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**Jayakumar K**  
Department of Botany, SVRNSS  
College, Vazhoor, Kottayam, India

**Murugan K**  
Plant Biochemistry and Molecular  
Biology Lab, Department of Botany,  
University College, Trivandrum,  
Kerala, India

## Purified solasodine and caulophyllumine: A from *Solanum mauritianum* Scop. Against MCF-7 breast cancer cell lines in terms of cell growth, cell cycle and apoptosis

**Jayakumar K and Murugan K**

### Abstract

85% of global population depends on herbal medicines for their primarily health needs and it is proved that a major part of ethnic therapy involves the usage of plant based active principles to cure or protect human beings from many human ailments. *Solanum mauritianum* Scop. a native of South America commonly known as Bug weed get dominated in many hill stations of Kerala. No medicinal utility related with this plant is reported so far. But recently, tribes use the plant to cure skin borne disorders. *Solanum*, the type genus known for alkaloids. Many alkaloids were isolated from herbals which display antiproliferation and antimetastasis effects on diverse types of cancers both under *in vitro* and *in vivo* conditions. In this juncture, present study was designed to fractionate, purify the alkaloids from *S. mauritianum* and to analyze its antiproliferative potentials against MCF-7 cells (breast adenocarcinoma). Crude alkaloid was isolated, purified by column chromatography yielded a bluish and yellowish coloured fraction caulophyllumine-A and solasodine respectively and was further confirmed by NMR. Both the alkaloids inhibited the growth of MCF-7 cell lines; the most potent one being solasodine with remarkable IC<sub>50</sub> values. Further, assays were carried out to analyze the mechanism of antiproliferation using flow cytometry. Interestingly, 25 and 50 µg/mL solasodine displayed an increase in the percentage of cells in the G1 phase and a decrease of cells in S phase of the cell cycle. In addition, Annexin V-FITC/PI analysis revealed a concentration and time dependent increase in the percentage of apoptotic and necrotic cells. In conclusion, purified solasodine obtained from *S. mauritianum* was more potent against MCF-7 cells, altering the cell cycle progression and inducing apoptosis. Further work is planned to analyze its molecular mechanism of anticancer potentiality.

**Keywords:** *Solanum mauritianum*, Inhibition of tumor cell growth, Alkaloids, MCF-7 tumor cell line, Cell cycle, Apoptosis

### 1. Introduction

Breast cancer is one of the major causes of death in the female population globally and was substantiated by WHO worldwide data analysis.<sup>[1]</sup> Generally, breast cancer was treated by radiotherapy, hormonal or chemotherapy. In most of the case, the patients experience adverse side effects from the therapy. Intensive radio or chemo therapeutical agents usually end in to nausea and bone marrow failure<sup>[2]</sup>. In addition, cancer cells are acclimatized resistant to chemical drugs<sup>[3]</sup>. In this scenario, secondary metabolites from herbals may be the best alternative for curing cancer.

*Solanaceae*, a pantropical family possesses ca. 2780 species and *Solanum* represents the type hyper genus. The species are known for the presence of diverse secondary metabolites of therapeutical importance<sup>[4]</sup>. *Solanum* is significant with steroidal glycoalkaloids and polyphenols. These forms the precursors for the synthesis of steroidal drugs. Many species of *Solanum* like *S. khasianum*, *S. lyratum*, *S. xanthocarpum*, *S. nigrum*, *S. gracile*, *S. tuberosum*, *S. laciniatum* were being wide scale used for the treatment of diseases like asthma, liver diseases and inflammation<sup>[5]</sup>. *Solanum mauritianum* Scop. of Solanaceae intruded species throughout India and mostly dominated all over the hill stations of Kerala. Many antifungal, antioxidant and cytotoxic compounds were isolated from *Solanum* species<sup>[6]</sup>. Jayakumar *et al.* already reported antioxidant activity of the alkaloids of the species<sup>[7, 8]</sup>. Routine treatments for breast cancer were costly and natural treatments have slowly recognized wide acceptance due to their promise of a cure with minimal side effects. No scientific validation of evidence exists regarding the herbal treatments. Cao *et al.* and Conforti *et al.* revealed a positive correlation between life style factors with cancer<sup>[9, 10]</sup>. For example epidemiological, biological and

**Correspondence**  
**Murugan K**  
Plant Biochemistry and Molecular  
Biology Lab, Department of Botany,  
University College, Trivandrum,  
Kerala, India

clinical research revealed that nutraceutical factors can defend cancers. Curcumin and epigallocatechin gallate were proven antimetastatic compounds without inhibiting normal healthy cells.<sup>11</sup> The antioxidant power of herbal resources i.e., their bioactive compounds, have been connected to their potentialities to inhibit multiplication of onco cells via minimizing oxidative stress, which may play pivotal role in the progression of cellular disintegration underlying tumour growth. In addition, it has been revealed that antioxidant supplementation may inhibit breast cancer recurrence and mortalities. Bioassay and animal studies indicates that many phytochemicals in the plants are potential antioxidants and possess anticancer properties. In this juncture, present analysis is undertaken to analyze antimetastatic potential of the purified alkaloids from *S. mauritianum* using MTT and flow cytometry assay.

