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Exploring the Antioxidant and Anticancer Activity of *Clematis heynei*, an Endemic Medicinal Plant of the Western Ghats

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INTRODUCTION

- Clematis heynei (Ranunculaceae), is a perennial climber, found mainly in the Deccan region of Karnataka, Madhya Pradesh and Maharashtra.
- Known locally in India as Morvela, Ranjai, Murhari or Churhar, Laghukarni, Madhulika, Madhumati etc
- In Ayurveda, root decoction are administered orally for treatment of bilious vomiting
- Medicinal value of leaves of Clematis hyenei is underexplored.

OBJECTIVES

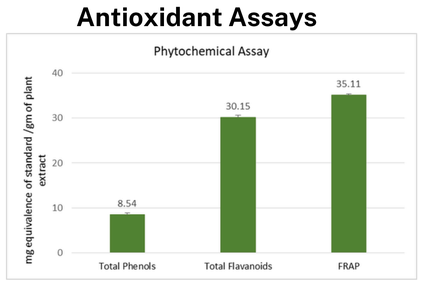
- Since the studies reporting the antioxidant and anticancer effect of leaves extract are few, the objective of our study was to evaluate the phytochemicals and antioxidant activity of ethanolic extract of *Clematis hyenei* and explore its anticancer activity against HeLa cervical cancer cells

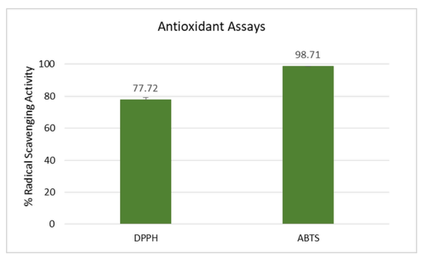
METHODOLOGY

- The Clematis heynei plant was collected from the Western Ghats (District: Satara) and authenticated before use.
- Ethanolic extracts were prepared by Maceration and Soxhlet method.
- Standard assays for determination of total phenols, flavanoids and radical scavenging activity by DPPH assay, ABTS assay and FRAP assay
- Cell based assays were done on Hela cells: MTT assay, Wound healing assay, Clonogenic assay, Methylene Blue staining and Hoecst staining assay

RESULTS

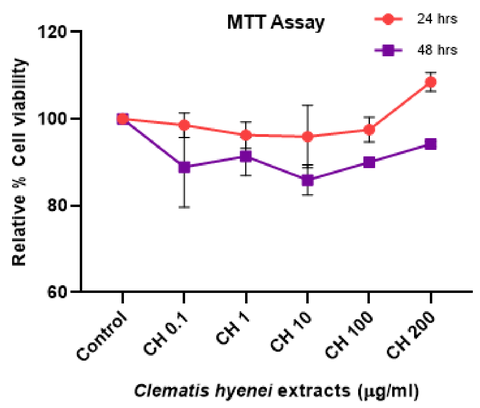
Antioxidant Assays





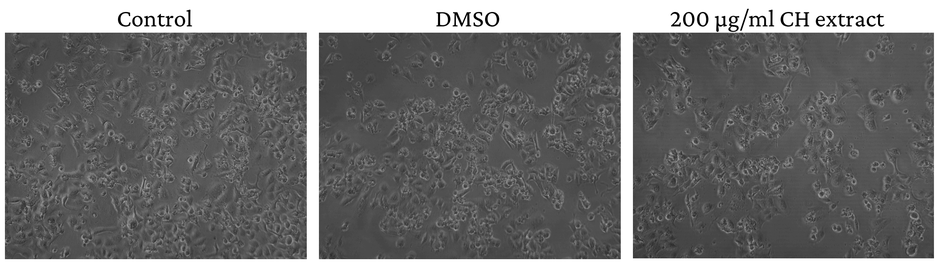
Total Flavanoids, FRAP activity

ABTS and DPPH activity



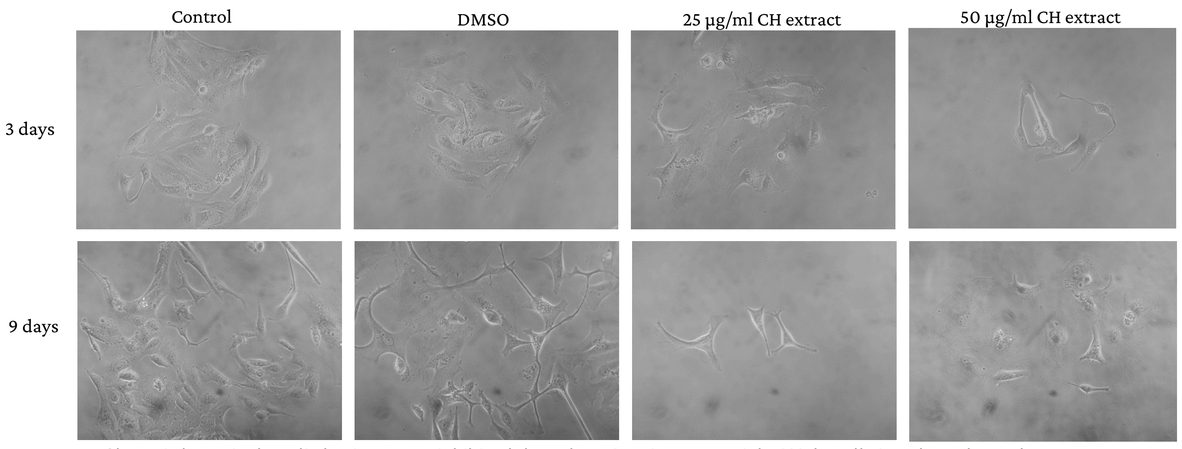
A marginal reduction in cell viability was observed after 48 hr of treatment with *Clematis hyenei* extracts

