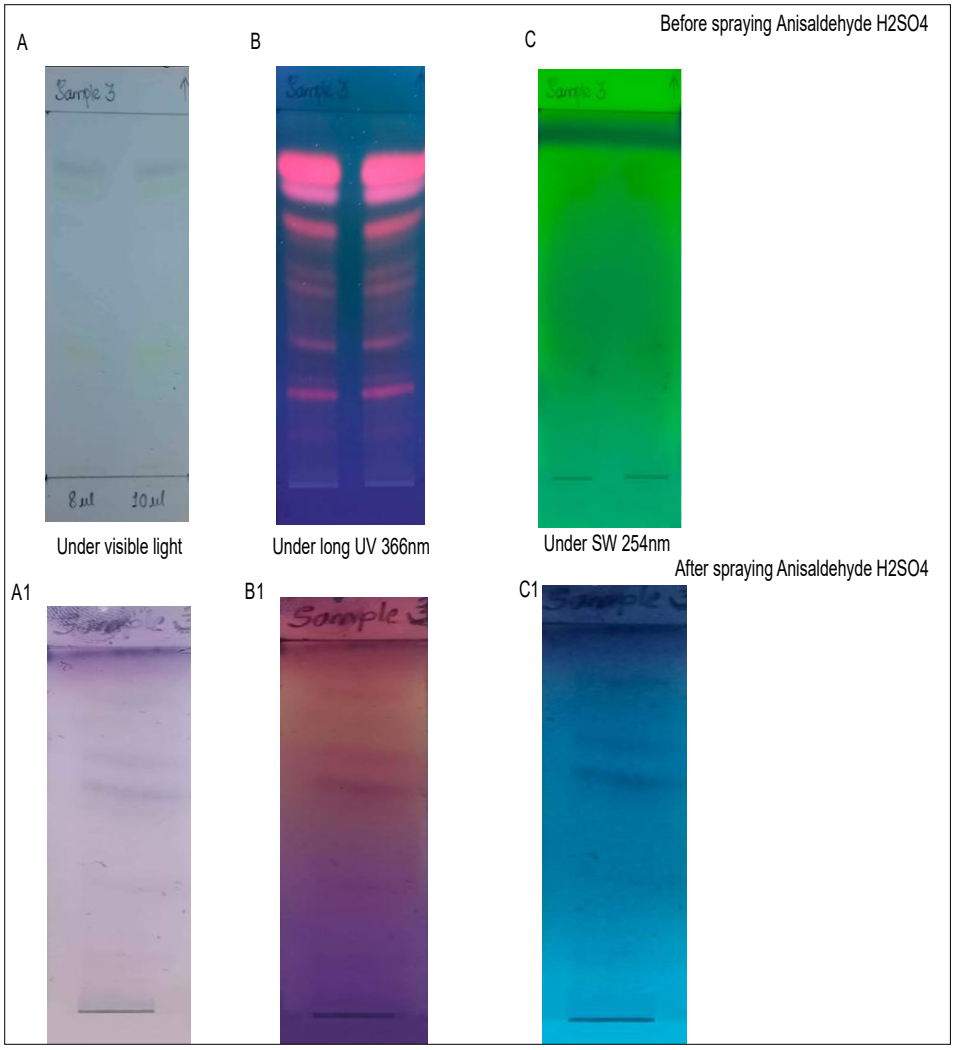

Fig 1: HPTLC chromatogram of *Parkinsonia aculeata* leaf methanol extract visualized under visible light (A & A1), short-wave UV (254 nm) (B & B1), and long-wave UV (366 nm) (C & C1). The chromatogram reveals three bands in visible light and short-wave UV, while twelve distinct bands indicating the presence of multiple phytoconstituents.