## 2. Materials and Methods

### 2.1 Plant material and extraction

The plant material *Solanum mauritianum* Scop. (UCB3211) (40 g) shade dried and subjected to continuous soxhlet extraction using non polar to polar solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water). Dragendorff's reagent test revealed the presence of significant alkaloids in chloroform and ethyl acetate fractions. 100% chloroform yielded bluish coloured fraction. Subsequently, it was then eluted using ethyl acetate and lyophilised and again subjected to column chromatography for further purification. Bluish and yellowish green fractions were eluted out of the column using ethyl acetate and ethanol respectively. These fractions were further reloaded at the top of freshly packed column for purification. Petroleum ether and chloroform mobile phase in the ratio 4:1 yielded highly purified bluish fraction. The elution time for bluish fraction was 120 h. Residual weight of the fraction was calculated after drying was 21.3 mg/g. Similarly, chloroform and ethyl acetate (6:4) solvent combination after 168 h resulted purified yellowish fraction. The residual weight was noted as 16.9 mg/g. Purity of the samples was first checked with FTIR. FTIR spectral peaks revealed the functional groups associated with alkaloids. Using proton NMR absorptions peaks, the identity and structure of the compounds were confirmed. Bluish coloured fraction was caulophyllumine-A and yellowish green fraction was identified as solasodine.

### 2.2 Cell culture conditions

Six major malignant cell lines such as human liver cancer cell line HLF (ATCC), human colon adenocarcinoma cell line (SW480), human ovarian carcinoma cell line (NIH-OVCAR3), human lung cancer cell line (PC-9) were grown in RPMI 1640 (Invitrogen) containing 10% fetal bovine serum (Invitrogen) with 50 U/mL of penicillin (Invitrogen) and 50 µg/mL of streptomycin (Invitrogen). Two human breast cancerous cells lines (MCF-7 and MDAMB-231) were grown in DMEM (Invitrogen) supplemented with 50 U/mL of penicillin (Invitrogen) and 100 µg/mL of streptomycin (Invitrogen). All cells were maintained in a humidified incubator at 37°C and 5% CO<sub>2</sub>.

### 2.3 Cytotoxic assay

Different malignant cells were seeded in 96-well plates at a density of  $2 \times 10^4$  cells/well. The cell lines were exposed to purified alkaloids caulophyllumine-A and solasodine in a concentration range of 5-100 µg/mL for 24, 48 and 72 h. Then the cells were washed with 1X PBS and incubated in 100 µL

of 0.5 mg/mL MTT at 37°C for 30 min. Under light protection, the dark blue crystals of formazan (MTT metabolites) were dissolved with 100 µL DMSO and incubated at 37°C for 30 min. Absorbance was measured at 570 and 650 nm using a microplate reader spectrophotometer. Viable cells were represented as a percentage of survival and calculated as  $\text{Survival (\%)} = \{(\text{As570} - \text{As650})/(\text{Ac570} - \text{Ac650})\} \times 100$ , where As570, As650, Ac570, and Ac650 are the absorbance of the test samples (at 570 and 650 nm) and negative control (at 570 and 650 nm), respectively.

According to US NCI plant screening program, drug is generally considered to have *in vitro* cytotoxic activity with IC<sub>50</sub> value  $\leq 20$  µg/mL, while this value was deemed at  $\leq 4$  µg/mL for a pure compound<sup>[12]</sup>.

### 2.4 Cell-cycle analysis

Cell-cycle analysis was performed using the standard protocol of Cheng *et al.* with some modifications<sup>[13]</sup>. Briefly,  $5 \times 10^5$  cells/well were seeded in 6-well plates and starved in serum-free medium at 37°C. After 12 h starvation, the cells were treated with solasodine and caulophyllumine-A and the complete medium for 24 or 48 or 72 h. The cells were then trypsinized, washed with cold PBS and fixed overnight with 70% cold ethanol containing 3% FBS at -20°C. Subsequently, the next day, the fixed cells were centrifuged at 1200 g for 1 min and washed with PBS twice. After that, the cell plates were resuspended with 200 µL RNase A (1 mg/mL) at 37°C for 10 min, followed by the addition of 300 µL propidium iodide (PI, 100 µL/mL) to stain the DNA of cells in the dark. After 20 min incubation at room temperature, the DNA contents of the cells were analyzed in a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and the data was analyzed by ModFitLT V2.0 software (Becton Dickinson).

