IN VITRO ANTICANCER ACTIVITY OF LEAVES AND STEM OF AZIMA TETRACANTHA LAM.

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ABSTRACT
The present study deals with anticancer activity of *Azima tetracantha*. The leaves and stem of the plant material were extracted with hexane and ethanol using soxhlet extraction. Anticancer activities of various extracts were assayed with standard MTT colorimetric procedure against MCF-7 cell lines. From the analysis it was found that ethanolic leaf extract of *Azima tetracantha* showed MCF-7 cell line inhibition at ng range tested dose and other three extracts (hexane - leaf, stem and ethanolic stem) also displayed anticancer activities against MCF-7 cell line. Further work is in progress to evaluate the chemical constituents present in the extract.

KEYWORDS: *Azima tetracantha* Lam., Antioxidant, Anticancer, MTT Assay, MCF-7 cell lines.

INTRODUCTION
The word ‘cancer’ originated from ‘cancrum’ which is a Greek word for crab as it furnishes with claws on both sides of its body, so does the disease.[1] Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and in some cases, to metastasize, invade and spread to distant sites of the body.[2] Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism).[3,4] It
can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy.\textsuperscript{[5]} Natural products have had a major impact on longevity and quality of life is in the chemotherapy of cancer. In fact, most major anticancer drugs are derived from plants or microorganisms. Examples include, Bleomycin, Doxorubicin, Daunorubicin, Vincristine, Mitomycin, Streptozocin, Paclitaxel (Taxol \textsuperscript{TM}), Irinotecan (a camptothecin derivative), Etoposide and Tenoposide (podophyllotoxin derivatives).\textsuperscript{[6]}

Azima tetracantha Lam., is a unique folk medicinal plant known as Mulsangu in Tamil, belonging to the family “Salvadoraceae”, which has various medicinal properties like stimulant, antispasmodic, anti rheumatism, diuretic, anti inflammatory, anti microbial, hypoglycemic, antioxidant and hypolipidemic activity. It is also used in the treatment of cancer, dyspepsia and chronic diarrhoea.\textsuperscript{[7,8]} Phytochemical composition of the plant like alkaloids\textsuperscript{[9]}, triterpenoids\textsuperscript{[10]} and flavonoids\textsuperscript{[11]} etc. were also reported. Clinical data showed that in the past there were herbs with anticancer property but the scientific validity of Azima tetracantha have not been established. Hence, the present study was designed to evaluate the anticancer activity of various extracts of leaves and stem of Azima tetracantha.

MATERIALS AND METHODS

Collection and identification of the Plant Material

The plant materials (leaves and stem) of Azima tetracantha Lam., were collected from Vellore district, Tamilnadu, India. The collected plant materials were botanically identified and authenticated by the Botanist Dr. Aravind, National Institute of Siddha, Tambaram, Chennai. The herbarium specimen was prepared and deposited at Department of Pharmacognosy, College of Pharmacy, Madras Medical College, for future reference.

Extraction of plant material

Freshly harvested leaves and stem were air- dried at room temperature for a period for 2 weeks. Dried materials were coarsely grounded and stored in an air- tight container. About 200g of dried powdered leaves and stem were packed well in soxhlet apparatus and was subjected for hot extraction with hexane and ethanol (99.9\%) at 18 hrs. The extract was filtered while hot and concentrated under Rotary vacuum evaporator in order to remove the solvent completely, dried and kept in a desiccator till further use.
IN VITRO ANTICANCER ACTIVITY

Chemicals and reagents
MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) invitrogen, USA. Acridine orange were obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture
MCF 7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37°C in a humidified atmosphere of 5% CO2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay[12]
Cell viability was measured with the conventional MTT reduction assay. Briefly, MCF 7 cells were seeded at a density of 5×10³ cells/well in 96-well plates for 24 h, in 200ul of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37° C for 4 h and then with DMSO at room temperature for 1h. The plates were read at 595nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments.

Cell viability (%) = Mean OD/Control OD x 100.

RESULTS
MCF-7 cell line inhibition of various extracts using MTT assay showed in Table 1 and Figure 1 and its graphical representation showed in Figure 2.

Table 1: In-vitro anticancer activity of the extracts of Azima tetracantha

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>MCF-7 Cell lines</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ethanol Leaf Extract</td>
</tr>
<tr>
<td>Control</td>
<td>4.29</td>
</tr>
<tr>
<td>1ng</td>
<td>30.95</td>
</tr>
<tr>
<td>10ng</td>
<td>31.01</td>
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<tr>
<td>100ng</td>
<td>41.98</td>
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<tr>
<td>1µg</td>
<td>54.14</td>
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<tr>
<td>10µg</td>
<td>56.41</td>
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<tr>
<td>100µg</td>
<td>59.09</td>
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</table>
DISCUSSION
Cancer is often associated with increased risk of death and the toxic side effects caused by the modern medicine, many cancer patients seek alternative and complementary methods of treatment such as usage of phyto medicine.

The present study shows a dramatic in-vitro anticancer activity of various extracts of A. tetracantha on human breast cancer cell line (MCF-7) at increasing concentrations. It showed that the ethanolic extract have more anticancer activity on MCF-7 cell line in the ng range.
CONCLUSION
The present study showed that *Azima tetracantha*, ethanolic leaf extract might be a potential alternative agent for human breast cancer therapy. Hence, it is anticipated that *A. tetracantha* would be a useful pharmaceutical material to treat breast cancer. Future research should focus on the molecular mechanism of *A. tetracantha* for anticancer action. There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s) to treat breast cancer.

REFERENCE