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Quantification Studies of Apoptosis for Aqueous Extracts of *Ipomoea Sepiaria* as Anticancer Agents towards Pc-3 Cell Lines

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Abstract

Ipomoea sepiaria (*I. sepiaria*), is an important and traditional ethanomedicinal plant associated in control of ageing diseases. Aqueous leaf extract of *Ipomoea sepiaria (*10 µM) were quantified using Annexin V and Propidium iodide staining technique using a flow cytometer against PC-3 Cell lines. The control used in the experimentation showed 25±6.2% of PE-positive cells and tested sample (10 µM aqueous leaf extract of *Ipomoea sepiaria)* shown 69±3.4% of PE-positive cells, revealed differences in percentage of PE-positive cells. This differentiation has distinct effect on apoptosis and cell cycle arrest.

Keywords: *Ipomoea sepiaria*, Annexin V, Propidium iodide, Quantification, Apoptosis

Introduction

The traditional and modern medicines from plant resources are the major elements of phytochemistry and pharmacognosy in the present decades [1]. Antioxidants from traditional medicinal plants are associated with anticancer, anti-diabetes, etc. used in the prevention and treatment of several ageing diseases [2]. India is having rich and diverse natural resources with strong traditions of nature conservation practices from ancient times [3].

Ipomoea sepiaria (I. sepiaria), is an important and traditional ethanomedicinal plant reported in Basavarajeeyam, an important handbook for the Ayurvedic physician from Andhra region [4]. It is commonly called as *Gollajiddaku* used as antimicrobial [5], diuretic, Leucorrhoea, uterine tonic, hypotensive, antidote to arsenic poisoning, hypotensive, cardiac depressant and spasmolytic [4].

Several methods for quantification studies of apoptosis using annexin v and propidium has been reported [6,7,8] using DNA flow cytometry (FCM). Several quantification methods experimented in plants has been shown in Table 1.

Table 1: Quantification methods experimented in plants using Annexin v and Propidium iodide

Plant/ plant derived agent	Dye	Cell line	Reference
Saffron [*Crocus sativus* L.]	Annexin V	A549	Samarghandian et al., 2011**[9]**
β-Elemene [herbs and spices]	Annexin V	H460 and A549	Li et al., 2009 [10]
Roots of *Astragalus mongholicus*	Annexin V/FITC and Propidium iodide (PI)	MG63 and K562	Yen et al., 2009 [11]
Resveratrol [grapes and red wine]	Annexin V-FITC and propidium iodide	A375	Niles et al., 2003 [12]
Curcumin [*Curcuma longa]*	Annexin V	AK-5	Bhaumik et al, 1999 [13]

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Material and Methods

COLLECTION OF PLANT MATERIAL

Fresh leaves of *Ipomoea sepiaria* were collected from surrounding areas of Visakhapatnam, India. The dust particles were removed by washing leaves of *Ipomoea sepiaria* with double distilled water. The leaves were shade dried and then grounded to powder using mortar and pestle. The obtained powdered samples were then stored in an airtight closed bottle and were used for further experiments.

PREPARATION OF PLANT EXTRACT OF *IPOMOEA SEPIARIA*

About 20gms of the plant powder of *Ipomoea sepiaria* was taken in 250 ml Erlenmeyer flask. The material was boiled with 100 ml of double distilled water, filtered with Whatman Filter paper no. 1 after cooling and was stored at 4°C for further experimentation.

TREATMENT WITH AQUEOUS LEAF EXTRACT OF *IPOMOEA SEPIARIA*

PC-3 cells (1×10^6) were plated in DMEM-FCS and treated with 10 µM aqueous leaf extract from *Ipomoea sepiaria*. The cells were collected at different time points, washed with PBS, fixed in 70% ethanol, stained with propidium iodide (PI) and analyzed by flow cytometry.

ANNEXIN V STAINING

PC-3 cells (2×10^6) were cultured in DMEM-FCS and treated with 10 µM aqueous leaf extract of *Ipomoea sepiaria* for 3 h. Control and treated PC-3 cells were suspended in 80 µl of binding buffer (100 mM HEPES/NaOH, pH 7.5, containing 1.4 M NaCl and 25 mM $CaCl_2$). Annexin V-FITC (fluorescein isothiocyanate) conjugate (5 µl) was added to each tube and incubated at room temperature for 15 min. Phycoerythrin (PE) is one of the most commonly-used fluorescent dyes for FACS (Fluorescence Activated Cell Sorting) analysis.

