DNA binding ability and MTHFR inhibitory activity of β Carboline harmaline of *Alangium salviifolium* on MCF-7

P. Bama

Departments of Biochemistry, Sri Sairam Siddha Medical College and Research Centre, Sai Leo Nagar, West Tambaram, Chennai-600 044, Tamilnadu, India.

**ABSTRACT**

Plant and plant products are the major source for the discovery of new drugs. *Alangium salviifolium* utilized in traditional medicine has resourceful medicinal properties. The aim of present study was to evaluate the biological activity of alkaloid β Carboline harmaline of *Alangium salviifolium* in cell growth activity inhibition. The potentiality of the alkaloid compound in inhibition of MTHFR with increased cytotoxicity in vitro was determined β Carboline harmaline of *Alangium salviifolium* at 320µm concentration exhibited MTHFR and cell growth inhibitory activity. The result suggested alkaloid β Carboline harmaline of *Alangium salviifolium* can be used as potential chemotherapeutic drug.

**Key words:** *Alangium Salviifolium*, β Carboline harmaline, chemotherapeutic drug

**INTRODUCTION**

*Alangium* is the only genus in the Alangiaceae, which is composed of ca 20 species (shrubs or trees) occurring in the Tropics of the Eastern Hemisphere (Benson, L. (1959). All parts of the *Alangium salviifolium* (L.f.) Wangerin used in folk medicine of India, China and Phillipines. The leaves, roots and flowers have a wide range of pharmacological activities and are used for diabetes, eruptive fevers and as cardiotonic. The powder of roots is given to treat dyspepsia, diarrhoea and piles. The flower has arid, bitter sweetish taste; removes impurities from blood, refrigerant and alleviative of cough, biliousness, aphrodisiac, vomiting, giddiness, worm infestation and burning of the skin. The seeds are said to be stomachic and restorative (Ashik Mosaddika *et al*., 2000). Biological activities for other *Alangium* plants are diverse and include DNA damaging activity (Xu *et al*., 2003). *Alangium species* contains alkaloids, iridoids and Terpenoids (Itoh *et al*., 1995). Cancer is a chronic degenerative disease, characterized by uncontrolled growth and spread of abnormal cells. Breast cancer is the most common malignancy in women. More importantly, approximately 30% of all patients with early stages of breast cancer will develop recurrent disease, which is predominantly metastatic and resistant to treatment (Longley and Johnston, 2005). The increasing incidence of breast cancer reported over the last a few years has led to development of new anticancer drugs. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al*., 2006). The present aim of the study was to evaluate the biological activity of alkaloid β Carboline harmaline of *Alangium Salviifolium* in inhibition of cancer cell growth and MTHFR enzyme activity.
MATERIALS AND METHOD

Plant Materials

Leaves of *Alangium salviifolium* were collected from Medicinal Plant Garden at Sri Sairam Siddha Medical College and Research Centre, West Tambaram, Chennai 600 044.

Screening isolation and Identification of alkaloid of Alkaloid

The leaves were air-dried and ground into uniform powder using mechanical grinder and the powder was sieved through 60# sieve. 50 g of powder was soaked in methanol for 24 hrs and extracted in Soxhelt extractor. The extract was distilled using rotary evaporator and concentrated. Screening for presence of alkaloid was determined using standard methods (Harborne, 1973; Treaseand Evans, 1989; Sofowara, 1993). Extraction of alkaloids was done by using Surya and John, 2001 method. Dry leaves (3.0 kg) were soaked in methanol for 24 h. The methanol extract was fractioned by dry flash chromatography on silica gel using chloroform and methanol of increasing polarity, yielding fractions which were reduced to alkaloid after comparison by TLC. The purified compound was identified by two dimensional correlated Proton Nuclear Magnetic Resonance (\(^1\)H NMR) spectra, \(^13\)C NMR spectra and the IR spectrum.

Cytotoxicity assay

The cytotoxicity effect of *A. salviifolium* alkaloidal compound in various concentrations (20, 40, 80, 160, 320 µM/ml) was determined on MDA-MB 468 cells in 24 hrs in CO\(_2\) filled incubator at 37 °C. The cell viability was assessed using MTT assay (Selvakumaran et al., 2003).

Inhibition assay of colony formation

Colony-forming ability of the MDA-MB 468 cells was assayed by replating them in specified numbers (300–400/well) in 6-well plates treated with 20, 40, 80, 160, 320 µM/ml of *A. salviifolium* alkaloidal compound. The cells were stained with 0.5% crystal violet in absolute ethanol after 12 days of incubation, and colonies with >50 cells were counted under a dissection microscope.

Determination of cell proliferation

MDA-MB 468 cells were seeded onto glass coverslips at an initial density of 4.0 X104/cm\(^2\) and allowed to grow for 12 hr, then treated with 20, 40, 80, 160, 320 µM/ml *A. salviifolium* alkaloidal compound for 48 hr and then Cells were incubated with BrdU in medium (20 mg/ml) for 12 hr. Further Inhibition of cell proliferation was analyzed by Thor et al.,1999 method.

