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Cytotoxicity activity of folklore medicinal plants of India

Dora Babu Neerugatti, Mallikarjuna Rao Talluri and Ganga Rao Battu

Abstract
The present study, cytotoxicity activity of the methanolic extracts of some folklore medicinal (*Buchanania axillaris* Desr, *Tamilnadia ulignosa* Retz, *Phaseolus semierectus* L and *Stylosanthes fruticosa* Retz) was evaluated on human cancer cell lines such as colon cancer (HT-29) and breast cancer (MCF-7 and MDA-MB) using MTT assay method. The selected plant extracts showed the dose dependent cytotoxicity activity on the tested cell lines. The cytotoxicity variations on different cell lines were also observed for selected plants extracts. The cytotoxicity of the extracts were increased as the concentration of them was increased. Among all tested plants extracts *Phaseolus semierectus* showed the better cytotoxicity activity on tested cell lines. The results support the folkloric usage of the studied plants and confirmed that the studied plant possesses the constituents with cytotoxic properties that can be useful for developing new anticancer agents.

Keywords: Cytotoxicity, Cancer, Cell lines and MTT assay

1. Introduction
Cancer is the second leading disease causing deaths around the world after cardiovascular disease. Cancer is the disease can affect any part of the body, in this the affected part produce the new cell abnormally rather than normal rate of growth and causes development of a lump, mass or tumor of the cells that are no benefit to the body. The cancerous cells can spread throughout the body from the infected part of the body through metastasis. The commonly affected parts are cervical, breast, stomach, oral, lungs, liver etc [1]. In these type of cancers breast, stomach cancers are leading diseases causing the deaths in India [2]. The healing of cancer consists of psychological support, surgery, radiotherapy and chemotherapy [3]. Presently, chemotherapy is the most commonly using treatment for cancer includes alkylating agents, antimetabolites, antitumor antibiotics, platinum analogs and natural anticancer agents [4]. However, due to the growing rate of mortality because of cancer and undesirable side effects of cancer treatment, the researchers are started investigation to discover new anticancer agents from nature [5], particularly plants because of their less side effects [6, 7] and they have been using in the treatment of diseases since the pre-historic time. In this pint of view the present study was carried out to evaluate the cytotoxic activity of four folklore medicinal plants of India [8, 9] on human cancer cell lines such as colon cancer and breast cancer.

2. Materials and Methods
2.1 Plant material collection and preparation of extracts
Buchanania axillaris Desr, *Tamilnadia ulignosa* Retz, *Phaseolus semierectus* L and *Stylosanthes fruticosa* Retz were collected from of the Thalakona region, Chittoor district, India. The plant specimen was authenticated by Dr. K. Madhava chetty, Department of Botany, Sri Venkateswara University, Thirupati. The plant materials were shade dried, then powdered in mill and extracted separately with methanol using soxhlet extraction process.

2.2 Cell lines
HT-29 cell lines for colon cancer, MCF-7 and MDA-MB cell lines for breast cancer were used for the present study.

2.3 Cytotoxic assay
MTT assay method is a Colorimetric, nonradioactive, fast and economical assay widely used to quantify cell viability and proliferation of mammalian cells.

So, the cytotoxicity of the selected plants methanolic extracts were tested using MTT assay [10, 11]. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely a higher absorbance rate indicates an increase in cell proliferation. Evidence of cell death may be inferred from morphological changes.

3. Results and Discussion

Plants are the base for the treatment for various diseases before the modern medicine. The chemical drugs using in modern medicine for treatment of different diseases including cancer are have the roots from naturally isolated compounds, especially medicinal plants [12]. But the long term use of the synthetic drugs are causing severe side effects to humans [13]. Still natural products, mainly plants are significant source of new drugs [14]. In this point of view, the present study was done to identify the cytotoxicity (Anticancer) activity of *Buchanania axillaris*, *Tamilnadia ulignosa*, *Phaseolus semierectus* and *Stylosanthes fruticosa* on breast cancer (MCF-7 and MDA-MB) and colon cancer (HT-29) cell lines [15].

The selected plant extracts showed the dose dependent cytotoxicity activity on the tested cell lines (Fig. 1-3). The cytotoxicity variations on different cell lines were observed for selected plants extracts. The IC_{50} values are showed in the table 1. The cytotoxicity of the extracts were increased as the concentration of them was increased. The selected plants *B. axillaris*, *T. ulignosa*, *P. semierectus* and *S. fruticosa* have been using by people around the southern parts of the India for treating the diseases [8, 9, 16, 17].