### 2.5 Identification of apoptosis by PI-Annexin-V staining

This assay was performed to detect cell apoptosis with an Annexin V-FITC Apoptosis Detection Kit, following the manufacturer's instruction. In brief, harvested cells were resuspended in 100 µL of the binding buffer to achieve a concentration of  $1 \times 10^6$  /mL. Then, 5 µL Annexin V-FITC and 10 µL propidium iodide (PI) (20 µg/mL) were added and incubated in the dark for 15 min at room temperature. Finally, 400 µL of the binding buffer was added to each reaction tube before the cells were analyzed by FACScan flow cytometry. The data was analyzed by WinMDI V2.9 software.

### 2.6 Analysis of Cellular DNA Content Using Propidium Iodide

MCF-7 cells at a concentration of  $1 \times 10^6$  cells/mL were seeded into 6-well plate in 2 mL culture medium with a concentration of IC<sub>50</sub> value of alkaloids and were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 24, 48 and 72 h. Some wells were left with no treatment to be used as a control. After the incubation period, the cultured cells were harvested using trypsin and centrifuged. After incubation, the cells were detached and stained by using the Cycle TEST TM PLUS DNA Reagent Kit. Cell cycle was read using the Cell Quest software within 3 h.

### 2.7 Statistical analysis

All analysis was performed in triplicate and was subjected to analysis of variance using the statistical analysis software. Comparisons among means were made using Duncan's multiple range tests.

### 3. Results and Discussion

#### 3.1 Cell proliferation assay

Purified solasodine and caulophyllumine-A from *S. mauritanum* was screened against six major malignant cell lines such as human liver cancer cell line HLF (ATCC), human colon adenocarcinoma cell line (SW480), human ovarian carcinoma cell line (NIH-OVCAR3), human lung cancer cell line (PC-9) and two human breast cancerous cells lines (MCF-7 and MDAMB-231) for antimetastatic potentialities. Doxorubicin, the synthetic drug was used as

positive control.

Initially, caulophyllumine-A (C-A), solasodine (S) and their combinations (C-A + S) induced cellular responses against selected malignant cell lines were examined by MTT assay for its ability to inhibit the cancer cell proliferation. As shown in Table 1, caulophyllumine-A (C-A) and solasodine (S) significantly inhibited the proliferation of MCF-7 cells in a time and dose-dependent manner when compared with other malignant cell lines.

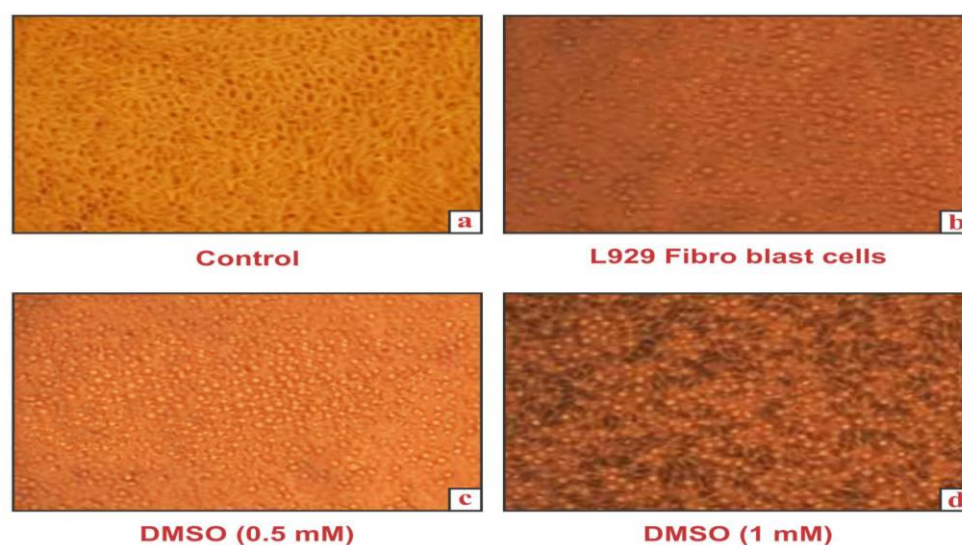
**Table 1:** Antiproliferative assay of solasodine (S), caulophyllumine-A (C-A) and S+C-A against MCF-7 cell lines at 24, 48 and 72 h

