

CYTOTOXICITY EFFECT OF ALKALOIDAL COMPOUND ISOLATED FROM THE LEAF OF *ALANGIUM SALVIIFOLIUM* ON IN-VITRO MCF-7 CELL LINE

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Received: 02 July 2012, Revised and Accepted: 12 Aug 2012

ABSTRACT

Alangium salviifolium (AS) a small deciduous tree has been identified as an important resource in traditional medicine due to its medicinal properties. The Phytochemicals in dried leaves of *A. salviifolium* was investigated. The cytotoxicity of alkaloidal compound isolated from leaves of *A. salviifolium* on invitro human breast cancer cell line MCF-7 was evaluated by MTT assay, Microscopic observation, DNA Fragmentation analysis. A total amount of 320µg/ml of alkaloidal compound showed 80% of viability on MTT assay and morphological changes in MCF-7 was observed. Further more alkaloidal compound exhibited its potential on inhibition of cell proliferation, cell cycle control, and DNA intercalation activity.

Keywords: *Alangium salviifolium*, Alkaloidal compound, Cytotoxicity

INTRODUCTION

Alangium salviifolium (Alangiaceae) is a tropical deciduous tree with strong, yellowish root. It is widely distributed in hotter parts in India. It is also distributed in Malaya, South China to Philippines, East Africa and Tenasserim. The plant is used as medicine in India, China and Philippines¹. Different parts of this plant are reported to possess acrid, astringent, emollient, anthelmintic diuretic and purgative properties. The leaves are used as a poultice in rheumatism^{2, 3}. *Alangium species* contains alkaloids, iridoids and terpenoids⁴. Breast cancer is one of the main life-threatening diseases that a woman may have to face during her lifetime⁵. The increasing incidence of breast neoplasia reported over the last few decades has led to development of new anticancer drugs, drug combinations, and scientific exploration in progress of synthetic, biological, and natural products⁶. The present aim was to study the effect of alkaloidal compound from leaf of *Alangium salviifolium* on in-vitro MCF-7 cell line.

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.

Phytochemical screening and isolation 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 phytochemicals was determined using standard methods^{7, 8, 9}. Extraction of alkaloids was done by using Surya and John¹⁰ method. Dry leaves (3.0 kg) were soaked in methanol for 24 h. The methanol extract was fractionated 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.

Identification of compound

The purified compound was identified by two dimensional correlated Proton Nuclear Magnetic Resonance (¹H NMR) spectra were recorded at 400MHz on JEOL GsX 400 Spectrophotometer and ¹³C NMR spectra were recorded at 100 MHz on JEOL GsX400 spectrophotometer as indicated, chemical shifts were reported in ppm(δ) using Tetramethylenesilane as internal standard and coupling constants were expressed in Hertz. Mass spectra was obtained using a VG 1250 instrument at 70 eV. The IR spectrum was

recorded on a Bio -Rad FTS - 7. An optical rotation was determined using a perkin- Elmer polarimeter model 241 set on the sodium line.

Cytotoxicity assay

The cytotoxicity effect of *A. salviifolium* alkaloidal compound in various concentrations (20, 40, 80, 160, 320 µM/ml) was determined on MCF-7 cells in 24 hrs in CO₂ filled incubator at 37 °C. Then the cell viability was assessed using MTT assay¹¹.

Inhibition assay of colony formation

Cells were assayed for colony-forming ability 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. After 12 days of incubation, cells were stained with 0.5% crystal violet in absolute ethanol and colonies with >50 cells were counted under a dissection microscope.

Determination of cell proliferation

Cells were seeded onto glass coverslips at an initial density of 4.0 X10⁴/cm² and allowed to grow for 12 hr, then treated 20, 40, 80, 160, 320 µM/ml *A. salviifolium* alkaloidal compound for 48 hr. Cells were incubated with BrdU in medium (20 mg/ml) for 12 hr. Further Inhibition of cell proliferation was analyzed by Thor et al.¹² method.