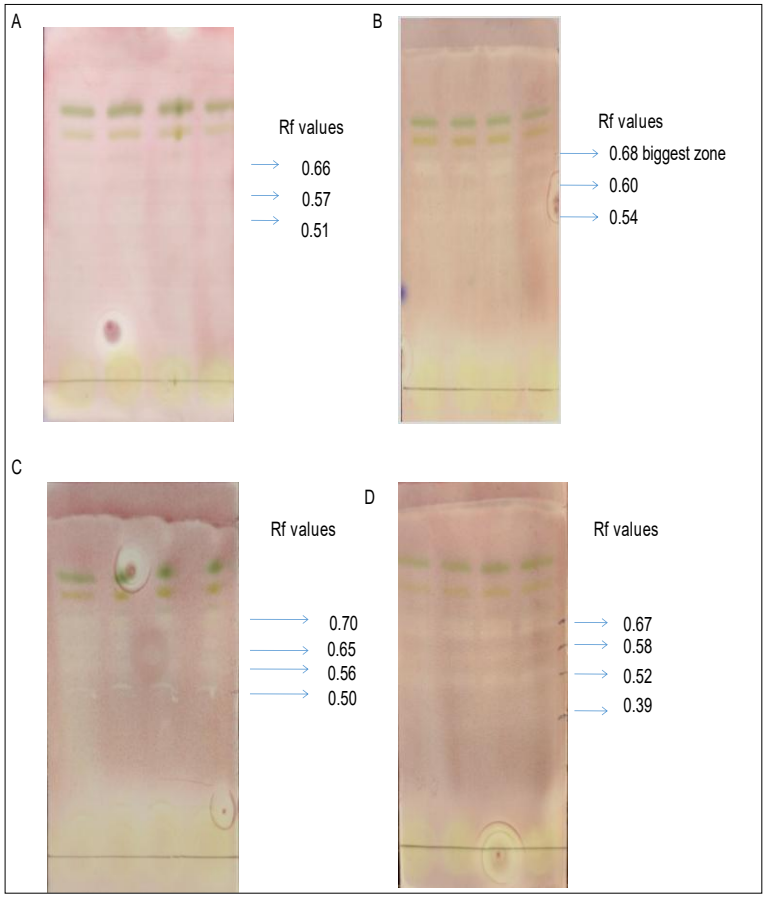

Fig 2: Antibacterial efficacy of *Parkinsonia aculeata* leaf methanol extract evaluated by bioautography technique against (A) *Pseudomonas aeruginosa*; (B) *Enterococcus faecium*; (C) *Staphylococcus aureus*; and (D) *Klebsiella pneumonia.* Rf values of zones of inhibition are indicated respectively

Fig 3: Antioxidant potential of *Parkinsonia aculeata* leaf methanol extract accessed by bioautography method exhibited the potency of ethanol fractions of *P. aculeate* to inhibit the DPPH free radicals. Images (A) represents fractionation of plant extract before treating with DPPH and (B) represents Radical scavenging activity of methanol fractions of *P. aculeate* and their Rf values.

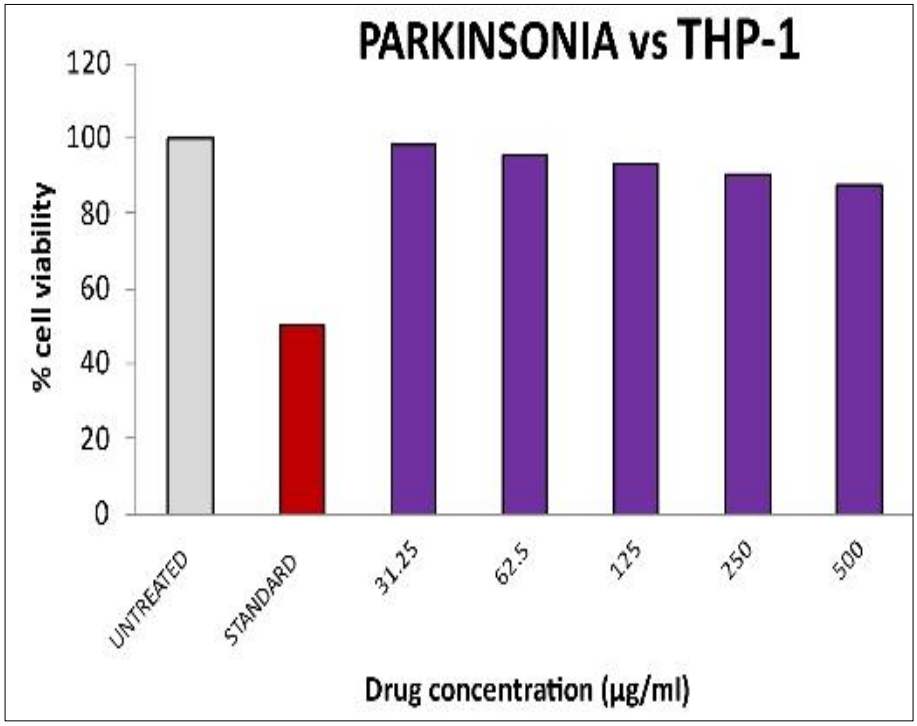

Fig 4: *Parkinsonia aculeata* leaf ethanol extract exhibited Anti-Leukemic Potential against THP-1 cell lines in a dose dependent manner

Conclusion

The findings of this study provide compelling evidence that *Parkinsonia aculeata* leaf methanol extract is a rich source of bioactive compounds with significant antimicrobial, antioxidant, and anticancer activities. The HPTLC fingerprinting revealed multiple phytochemicals, and bioautographic analysis confirmed the extract's potential against various bacterial pathogens. The DPPH assay further established its antioxidant capabilities, while the cytotoxicity study demonstrated its dose-dependent anticancer effects against THP-1 leukemia cells. These results support the traditional medicinal use of *P. aculeata* and underscore its potential as a natural therapeutic agent. Future research should focus on isolating and characterizing the active constituents and exploring their mechanisms of action for pharmaceutical applications.

Ethical statement

There is none to be disclosed.

Declaration of Competing Interest

The authors declare no conflict of interest in regards to this article.

Credit authorship contribution statement

- **Kavitha Sagar:** Conceptualization, Supervision.
- **Nitesh Kumar:** Methodology Data analysis, Manuscript writing, and Editing.

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