



***In-vitro* Cytotoxic Activity of *Orthosiphon thymiflorus* Roth.) Sleensen Leaf Extract against Dalton Lymphoma Ascites Cell Line**

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ABSTRACT

*In this study, different concentrations of the methanolic extract of leaves of the plant *Orthosiphon thymiflorus* were subjected to cytotoxic activity study against Dalton Lymphoma Ascites (DLA) cells using the MTT assay. Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extract. Cell viability was inhibited to different extents by different concentrations of the extract.*

Keywords: *Orthosiphon thymiflorus*; cytotoxic activity; crude extracts; MTT.

INTRODUCTION

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings [1]. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value [2]. The medicinal value of plants lies in some chemical substances (usually secondary metabolites), that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolics [3].

Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cell [4]. In developed nations at least one in five of the populations of Europe and North America can expect to die of cancer. After cardiovascular diseases, it is the second cause of death amongst the global population [5]. Cancer is the cause of more than six million deaths each year in the world. In 2001, about 1,268,000 new cancer cases and 553,400 deaths were reported in the United States [6]. For a long time, plants are being used in the treatment of cancer. Despite, the availability of rich synthetic drugs, plants remain— even today —a fundamental ingredient of health care [7]. According to an estimate, 50% of breast cancer and 37% of prostate cancer patients use herbal products [8, 9]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [10]. Some preclinical studies suggest that phytochemicals can prevent colorectal cancer and other cancers. [11-14]. More than 60% of currently used anticancer agents are derived in one way or another from natural sources [15, 16]. Studies with cell lines can serve as an initial screen for agents that might regulate drug resistance. The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment [17]. Plants based natural constituents can be derived from any part of plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components [18]. *Orthosiphon thymiflorus* is a medicinal plant native to South East Asia and some parts of tropical Australia. It is an herbaceous shrub which grows to a height of 1.5 meters. It is a popular garden plant because of its unique flower, which is white and bluish with filaments resembling a cat's whiskers [19-22]. *Orthosiphon* contains 9 species; Pharmacological properties like antidiabetic, diuretic, antihepatotoxic antibacterial, hypertensive and antitumor activity of this genus [23-26]. *Orthosiphon thymiflorus* aqueous extracts have found to be having anti-inflammatory and acetylcholine antagonistic action. Leaf juice has been used by the tribes as a lotion [27-30].

EXPERIMENTAL SECTION

Plant material and extraction: The leaves of *Orthosiphon thymiflorus* (Roth.) Sleensen (Labiatae), collected from the tribal areas of Attapady, Palakkad district, Kerala state, South India were authenticated by the Botanical survey of India, Coimbatore, Tamilnadu (BSI). A voucher specimen (no.BSI/SRC/5/23/10-11/Tech-936) was deposited in the departmental herbarium. The leaves were cut, air dried and ground into coarse powder. This powder was stored in air tight container and used for extraction. The dried and powdered material was extracted with Methanol using a soxhlet apparatus. The extraction was carried out for 24 h at room temperature with mild shaking. The extract was filtered, concentrated and the weight of the residue was recorded and percent yield calculated.

Preliminary Phytochemical Screening

Conventional standard protocols for detecting the presence of different chemical constituents in the plant extract was employed [31]. The tests for the secondary metabolites viz. alkaloids, tannins, sterols, saponins, amino acids, glycosides, proteins, sterols/terpenes, reducing sugars, non-reducing sugars, resins flavonoids and phenols were carried out with the methanolic extract of leaves of *Orthosiphon thymiflorus* using preliminary phytochemical screening.

Cell Culture

DLA (Dalton Lymphoma Ascites) cell lines were obtained from Amala Cancer Research Centre, Thrissur, Kerala. Each cell line was cultured in suitable medium for desired growth, plus 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37°C in an atmosphere of 95% O₂ and 5% CO₂. Then the growth curve of each cell line was plotted.

In-vitro cytotoxicity studies

Viability Staining by Trypan blue dye exclusion method

Principle

Trypan Blue is a blue acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan Blue is an Essential dye, use in estimating the number of viable cells present in a population.