Determination of cell cycle control

A total of 1X10⁷ cells were harvested from control culture and cells treated with 320 µM/ml *A. salviifolium* compounds for 48 h. Cell cycle distribution and apoptotic cells was analyzed in *A. salviifolium* compounds treated Human Breast Cancer cell MCF-7 by flow cytometry (FCM) analysis¹³.

Detection of DNA intercalation by Methyl green assay

Different quantity (20, 40, 80, 160, 320 µM/ml) of *A. salviifolium* alkaloidal compound was added to several aliquots of 15 µl of methyl green solution (1 mg/ml) and 1 ml of DNA solution (30 µg/ml in 10 mM phosphate buffer, pH 7.5). Optical Density 630 was obtained initially as well as after sample incubation at 37 °C for two hours in darkness¹⁴.

RESULTS

Phytochemical screening of methanol leaf extract of *A. salviifolium*

The phytochemical screening of the *A. salviifolium* studied showed the presence of alkaloids flavonoids terpenoids, saponins and tannins (Table 1). Many commercially proven drugs used in modern medicine was initially used in crude form in traditional or folk

healing practices, or for other purposes that suggested potentially useful biological activity¹⁵. The medical plant possesses variety of phytochemicals which help human from escaping from various diseases¹⁶. The phytochemical analysis results were in similar to previous reports of alkaloids flavonoids terpenoids, saponins and tannins from *Alangium species*^{17,18,19}.

Identification of compound

Isolated *A. salviifolium* alkaloidal compound as yellow crystals showed UV maxima at 248 nm and 348 nm 460 nm and IR bands at 2920, 2875,

1610,1600cm⁻¹. ¹H NMR showed the presence eighteen protons (Table-2). The ¹³C NMR spectra showed the presence of twenty carbons (Table-2). The multiplicities of the carbon were determined by DEPT pulse sequence which showed the presence of six CH, three CH₂ two CH₃ and nine quaternary carbon atoms. The EMIS spectrum of the compound showed the molecular ion peak at m/z 336.1180. Thus the structure analysis data characterized the compound as Deoxytubulosine (C₂₀H₁₈NO₄). The IR and UV spectrum,¹H-NMR shifts,¹³C-NMR data mass spectrum were identical to those described in the published literature for Deoxytubulosine alkaloids²⁰.

Table 1: Phytochemical screening of methanol leaf extract of *A. salviifolium*

S. No.	Constituents	Methanol leaf extract of <i>A. salviifolium</i>
1	Alkaloids Dragendorff's test	+
2.	Flavonoids Alkalai Reagent	+
3.	Glycosides Bornbager's test	+
4.	Tannin FeCl ₃ test	--
5.	Saponins Frothing test	--
6.	Terpenoids Nollers test	+

-- = Negative (absent); + = Positive (slightly present)

Table 2: ¹³C-NMR and ¹H-NMR spectra of Alkaloidal compound of *A. salviifolium*

Carbon No	¹³ C-NMR	Multiplicity (DEPT)	¹ H-NMR Multiplicities of proton (δ) Signals
1	10.5.4	CH	7.39 s
2	148.6	-C-	-
3	151.0	-C-	-
4	107.9	CH	6.38 s
4a	128.9	-C-	-
5	27.2	CH ₂	3.15-3.26 m
6	49.8	CH ₂	4.75-4.80 m
8	145.9	CH	9.40 s
8a	122.1	-	-
9	144.8	-C-	-
10	150.5	-C-	-
11	127.2	CH	7.90 ABd (J11, 12+9.0Hz)
12	121.8	CH	7.89 ABd (J11, 12+9.0Hz)
13	121.8	CH	8.35 s
14	138.2	-C-	-
14a	124.1	-C-	-
OCH ₂ O	101.1	CH ₂	6.00 s
OCH ₃	61.9	CH ₃	4.19 s
OCH ₃	56.3	CH ₃	4.07