Concentration (µg)	Solasodine (S)			Caulophyllumine-A (C-A)			Concentration (µg)	S + C-A		
	% of viability			% of viability				% of viability		
	24 h	48 h	72 h	24 h	48 h	72 h		24 h	48 h	72 h
Control	100 ±1.9	100 ±3.3	98.9±2	100 ±3.7	100 ±3	99 ±1.6	Control	100 ±3	100 ±2.6	99.3 ±0.54
5	88.6 ±6.3	71.2 ±2.6	64.3 ±3.3	96.2 ±2.8	90 ±6.9	87 ±1.7	10 + 10	97.4 ±4.8	94.6 ±1	80.7±0.2
10	80.1±2.5	67±1.8	59.1 ±4.8	86.3 ±7.4	78.5±3.7	71.2 ±2.5	25 + 25	95.5 ±6.6	85.3 ±1.7	76.2 ±0.8
25	74.4±4.1**	59.2±2.7**	50.2 ±6.6*	79.3±4.7*	65 ±2.8*	61.2 ±3.4*	25 + 50	89 ±1.8*	78±4.9*	59.6±0.4*
50	69.5±1.7**	43.4±1.6**	30.9 ±1.3*	63±1.3*	57.4±3*	48.6 ±2.5*	50 + 25	87 ±0.67	72.3±9.0	58.9±2
100	39.3±2.1**	35.7±0.8**	33.8 ±2*	60 ±5.7*	53 ±2.8*	45.1 ±6.2*	50 + 50	80±0.88	66±0.76	50.2±0.1

In addition, the results observed for solasodine against MCF-7 cell line viabilities at varying durations implying that the MCF-7 cells respond effectively within a span of 48 h. Interestingly, at the highest dose of solasodine (100 µg/mL), cell viability of MCF-7 cells appeared to be independent of duration. IC<sub>50</sub> (half maximal inhibitory concentration) is a measure of antagonist drug potency used to evaluate the calibre of a molecule i.e., the lowest the IC<sub>50</sub> value means the highest the potency of the molecule. According to the MTT assay results, the IC<sub>50</sub> values of solasodine for MCF-7 cells were 24.8, 47.3 and 87.4 µg/mL at 72, 48 and 24 h respectively, whereas for C-A the values were 35.6 (72 h), 54.1 (48 h) and 88.3 µg/mL (24 h). Meanwhile, insignificant inhibitory IC<sub>50</sub> values was noticed for other cell lines and also

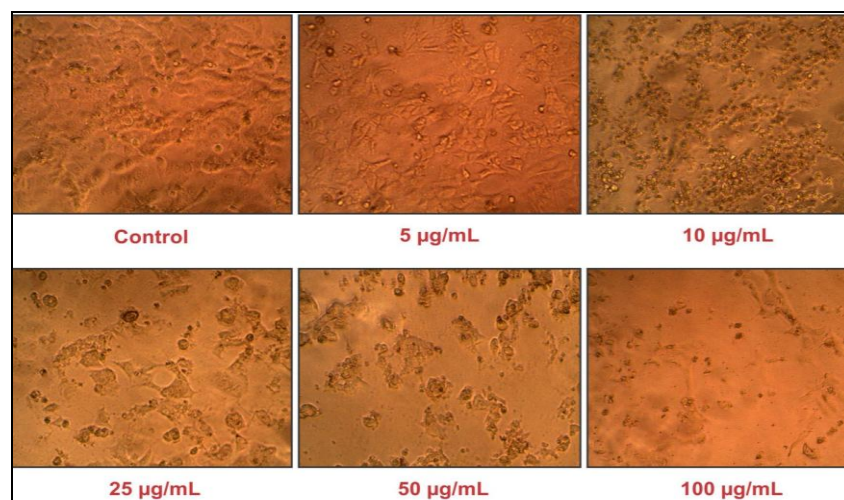
for the combination C-A + S. Interestingly, caulophyllumine-A (C-A), solasodine (S) showed negligible cytotoxicity against L929 fibroblast cells with IC<sub>50</sub> value ranged from 0.67 ± 0.01 to 1.3 ± 0.42 µg/mL. Further, positive control DMSO revealed marginal insignificant effects on MCF-7 and L929 fibroblast cell lines. Doxorubicin, the synthetic anticancer drug (with 25 µg/mL) showed 89.5% cytotoxicity on MCF-7 cells at 48 h (59% at 24 h).

The untreated and DMSO treated cells (A, B in Figure 1) served as control in the antiproliferative assay showed uniformly spread confluent layer of long, elongated cells whereas cells treated with different concentrations of solasodine and C-A in DMSO showed varying degrees of damage (Figure 2 and 3).