Procedure

The Methanolic extract was studied for short term in vitro cytotoxicity using Dalton's Lymphoma ascites cells. 10mg of the extract was taken in an Eppendorf vial of capacity 1ml and dilute to six different concentrations with its duplicate and control (50%) using DMSO as a solvent and mixed with the help of a vortexing machine. Aspirated tumor cells from peritoneal cavity of mice were obtained from Amala cancer research centre, Amala nagar Thrissur, Kerala. The procedure was approved by institutional animal ethics committee. The cell viability was checked by trypan blue dye (1%) [32,33]. The cell suspension (1x 10⁶ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hour at 37°C. After incubation 0.1 ml trypan blue was added and number of dead cells determined by using haemocytometer. The percent viability was calculated by using formula:

$$\% \text{ viability} = (\text{live cell count} / \text{total cell count}) * 100$$

Micro culture tetrazolium (MTT) assay

Cell viability was assessed by MTT assay (Micro culture tetrazolium/formazan assay) in the presence and absence of different concentrations of the plants extract. The cells were seeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used the cell line. Then, the cells were incubated at 37°C. After 24 h the medium was replaced by fresh medium containing different concentrations of the plants extract. Then, the medium was changed by fresh medium containing MTT ([3-(4, 5-dimethylthiazol-2-yl)-2, 4-diphenyltetrazolium bromide]) with a final concentration of 0.5 mg/ml (after 24 h). The cells were incubated for another 4 h in a humidified atmosphere at 37°C and after that the medium containing MTT was removed and remaining MTT formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm immediately using an ELISA reader. IC₅₀ was defined as the concentration of the extract that produced a 50% decrease in cell viability relative to the negative control which was wells exposed to the solvent without any extract [34, 35].

Statistical Analysis

Data are reported as the mean ± SD for at least three replicates. Statistical analysis was performed using the Student t-test, with significance level set at P < 0.05.

RESULTS AND DISCUSSION

Percentage cell viability of cell lines was carried out by using Trypan blue dye Exclusion technique.

Table 1: Determination of cytotoxicity by MTT Assay

Plant extract	Conc Mcg/ml	Absorbance	% inhibition	IC ₅₀ Mcg/ml	R ²
<i>Orthosiphon thymiflorus</i>	62.5	0.427	14.25	266.8	0.9617
	125	0.380	23.69		
	250	0.268	46.05		
	500	0.165	66.73		
	1000	0.00166	99.66		
Control		0.498			