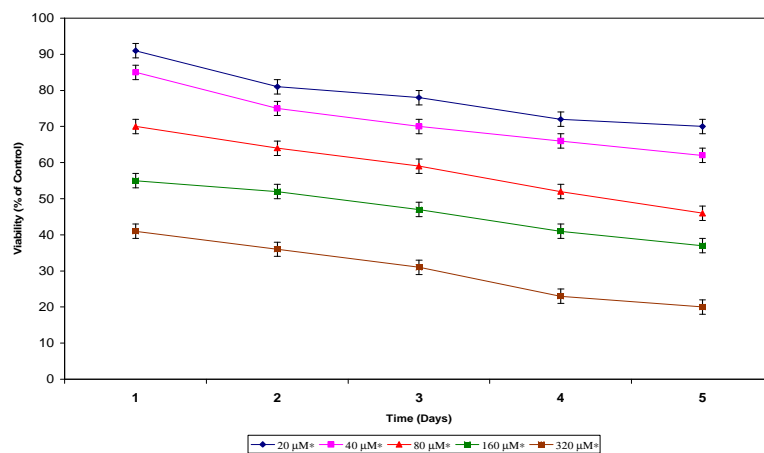


Fig. 1: Cytotoxic Activity of *A. salviifolium* Alkaloidal Compound against Human Breast Cancer Cells MCF-7

Cytotoxic activity of *A. salviifolium* compound against human breast cancer cells

A. salviifolium alkaloidal compound showed a dose- and time-dependent inhibitory effect on the growth of MCF-7 breast cancer cells ($P < 0.05$). IC_{50} was determined, and the maximal inhibition of cell growth (>80%) was obtained at 320 $\mu\text{M/ml}$ (Fig-1). Plants and their products both as extracts and isolated compounds extensively used as chemo preventive agents against various types of cancers²¹. Results from previous studies have shown similar effects of alkaloidal compound of *Uncaria tomentosa* on MCF-7 cancer cell with IC_{50} value ranging between 29.86 μml^{22} .

IC_{50} values of cancer cell MCF-7 determined after treated cell were incubated for 72h with *A. salviifolium* alkaloidal compound. OD values of each treated group were compared with that of the Control

at the same time point, the single (*) indicates a significant difference from the control ($P < 0.05$), one way Anova Dunnett C Test. Results are mean value \pm standard deviation of independent experiments performed in triplicate.

Effect of *A. salviifolium* alkaloidal compound on human breast cancer cells for inhibition of colony formation

Untreated MCF-7 produced 385 ± 1 colony numbers whereas *A. salviifolium* alkaloidal compound suppressed the colony numbers of MCF-7 cells to 109 ± 1 ($P < 0.05$), at 320 $\mu\text{M/ml}$ (Fig-2). Effect of *A. salviifolium* compounds on colonogenic survival assay closely paralleled with the previous report that suggested the efficient MCF-7 cancer cell killing of (trans-amminedichloro (thiazole) platinum (II[TV1]) with decreased colony formation²³.

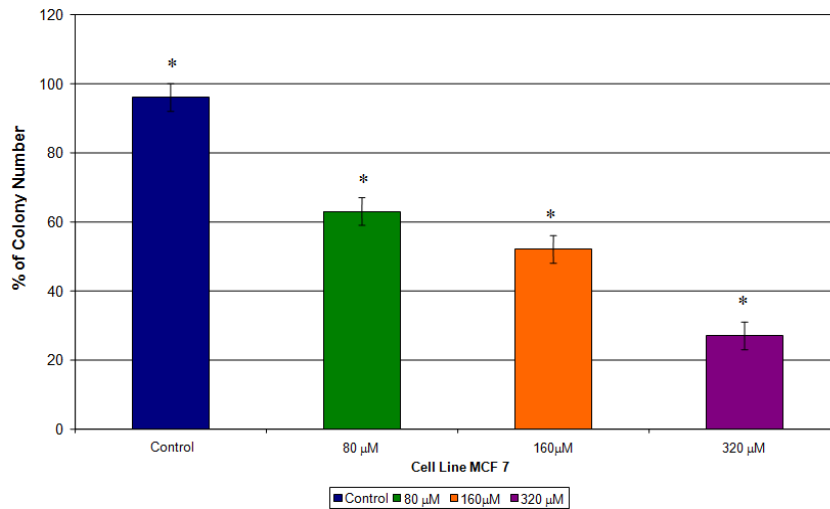


Fig. 2: Colony Formation inhibition of *A. salviifolium* alkaloidal compound on Human Breast Cancer Cells MCF-7

The Colony Number of *A. salviifolium* alkaloidal compound treated cells were compared with that of the control, the single (*) indicates a very significant difference from the Control.

