ARTICLE INFO

Antiproliferative Potential of *Callistemon lanceolatus* (Sm.) Seeds Against HeLa Cell Line

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**ABSTRACT**

*Callistemon lanceolatus* (Sm.) is a common ornamental plant native to Australia and Asia and demonstrate a spectrum of bioactivities. Only a scant literature is available on its anticancer prospects. Our previous findings reported a strong antimicrobial potential of aqueous extract of *C. lanceolatus* seeds, not only against the reference pathogenic microorganisms but also the resistant clinical isolate, MRSA. The current report documents another bioactivity of the *C. lanceolatus* ethyl acetate extract and partially purified phytoconstituents in terms of antiproliferative activity against HeLa cell line. This is apparently the first report on *C. lanceolatus* against HeLa cell lines, an oldest cell line against which both the test extract and phytoconstituents demonstrated good antiproliferative potential as compared to curcumin. Thus, further evaluation of the above may give some good insight in the field of cervical cancer research.

**Keywords:** Antiproliferative, Antimicrobial, *Callistemon*, Phytoconstituents

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INTRODUCTION:
Since ages, the plants have been a rich source of bioactive compounds with a potential in the field of healthcare and medicine. Various components of plant origin viz. Alkaloids, saponins, terpenoids, phytosterols and cardiac glycosides have shown antitumor activity in different experimental model systems (in-vitro and in-vivo) \[1\]. *Callistemon lanceolatus* (Sm.), commonly known as Bottle brush belonging to Myrtacea family, is cultivated for ornamental purposes. The genus *Callistemon* is a folk medicine with anticough, antibronchitis and insecticidal effects \[2\]. Some of the important bioactivities reported for this plant includes antibacterial \[3,4\], antifungal\[5\], antioxidant\[6\], anti-aflatoxin \[6\] and antihelminthic[2] etc. The antimicrobial activity of *C. lanceolatus* (Sm.) seeds has been previously reported \[3\] by our lab and in this paper it has been further screened for its antiproliferative potential which has earlier been reported for *Callistemon viminalis* against various cancerous cell lines\[7\]. Apparently, the antiproliferative potential of *C. lanceolatus* has not been reported so far which provides relevance to the study, where both ethyl acetate extract and partially purified phytoconstituents of the seeds have been used to explore their antiproliferative potential against cancerous HeLa cell line.

MATERIAL AND METHODS
Human cancer cell line HeLa (Cervix) was procured from the National Centre for Cell Science, Pune, India. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with streptomycin (100 U ml\(^{-1}\)), gentamicin (100 µg/ml), amphotericin B (0.25 µg ml\(^{-1}\)) and 10% fetal bovine serum (Himedia) in a CO\(_2\) incubator (5% CO\(_2\); 90 % Relative Humidity) at 37°C. The *in vitro* cytotoxicity of ethyl acetate extract and partially purified phytoconstituents was determined against HeLa (Cervix) cell line by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide) assay\[8\] where 5 x 10\(^3\) cells were coated in each well of 96 well microtiter plate and incubated at 37°C, 5% CO\(_2\) for 24 h. Thereafter, the cells were given the relevant treatment with ethyl acetate extract and partially purified phytoconstituents for 48 h, followed by washing and addition of 100 µl of fresh medium with 20 µl MTT solution (5 mg ml\(^{-1}\)) to each well. The cells were incubated at 37°C, 5% CO\(_2\) for 4 h. After incubation the medium was removed and formazan product was dissolved in 100 µl of DMSO (Dimethyl sulfoxide) and shaken for 10 min \[9\]. The optical density was measured at 550 nm by microplate reader. Percentage of cell growth inhibition was calculated by using formula:

\[
\% \text{ cell growth} = \frac{\text{OD of treated cells}}{\text{OD of control}} \times 100
\]

RESULTS AND DISCUSSION
The cellular response of Ethyl acetate extract and partially purified phytoconstituents (cardiac glycosides and phytosterols) from *C. lanceolatus* seeds on the HeLa (Cervix) cell lines is represented in Fig 1 where the ethyl acetate extract of *C. lanceolatus* seeds demonstrated the best antiproliferative potential followed by the partially purified cardiac glycosides and phytosterols which in turn showed better potential as compared to curcumin. The better potential of ethyl acetate extract may be attributed to the cumulative effect of different phytoconstituents working synergistically thereby showing better efficacy in comparison to partially purified phytoconstituents. The proliferation of the cells in case of ethyl acetate extract at 125 µg/ml was 5.43% closely followed by partially purified cardiac glycosides i.e. 6.38 %. While in case of phytosterols it showed a slight increase in proliferation i.e. 17.65 %. The antiproliferative potential of both ethyl acetate extract and partially purified phytoconstituents showed better efficacy as compared to curcumin which showed 19.67% inhibition at 100 µM. Also, the efficacy of the ethyl acetate extract and partially purified phytoconstituents of *C. lanceolatus* seeds was better in comparison to previous reports on ethanolic extracts of *Markhamia tomentosa* leaves at 189 µg/ml \[10\].
CONCLUSION
The current work, apparently for the first time proposes ethyl acetate extract and partially purified phytoconstituents isolated from C. lanceolatus seeds, an important natural remedy as antiproliferative agents against HeLa cell line, by in vitro MTT assay. Therefore further scaling up of these upto clinical trials may provide useful information in the field of cervical cancer research. Further studies on the antiproliferative mechanism of these constituents needs to be worked out in order to provide more convincing evidence.

DECLARATIONS
Ethics approval and consent to participate
As the study does not involve any animal model system so no such approval is required.
Consent for publication
Both the authors hereby declare their consent for publication
Availability of data and materials
The study does not involve any such data and will be provided if required.

Competing interests
Both the authors declare that they have no competing interests.

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Author contributions
DSA, being the Ph.D supervisor contributed in analysis and drafting of the manuscript. Experimental work was done by Lovedeep Nim. The design of the experiments as well as the manuscript was contributed equally by both the authors.

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REFERENCES