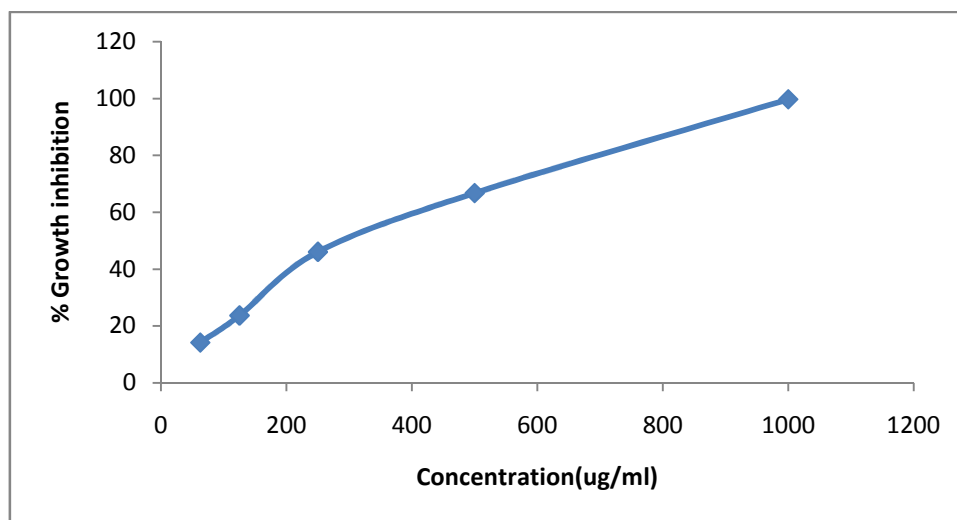
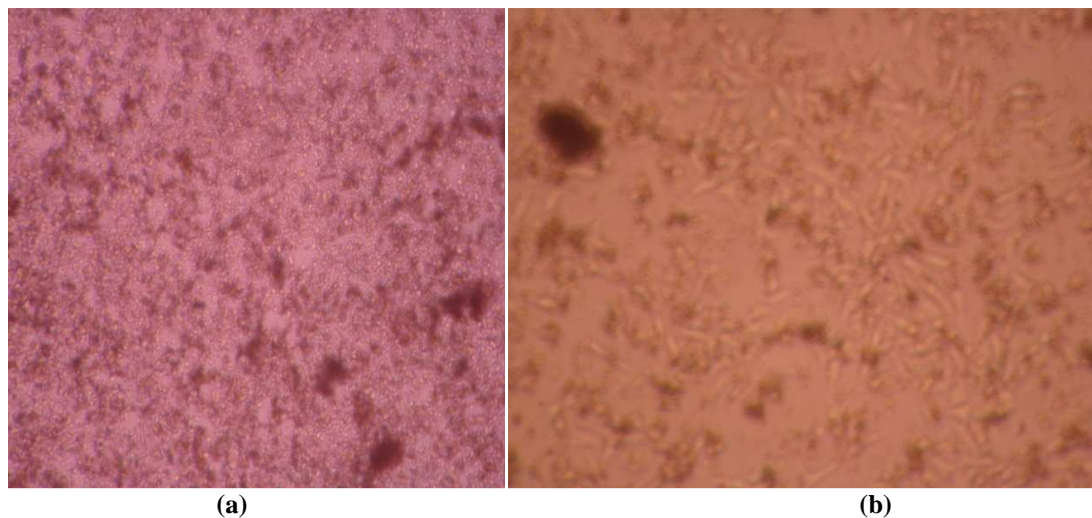
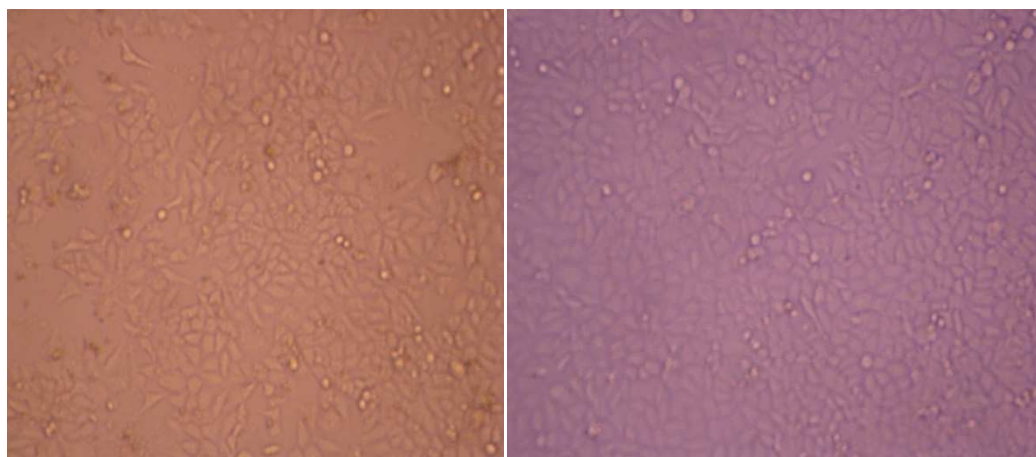


Figure 2: Percentage growth inhibition of methanolic extract of *Orthosiphon thymiflorus* against DLA cell line.

The results show dose dependent response. The cytotoxic activity may be due to the presence of alkaloids and terpenoids present in the leaves of *Orthosiphon thymiflorus*.

From the Table1, it showed that the % viability of DLA cell line is 51-96%, which is most suitable to perform cytotoxicity studies.





(c) (d)
Figure 1: Photomicrograph (Pictorial view) of DLA cell lines.

DLA cell lines-(a) *O.thymiflorus* 1000µg/ml (b) *O.thymiflorus* 500µg/ml (c) *O.thymiflorus* 250µg/ml (d) Control
 The *in vitro* screening of the methanolic extract showed that *Orthosiphon* had cytotoxic effect on DLA cell lines. The cytotoxicity activity was carried out by using MTT assay. The inhibition percentage with regard to cytotoxicity was found to be 99.66 % at 1000 µg/ml with IC₅₀ value of 266.8µg/ml (Table-2)

CONCLUSION

Studies have shown differential sensitivities to several natural compounds between tumor and normal cells *in vitro* or *in vivo*, and the results obtained from the present study show that the methanol extract from *Orthosiphon thymiflorus* is cytotoxic to DLA cell lines. Our phytochemical screening revealed the presence of terpenoid, flavonoids and alkaloids in the methanolic extracts of *Orthosiphon thymiflorus*, which could be responsible for this activity. Flavonoids have been found to possess antimutagenic and antimalignant effects [36, 37]. Moreover it has protective effect against cancer by their effect on signal transduction in cell proliferation and angiogenesis [38,39].It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent against cancer and we therefore, suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *in vivo* cytotoxic activity of the plants with a view to obtaining useful chemotherapeutic agent.

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