($P < 0.05$), one way ANOVA Dunnett C Test. Results are mean values \pm standard deviation of independent experiments performed in triplicate.

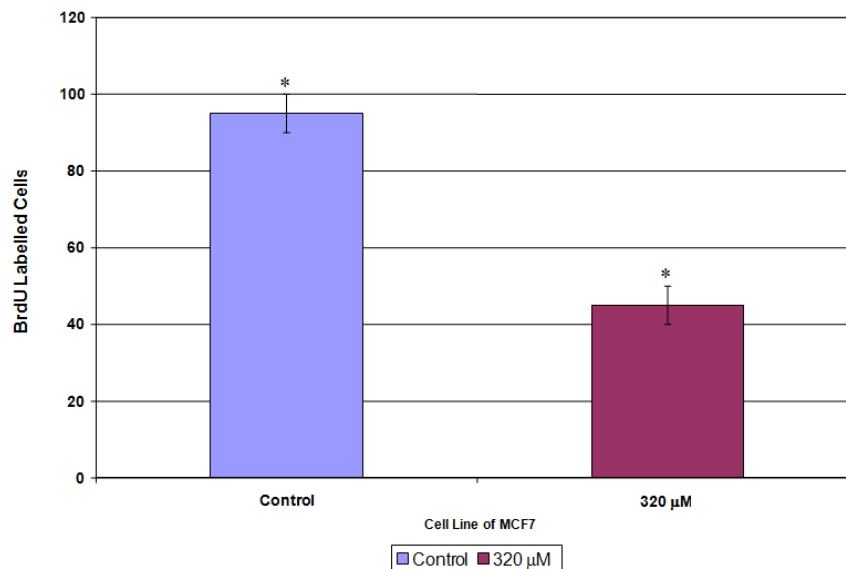


Fig. 3: Cell Proliferation inhibition of *A. salviifolium* alkaloidal compound on Human Breast cancer cell MCF-7

BrdU incorporation of *A. salviifolium* alkaloidal compound treated cells were compared with that of the control, the single (*) indicates a very significant difference from the Control.

($P < 0.05$), one way ANOVA Dunnett C Test. Results are mean values \pm standard deviation of independent experiments performed in triplicate.

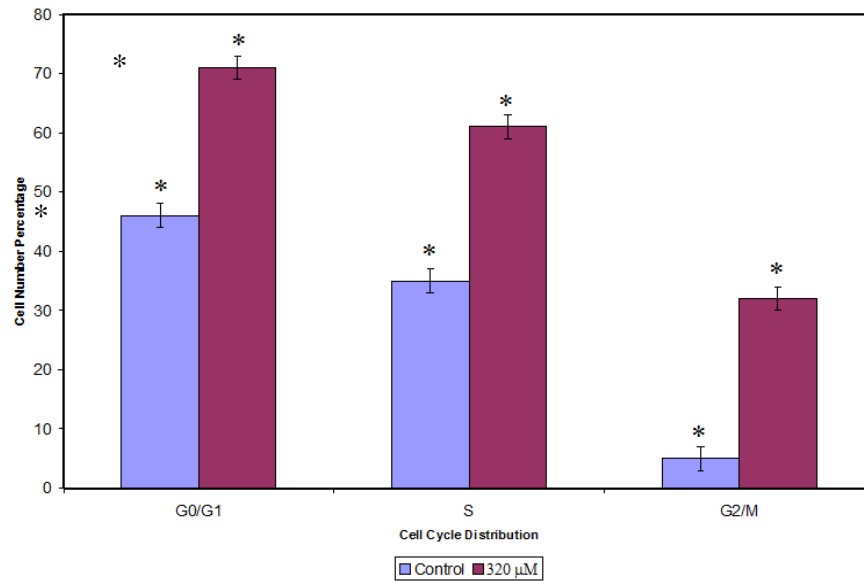


Fig. 4: Cell Cycle Analysis of *A. salviifolium* alkaloidal compound on Human Breast Cancer Cell MCF-7