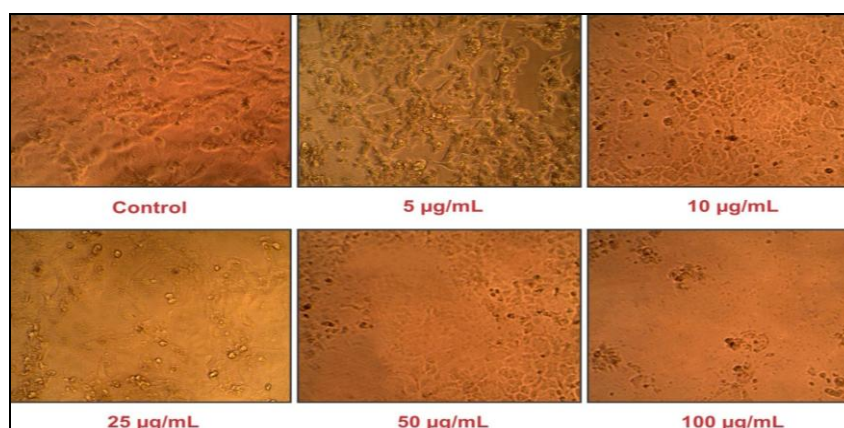


**Fig 1:** Antiproliferative assay (MTT) after 72h of culture

A-control MCF-7 cells (untreated), b-solasodine treated L929 Fibro blast cells, c&d-DMSO treated MCF-7 cells



**Fig 2:** Cell viability of caulophyllumine-A treated MCF-7 cells after 72 h



**Fig 3:** Cell viability of solasodine treated MCF-7 cells after 72 h

The cells were rounded and the number of normal cells was found to be less in treated samples. The results were comparable with that of alkaloid extracts of leaf from *Excoecaria agallocha*.<sup>[14]</sup> Thus, MTT assay results suggest that MCF-7 cell lines was significantly growth inhibited than other cell lines by solasodine (S) and caulophyllumine-A. Therefore, further analysis of solasodine (S) and caulophyllumine-A (C-A) was restricted with MCF-7 cell lines only. Al-Oqail *et al.* reported that 10–1000 µg/mL of *Verbesina encelioides* extract for 24 h, showed antiproliferative potential against MCF-7 and HepG2 cells, but not with A-549 cells<sup>[15]</sup>. Howard *et al.* also revealed anticancer potential of aqueous extracts of *Vernonia amygdalina* in terms of cytotoxicity<sup>[16]</sup>. Banerjee *et al.* analyzed the efficacy of andrographolide in terms of cytotoxicity and cell cycle arrest leading to programmed cell death of MDA-MB-231 breast cancer cells<sup>[17]</sup>. Latifah *et al.* reported the inhibitory effect of extract from *Dillenia suffruticosa* against MCF-7 and MDA-MB-231 via induction of G (2)/M arrest and apoptosis.<sup>[18]</sup> Similarly, the aqueous extract of *Tuberaria lignosa* inhibits cell growth by altering the cell cycle and triggers apoptosis against NCI-H460 tumor cell lines<sup>[19]</sup>. All the above results substantiate the role of phytochemicals as antimetastatic compounds interms of cytotoxicity.

### 3.2 G1/S cell cycle arrest in MCF-7 cells

Cell cycle arrest by flow cytometric analysis was carried out to gain an insight in to the antiproliferative mechanism of the alkaloids against the MCF-7 cell lines. Results showed that solasodine (S) induced a time and dose dependent growth

inhibition in the G1 period of the mitotic cycle, accompanied with a notable decrease in the S phase cells. The values are statistically significant at 1% level ( $p < 0.01$ ). Further, 100 µg/mL exposure of solasodine (S) for 24 h, the G1 phase of MCF-7 cells showed a marked increase to  $55 \pm 0.06\%$  whereas the percentage of cells in the S phase decreased to  $23.1 \pm 0.47\%$ . Time-dependent analysis of the MCF-7 cells treated with 25 µg/mL solasodine (S) in terms of G1 phase showed a proportional increase i.e., 45, 55.8 and 64% with 24, 48 and 72 h respectively. Similarly, with 50 µg/mL solasodine (S) also caused G1 phase growth arrest across time i.e., an increase in the percentage of cells from 57.9 (24 h) to 72% (72 h). It is noteworthy that, following 100 µg/mL solasodine (S) treatment, cells began clumping in the sub-G1 phase and the percentage of sub-G1 phase cells increased at 72 h. This imparts that the cells experienced apoptotic impact rather than cell cycle arrest i.e., the damage caused by solasodine (S) was drastic to be recouped by the usual DNA repair mechanisms in the cells. This may be justified by recording the decline of cells in the G1 phase with the concentration of 50 µg/mL of solasodine for 72 h. Overall, the results indicated that 25 and 50 µg/mL solasodine (S) treated MCF-7 cells were halted in the G1 phase of cell cycle at 24 h and 48 h, after which apoptosis progressed with increasing duration of treatment. Thus, it tempts to suggest that solasodine (S) is able to inhibit the proliferation of MCF-7 cells by fostering cell cycle block at the G1/S phase. On the contrary, the effect of C- A on cell progression in the treated MCF-7 cell lines was less, and no remarkable dose or time-dependent growth arrest could be clearly noticed. Increasing doses of C-A does not result in the accumulation of G1 cells even after 72 h exposure (Table 2).