QUANTIFICATION STUDIES OF APOPTOSIS FOR AQUEOUS EXTRACTS OF *IPOMOEA SEPIARIA*

Apoptotic population of PC-3 cell lines were quantified using Annexin V and Propidium iodide staining technique using a flow cytometer, polo *et al*., 2015 [14]

Result and Discussion

In the present studies, Untreated and treated cells of PC-3 cell lines have been evaluated for apoptosis by measuring the amount of hypo-diploid cells using of DNA flow cytometry (FCM) (Figure 1)

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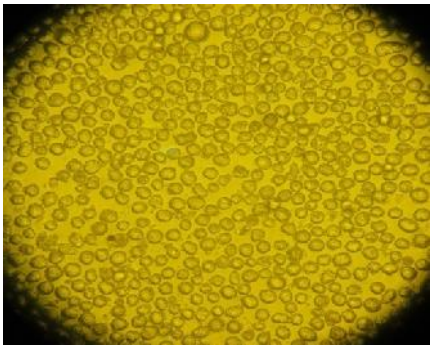
Figure 1: Cultured Mononuclear cells (Control)



The control used in the experimentation showed 25±6.2% of PE-positive cells and tested sample (10 µM aqueous leaf extract of *Ipomoea sepiaria)* shown 69±3.4% of PE-positive cells, revealed differences in percentage of PE-positive cells (Figure 2 and 3). This differentiation has distinct effect on apoptosis and cell

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cycle arrest. The test described, discriminates intact cells ($FITC^-/PE^-$), apoptotic cells ($FITC^+/PE^-$) and necrotic cells ($FITC^+/PE^+$). In comparison with existing traditional tests the Annexin V assay is sensitive and easy to perform. The Annexin V assay presents the possibility of detecting early phases of apoptosis.

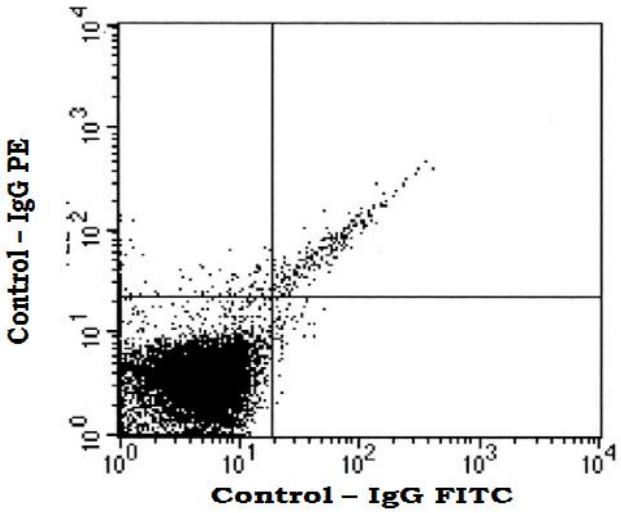

Figure 2: Effects of 10 µM concentration of extract on cancer cells

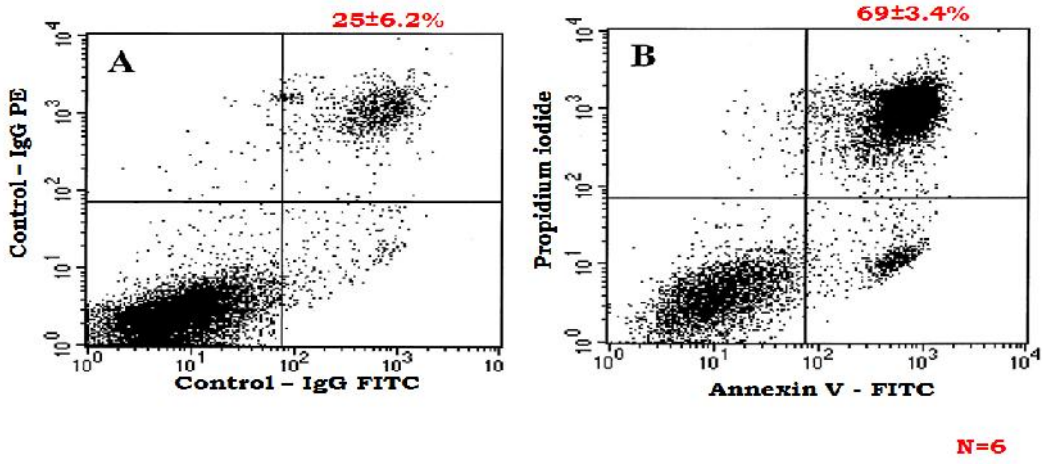

Figure 3: Effects of 10 µM concentration of extract on cancer cells using Annexin V and Propidium iodide

In both diagnostic and research, developments in immunhistochemistry using flow cytometry is an effective method used for characterization of tumor cells in clinical effusion specimens [15]. A refined biological process in regulation of cell cycle depends on a series of expression in cellular molecules with complex system network [16]. Mutating from the normal to abnormal molecule in the component of a system results in disorder of cell cycle control and lead to tumorigenesis.

Conclusion

Cell and system differentiation has distinct effect on apoptosis and cell cycle arrest. The present experimentation shows that flow cytometry is an effective method used for characterization of tumor cells. 10 µM aqueous leaf extract of *Ipomoea sepiaria* has shown anticancer activity withPC-3 cell lines.

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