Effect of *A. salviifolium* alkaloidal compound on human breast cancer cells for Inhibition of cell proliferation

BrdU-labeled cells in 320 µM/ml. *A. salviifolium* alkaloidal compound treated cells were $45 \pm 1.5\%$ ($P < 0.05$), where as untreated cells showed $> 90\%$ cell proliferation (Fig-3). Until now many investigators used many useful methods to analyse cell kinetics and various markers of cell proliferation²⁴. *A. salviifolium* compounds colonogenic survival assay closely parallel with the previous report that suggested a strong suppression in cell growth and proliferation in the human breast cancer cell lines by celecoxib²⁵

A. salviifolium alkaloidal compound Methyl green assay for detection of DNA intercalation

By using methyl green expelling test, it was shown that *A. salviifolium* alkaloidal compound displaced that dye from its complex with DNA, and indicating that possessed the lowest affinity to DNA (Fig-5). The potency of *A. salviifolium* alkaloidal compound to cause DNA damage was analogous to the previous report on the effect of *Alangium javanicum* induce DNA damage in yeast tester strain and also revealed that biological activities for other *Alangium* plants are diverse and include DNA damaging activity²⁷.

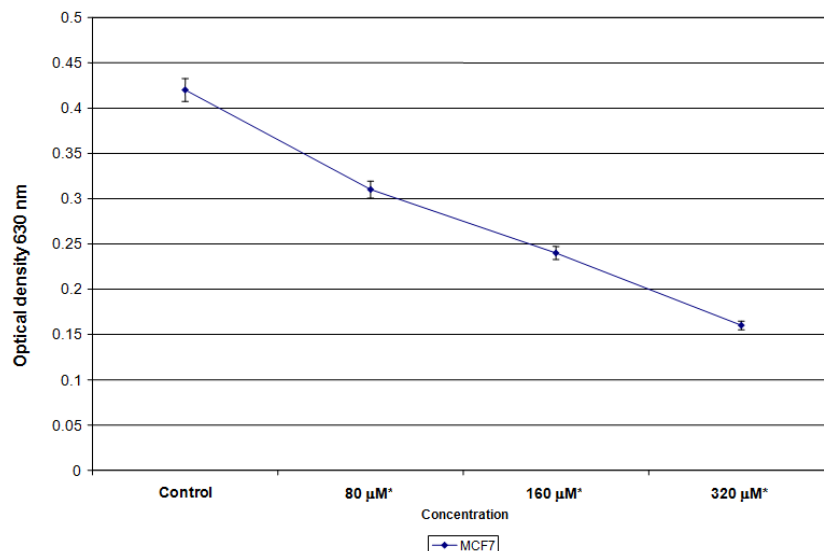


Fig. 5: Effect of *A. salviifolium* alkaloidal compound on DNA intercalation in MCF-7 Cell by Methyl Green Assay

CONCLUSIONS

A. salviifolium is a small deciduous tree used in traditional medicine due to its medicinal properties. The leaves has considerable amount of secondary metabolites. The screening of the phytochemical analysis on leaf exhibited the presence of alkaloid, flavanoids, glycosides and phenolic compounds. The compound Deoxytubulosine was characterized and tested for its toxicity against cancer cell line MCF- 7. Experimental investigations demonstrated that the *A. salviifolium* alkaloidal compound has potential cytotoxic activity and can be utilized for chemopreventive agents against Cancer.

ACKNOWLEDGEMENT

The authors thank Gloris Biomed Research Centre (P) Ltd for providing financial and Technical support to carry out the present work.

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