**Table 2:** Flow cytometry analysis of MCF-7 cells against the purified alkaloids at 25, 50 and 100 µg/mL with different durations (24 to 72 h)

Duration (h)		Control	Solasodine (S)			Caulophyllumine-A (C-A)			S + C-A
			25	50	100	25	50	100	
24	G1	54 ± 0.43	50 ± 1.6*	50.9 ± 0.99*	58 ± 0.06*	60 ± 0.98	57 ± 0.27	42 ± 0.91	48 ± 0.98
	S	18 ± 0.22	28 ± 0.67*	23.4 ± 0.34*	15.1 ± 0.97*	30 ± 0.28	29 ± 0.01	28 ± 0.12	28 ± 0.28
48	G2	23 ± 0.31	16 ± 0.88*	18 ± 0.39*	19 ± 0.22*	8.5 ± 0.33	12 ± 0.98	16.7 ± 0.22	20 ± 0.33
	G1	52.3 ± 0.27	30.8 ± 0.54*	34 ± 3.1*	40 ± 0.03*	52 ± 0.76	48.8 ± 0.34	40 ± 0.98	39 ± 0.76
72	S	17 ± 0.4	20.1 ± 0.22*	20.2 ± 0.12*	14.8 ± 0.65*	30 ± 0.27	26 ± 0.67	26.2 ± 0.90	26 ± 0.27
	G2	24 ± 0.09	40.3 ± 0.32*	36 ± 0.04*	35.6 ± 0.03*	15 ± 0.33	18.4 ± 0.98	25.5 ± 0.03	23 ± 0.33
72	G1	53 ± 0.68**	28 ± 0.71**	30 ± 2.1**	32.6 ± 0.02**	42 ± 0.99**	45.4 ± 0.05**	39 ± 0.08**	38 ± 0.99**
	S	19 ± 1.0**	16.5 ± 0.55**	15 ± 0.77**	12 ± 0.83**	30 ± 0.05**	23 ± 0.06**	21 ± 0.04**	22 ± 0.05**
72	G2	22 ± 0.05**	45 ± 0.27**	42.6 ± 0.03**	45 ± 0.72**	21.4 ± 0.07**	23 ± 0.03**	38 ± 0.29**	29.4 ± 0.07**

Thus, the results imply that solasodine (S) is more potential in imparting growth arrest in the G1/S phase of the cell cycle in MCF-7, compared to C-A, indicating the differential mode of action of S and C-A alkaloids on cell cycle progression within MCF-7 cell lines. Doxorubicin affected cell cycle more significantly in G1 phase followed by S phase i.e., at 25 µg/mL cells at G1 phase was 54.5% (24 h) and 60.8% (48 h). Similarly, at S phase 30 and 35.4% respectively.

#### *Solasodine (S) and caulophyllumine-A (C-A) induced apoptosis in MCF-7 cells*

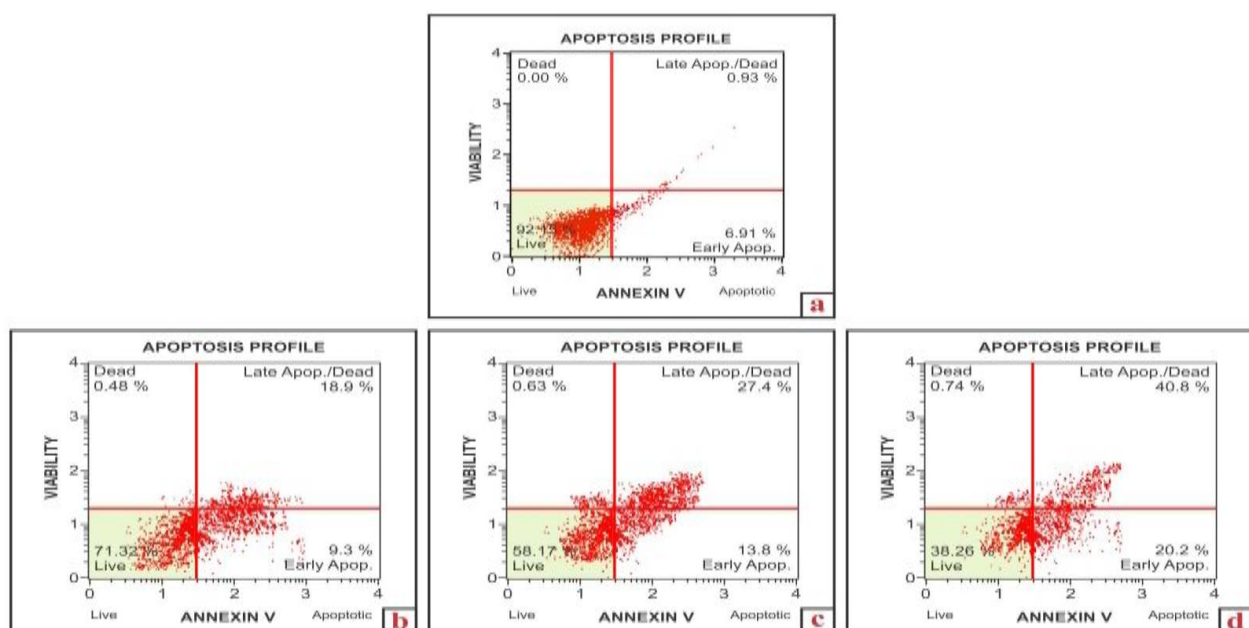
Annexins, a group of calcium dependent phospholipid binding proteins. These binds with phosphatidyl serine to demark the cells as apoptotic. The cells with hypodiploid DNA content in the sub-G1 phase of the cell cycle also suggests apoptosis. Phosphatidylserine exposure was analyzed by Annexin V-FITC/PI assay using flow cytometry to further substantiate whether, the alkaloids could induce apoptosis in the MCF-7 cells. Interestingly, a concentration and time dependent apoptosis in MCF-7 cell lines induced by solasodine was detected (Figure 4).