Assay for Methylene tetrahydrofolate reductase

A. 50 mM Potassium Phosphate Buffer with 100 mM Potassium Chloride, pH 7.5 at 25°C
B. 0.002% (w/v) Tetrahydrofolic Acid with 0.002% (v/v) Formaldehyde and 0.1% (v/v) 2-Mercaptoethanol Solution (FH4)
C. 20 mM ß-Nicotinamide Adenine Dinucleotide Phosphate (ß-NADP)
D. 5.10-Methylenetetrahydrofolate Dehydrogenase Enzyme Solution

Procedure:
Pipette (in milliliters) the following reagents into suitable cuvettes:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>2.80</td>
<td>2.80</td>
</tr>
<tr>
<td>D</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Mix by inversion and equilibrate to 25°C. Monitor the A340nm until constant using a suitably thermostatted spectrophotometer. Then add:

<table>
<thead>
<tr>
<th>Reagent C (ß-NADP)</th>
<th>0.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Immediately mix by inversion and record the increase in A\(_{340}\)nm for approximately 5 minutes. Obtain the r A\(_{340}\)nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

\[ \Delta A_{340\text{nm/minute Test}} - \Delta A_{340\text{nm/minute Blank}} \]

Units/mg enzyme = \[
\frac{(7.1) (mg enzyme/ml RM)}{2 \ RM} \]

7.1 = Millimolar extinction coefficient of ß-NADPH at 340 nm under the conditions of the assay.

2 RM = Reaction Mix
RESULT AND DISCUSSION

Phytochemical screening of methanol leaf extract of A. salviifolium

The presence of alkaloid in A. salviifolium was screened. The presences of alkaloids iridoids and terpenoids have been reported in Alangium species (Nobuko et al., 2006). The phytochemicals of plants possessed variety of medicinal properties to cure diseases (Sankaranarayanan et al., 2010).

Table 5. Phytochemical Screening of Leaf extract of A. salviifolium

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Phytochemical Constituents</th>
<th>Observation</th>
<th>Methanol extract of Alangium salviifolium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragenorff’s test Orange / red precipitate +</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayers test</td>
<td>Yellowish precipitation +</td>
</tr>
</tbody>
</table>

Identification of the compound

The alkaloidal compound had the molecular formula of 230.1059 by m/z. the compound was identified as β Carboline Harmane by comparison of its $^1$H-13C NMR spectroscopic data with those in the literature (Duan et al., 1998). The $^1$H-NMR spectrum showed three one-proctor. Aromatic signals at $\delta$ 7.60 (d, J = 8.9 Hz, H-9), $\delta$ 6.86 dd, J = 8.9, 1.8 Hz, H-10) and $\delta$ 6.95 (d, J = 1.8 Hz, H-12). The appearance of C-2 and C-13 ($\delta$ 145.0, 138.9) in AS2 Harmaline was compared to that of tetrahydroharmine ($\delta$ 137.7, 138.0). The upfield resonance of C-3, is due to the gauche effect of the carbonyl function (Bolle et al., 1996).

Cytotoxic activity

A. salviifolium alkaloidal compound showed a dose- and timedependent inhibitory effect on the growth of MDA MB-468 breast cancer cells (P < 0.05). IC$_{50}$ was determined, and the maximal inhibition of cell growth (>80%) was obtained at 320 µM/ml (Fig-1). curcumin and carvacrol exhibited toxicity acted as an apoptotic inducer in MDA-MB 231 cells (Prasad et al., 2009). A. salviifolium leaves alkaloidal extract revealed the antioxidant and anticancer properties and also inhibited cell proliferation (Kothapalli and Raman, 1999).

Inhibition of colony formation

The inhibitions of colony formation of A. salviifolium compound MDA-MB-468 were determined. The A. salviifolium alkaloidal compound at 320 µM concentration suppressed maximum number of colonies. A. salviifolium alkaloidal compound suppressed the colony numbers of MDA-MB-468 cells to 190±1.1 (P < 0.05), at 320 µM/ml where as the Untreated MDA-MB-468 cell produced 381±1.6 colony numbers. Plant extracts exhibit potential anticancer properties with inhibited colony formation of multiple human cancer cell (Ju et al., 2004). Achyranthes aspera leaves extract efficiently decreased 77% of colony formation of human breast cancer cell line (Pochi et al., 2010).

Inhibition of cell proliferation

Brdu labeled A. salviifolium alkaloidal compound treated cells at 320 µM/ml exhibited 60 ± 2.0% cell proliferation. Deoxy tubulosine compound of A. salviifolium efficiently inhibited cell proliferation and suppressed cell growth (Bama et al., 2012) Trans-[PtCl$_2$(NH$_3$)] (thiazole)] (trans-ammendedichloro (thiazole) platinum (II[TV1]) (ATZ) powerfully killed the cancer cell line and decreased cell proliferation (Nicholas et al., 2004).

Inhibition of MTHFR enzyme activity

MTHFR, elevate plasma levels of homocysteine and decreased 5-methyltetrahydrofolate levels there by increasing the risk of breast cancer (Frosst et al., 1999). The investigation on the MTHFR Enzyme inhibitory activity in A. Salviifolium treated MDA-MB-468 cells resulted in maximum inhibition of Enzyme activity at 320 µM concentration. About 80% of inhibition activity was determined in treated cells. MTHFR is a critical enzyme in one carbon metabolism redirecting the pool of folate from DNA synthesis / repair to methylation (Sonia et al., 2009). β-carboline benzoquinolidine plant alkaloid inhibited the DTHFR Enzyme activity and controlled the cancer cell growth (Kothapalli and Raman, 1999).

CONCLUSION

Traditional medicine is one of the most inexpensive and easily accessible sources of treatment in the healthcare. Selection of natural products like plants provides new drugs for the treatment of various diseases. Alangium salviifolium is a shruby tree grown in tropical parts of the world. The plant is used in traditional medicine due to its medicinal properties. Alangium salviifolium leaf methanolic extract was screened for the presence of alkaloid and the alkaloid compound was isolated and characterised as β carboline harmaline. Investigation on anticancer properties of the plant suggested that the plant inhibited the cancer cell growth and MTHFR enzyme activity. The result revealed that...
Alangium salviifolium β carboline harmaline can utilized as chemopreventive drug.

ACKNOWLEDGEMENT

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REFERENCE


3. Benson L. Plantes classification 1959;277-278.


