## Exploring Cytotoxic and Atioxidant Properties of $Heliotropium\ calcareum$ in Polar and Non-Polar Extracts

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## **Article Information**

## **Identifiers and Pagination:**

Year: 2021 Volume: 2 Issue: 3

First Page: 239 Last Page: 247

**Publisher ID:** CNT-2-239

**DOI:** 10.2174/2665978602666210505104356

**Article History:** 

**Received Date: 21/09/2020** 

Revision Received Date: 13/02/2021 Acceptance Date: 24/02/2021 Electronic publication date: 2021

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**Background:** Plants are a vital source of natural drugs as the traditional use of plants as therapeutic agents for a variety of ailments has been traced back thousands of years. The utilization of *Heliotropium calcareum* has been evident since ancient times for treating various disease states like inflammation associated with gout and rheumatism, poisonous bites, and other skin disorders. The current research work was carried out to determine the phytochemistry and biological activities of the crude methanolic extract obtained through maceration from the aerial parts of *Heliotropium calcareum*.

*Methods*: The plant was collected from district Bhakkar, Punjab, Pakistan. Maximum phenolic (74.5 μg GAE/mg) and flavonoid contents (58.99 μg QE/mg) were observed in ethyl acetate fraction. Significant

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antioxidant potential was observed in ethyl acetate fraction with the highest free radical hunting activity of 92.6  $\pm$  6.7  $\mu$ M.

**Results:** Cytotoxicity assay using MTT dye was performed where non-polar (n-hexane) and polar (ethyl acetate) fractions displayed excellent cytotoxicity against HeLa cells (IC<sub>50</sub> = 79.95 ± 3.7<sup>1</sup>8 & 164 ± 4 µg/mL, respectively). Furthermore, the above fractions showed momentous results in cell cycle analysis and promising proapoptotic effect against cervical (HeLa) cancer cell lines. An n-hexane and ethyl acetate fraction were selected for cell cycle analysis to determine the quantitative measurement of the degree of apoptosis. According to the results given below in the figure, the cervical (HeLa) cancer cells were treated with n-hexane and ethyl acetate fractions at various concentrations. An increase in the cell population at G0/G1 phase and a decrease in the S-phase population concerning untreated cells suggested the G0/G1 phase arrest in n-hexane and ethyl acetate fractions treated HeLa cells.

**Conclusion:** Taken together, *n*-hexane and ethyl acetate fractions were found to be the most promising and active elements of *H. calcareum* and may be utilized to explore their cytotoxic effects further in the animal model.

**Keywords:** *Heliotropium calcareum*, breast cancer, cervical cancer, flow cytometry, non-polar extracts, Reactive Oxygen Species (ROS).