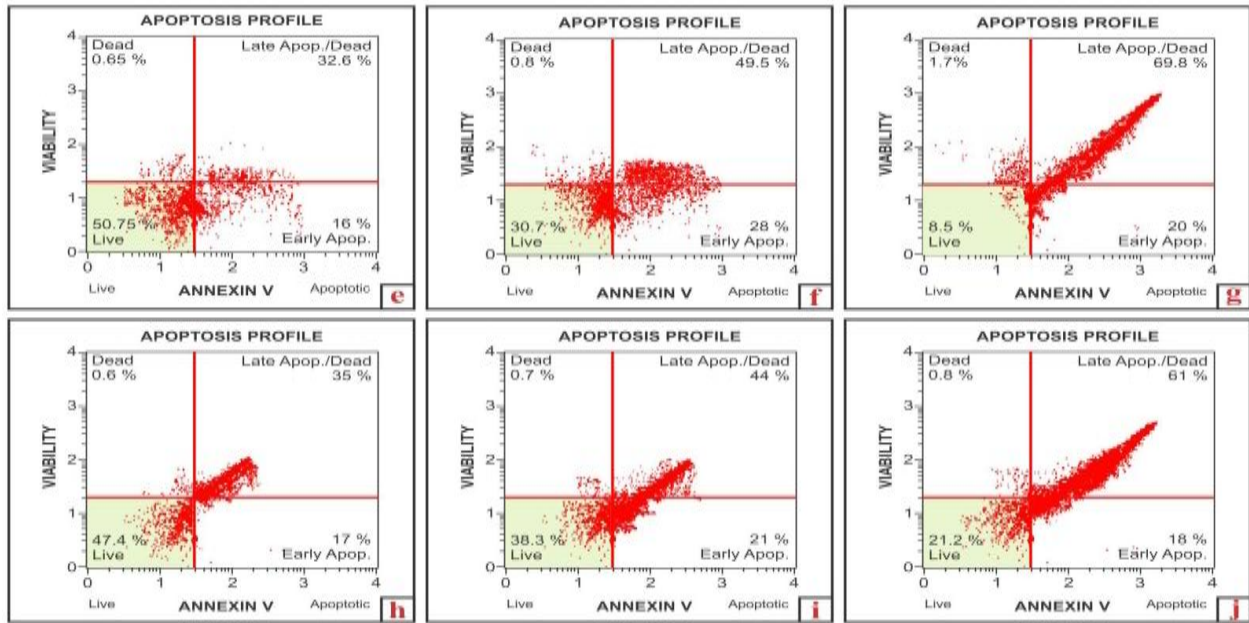
Fluorescence microscopy was employed to visualize morphological transforms of cell death mode induced by

solasodine and caulophyllumine-A 25, 50 and 100 µg/mL concentrations after 24, 48 and 72 h using fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining. Living cells were circular, round, light green coloured nuclei with intact DNA. Early apoptotic cells have degraded DNA with cell blebbing. Late apoptotic and necrotic cells show fragmented DNA with reddish and green staining.

In addition, the percentage of viable, apoptotic and necrotic cells were recorded. In untreated cells the percentage of apoptotic cells were ranged from 0.48% (24 h) to 4.7% (72 h) (Table 3). Meanwhile, the percentage of apoptotic cells increased rapidly from 32.6 to 69.8% (72 h) in 50 µg/mL concentration solasodine treated cells. Necrotic cells also showed a more or less similar trend among the treated and control cells. The data of DNA content from MCF-7 cells revealed by flow cytometry using PI staining nuclei corroborates with the antiproliferation data.