Cell morphology studies



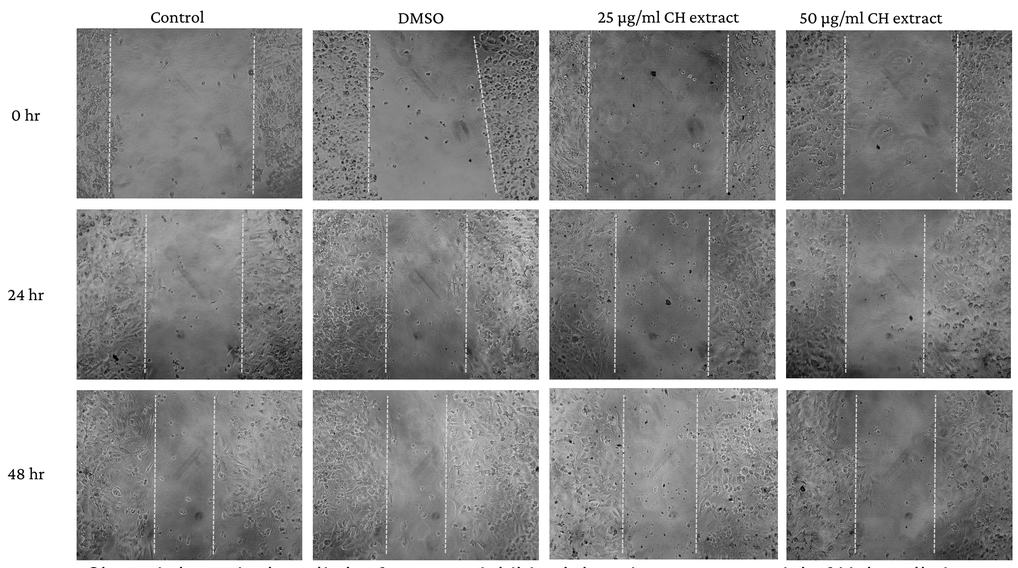
Clematis hyenei extracts induced morphological alterations in Hela cells (Total magnification 100X) post treatment of 48 hours

Clonogenic Assay



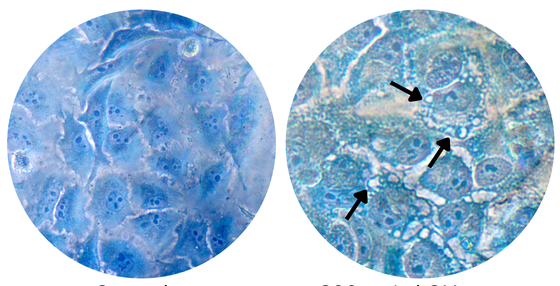
Clematis hyenei ethanolic leaf extracts inhibited the colony forming potential of Hela cells in a dose-dependent manner

Scratch Assay



Clematis hyenei ethanolic leaf extracts inhibited the migratory potential of Hela cells in dose-dependent manner

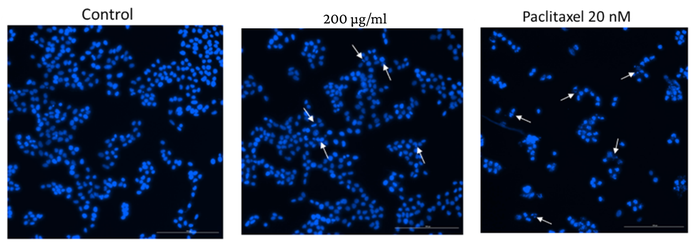
Methylene Blue Staining



Control 200 µg/ml CH extract

Cytoplasic granularity, membrane blebbing is very much evident in Hela cells treated with 200 µg/ml of ethanolic leaf extracts

Hoecst Staining



Clematis hyenei ethanolic leaf extracts at 200 µg/ml concentration induced apoptosis in Hela cells

CONCLUSION

- Invitro assays showed increased levels of total flavanoids, FRAP activity and significant amount of DPPH and ABTS radical scavenging potential of ethanolic leaf extracts of *Clematis hyenei*
- MTT assay showed a marginal growth inhibition of Hela cells post 48 hr of treatment, however at 200 µg/ml concentration, alterations in cell morphology were observed
- Migratory potential and colony forming ability of Hela cells was inhibited by Clematis ethanolic extracts in dose dependent manner. Extract at 50 µg/ml concentration completely inhibited cell migration and colony formation
- Methylene Blue Staining revealed cellular disruption and membrane blebbing.
- 6.1% apoptotic cell population was observed on Hoecst staining indicative of potential of the extracts to cause cellular stress and sensitize the Hela cells to apoptotic cell death.

FUTURE SCOPE

- To confirm the potential of ethanolic leaf extracts of *Clematis hyenei* to induce apoptotic cell death in varied cancer cell lines
- Deliniate the bioactive compounds in the leaf extract and study their anticancer activity
- Understand the molecular mechanism underlying the action of extract components

REFERENCES

- Estimation of bioactive compound using RP-HPLC and antioxidant, antidiabetic activity of aerial parts of Clematis heynei and Solanum virginianum. Pravin Morankar and Alok Pal Jain. Journal of Pharmacognosy and Phytochemistry 2019; 8(3): 3219-3223
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