After 24 h exposure of MCF-7 cells to solasodine (S) at 100 µg/mL resulted an increased levels of apoptotic cells i.e., nearly 20 fold, (from 4.7% of the control to 61% in the treated cells).





**Fig 4:** Apoptosis activity of MCF-7 cells control and solasodine with varying concentrations (25, 50, 100  $\mu\text{g}/\text{mL}$ ) and time durations (24, 48, 72h) by annexin V-FITC/PI assay using flow cytometry

Following 24 h and 48 h solasodine (S) treatment, Annexin V-FITC-stained positive cells increased to 35 and 44% respectively. For caulophyllumine-A (C-A), apoptotic cells were found to be pronounced after treatment with the highest concentration (100  $\mu\text{g}/\text{mL}$ ) only. The Annexin V-FITC-stained positive cells increased from 0.48 in the control to 29% at 24 h, from 3.1 to 36% at 48 h, and from 4.7 to 53% at 72 h treatment. However, the apoptotic effect induced by C-An appeared to be less effective in MCF-7 cells when compared with solasodine (S). Doxorubicin, the synthetic drug showed remarkable % of apoptotic, necrotic and normal cells at 25  $\mu\text{g}/\text{mL}$  (52, 18, 30% at 24 h; 70, 22 and 8% at 48

h). Thus, the results confirm the ability of solasodine (S) to induce cell death in MCF-7 cells through apoptosis, in varying degrees with concentration and duration. Apoptosis induced by anticancer drugs constitutes one possible of treatment effect. Two different pathways involved in the process have been investigated. One mechanism is the death-receptor pathway and the other is the mitochondrial pathway; the latter has been considered an important mediator of cell apoptosis in mammals. Zhang *et al.* reported anti-proliferation of breast cancer cells by inducing apoptosis using huaier aqueous extract [20].

**Table 3:** Fluorescence microscopic analysis of MCF-7 cells against the alkaloids at different concentrations and durations

	Control			Solasodine ( $\mu\text{g}/\text{mL}$ )								
				25			50			100		
	24	48	72	24	48	72	24	48	72	24	48	72
Apoptotic cells	1.48±0.02	3.1±0.8	4.7±0.6*	27±0.5	41±0.4	61±1.5*	48±0.76	68±0.65	89.2±3.1*	52±1	61±3.3	78±2.6*
Necrotic cells	0.1±0.03	0.1±0.3	0.1±0.4*	0.48±0.01	0.63±0.03	0.74±0.08*	0.65±0.01	0.8±0.02	1.7±0.07*	0.6±0.03	0.7±0.03	0.8±0.12**
Viable cells	98.2±1.2	95±1.4	94±0.3*	71.2±2.2	58.2±2.5	38.26±0.2**	50.75±0.3	30.7±0.7	8.5±0.3*	47±2.1	38±0.99	21±0.84**
	Control			Caulophyllumine-A ( $\mu\text{g}/\text{ml}$ )								
				25			50			100		
	24	48	72	24	48	72	24	48	72	24	48	72
Apoptotic cells	1.48±0.02	3.1±0.8	4.7±0.6*	16.9±2	30.5±0.23	40.9±1.5*	34.6±0.76	44.4±0.7	58.5±3.6*	38±0.8	46±0.99	64±0.33**
Necrotic cells	0.1±0.03	0.1±0.3	0.1±0.4*	0.34±0.01	0.38±0.01	0.41±0.03**	0.48±0.04	0.6±0.01	0.67±0.02**	0.6±0.04	0.8±0.03	0.9±0.02**
Viable cells	98.2±1.2	94±1.4	94.4±0.3**	82.8±2.3	69±3.4	58±2.1**	64.9±0.33	55±2.4	40.6±0.9**	61.4±0.6	53±1.4	35±1.3*

Similarly, Abedian *et al.* revealed antioxidant and apoptotic impacts of aqueous extract of *Urtica dioica* against MCF-7 cell lines. [21] These reports concurred with the present results to suggest that alkaloids induce apoptosis on MCF-7 cells more extensively with increasing concentration and time.

#### 4. Conclusion

The present results showed that the alkaloid solasodine could inhibit cell proliferation by inducing apoptosis and cell-cycle arrest in breast cancer cells effectively than caulophyllumine-A. Creating awareness related with the usage of herbals and unraveling phytochemicals properties can play remarkable

clinical roles as knowledge resources for common man. Limited plants have been screened for biological activity and available investigations into anticancer activity of herbals showing promising activity, must be undertaken. Antimetastatic process of alkaloids must be further identified using new technologies, the effective combinational therapy

methods may be explored, the effective drug delivery systems need to be developed and the additional clinical anticancer trials for these alkaloids need to be performed.

## 5. References

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