P. semierectus methanolic extract showed more cytotoxic activity on tested cell lines compared to other plants methanolic extracts. The IC_{50} values are 25.50±4.64, 35.56±4.07, 122.06±3.45 on MCF-7, MDA-MB and HT-29 cell lines respectively. The variation of activity on breast cancer cell lines was observed because of their response of action on cells [15]. The *T. ulignosa*, *P. semierectus* and *S. fruticosa* extracts showed the more activity on the MCF-7 cell lines and HT-29 cell lines but surprisingly *B. axillaris* extract showed the better activity on MDA-MB cell lines (Table 1). Among all tested plants extracts *P. semierectus* showed the better cytotoxicity activity on tested cell lines. The cytotoxicity of the medicinal plants is may be due to the apoptosis or necrosis [18, 19]. Apoptosis include cell shrinkage, activation of caspases, DNA cleavage, chromatin condensation, and nuclear fragmentation. During apoptosis, activation of endonucleases causes double-strand breaks in DNA between nucleosomes leading to that DNA is fragmented into multiples less than 200 base pair pieces [20]. Necrotic cell death is an unregulated process resulting from severe damage, such as ATP depletion, hypoxia, various toxins and hyperthermia and characterized by cell swelling, lysis, and the release of intracellular contents associated with pathological tissue injury [21]. The cytotoxicity activity of the selected plants extracts is may be these process [5, 22]. Plants have different chemical constituents in them for their metabolic activities at the same time protection from their predators i.e. the compounds which are protect the plants are may be responsible for increasing the cancer cells mortality. The further studies are needed to isolate the pure compounds and their derivatives from the selected plants which are responsible cytotoxicity.

Table 1: IC_{50} values for test extracts (*B. axillaris*, *T. ulignosa*, *P. semierectus* and *S. fruticosa*) after performing cytotoxicity assay (MTT assay) for 24h on MCF-7, MDA-MB and HT-29 cell lines.

	MCF-7	MDA-MB	HT-29
Bam	350.13±27.03	118.97±0.99	NA
Tum	42.75±4.89	98.24±2.20	138.38±7.33
Psm	25.50±4.64	35.56±4.07	122.06±3.45
Sfm	81.46±21.05	95.07±1.21	142.54±2.86

Values are mean ±SD, n= triplicate experiment.

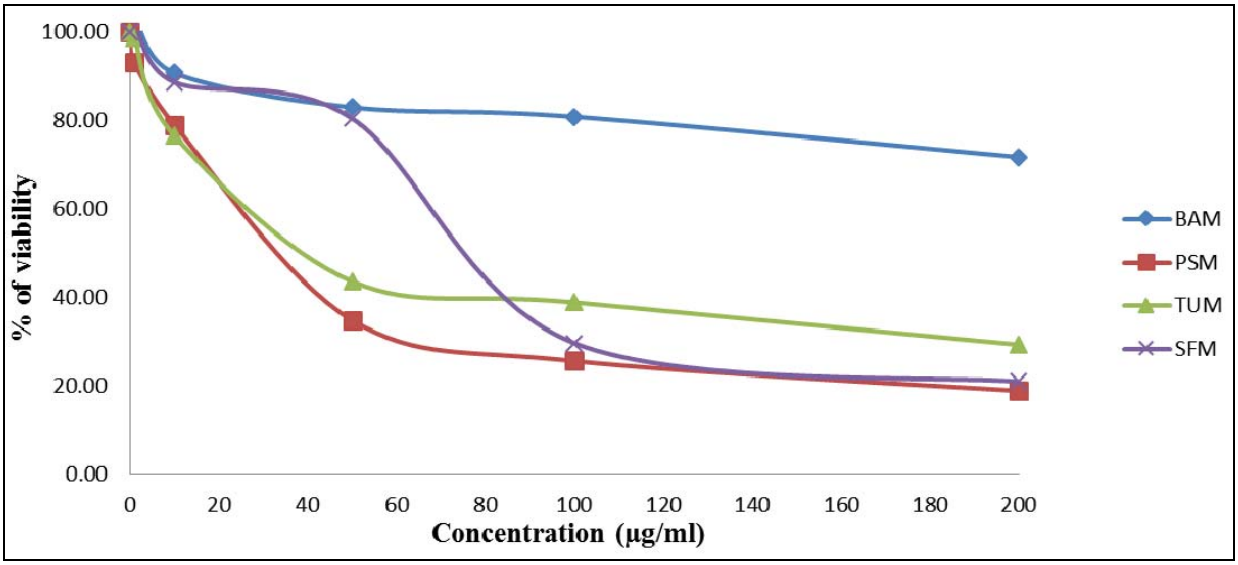

Fig 1: Graphical representation of Cytotoxicity of the test extracts (*B. axillaris*, *T. ulignosa*, *P. semierectus* and *S. fruticosa*) on MCF-7cell line. All values are mean ± SD, n= triplicate experiment after 24h exposure

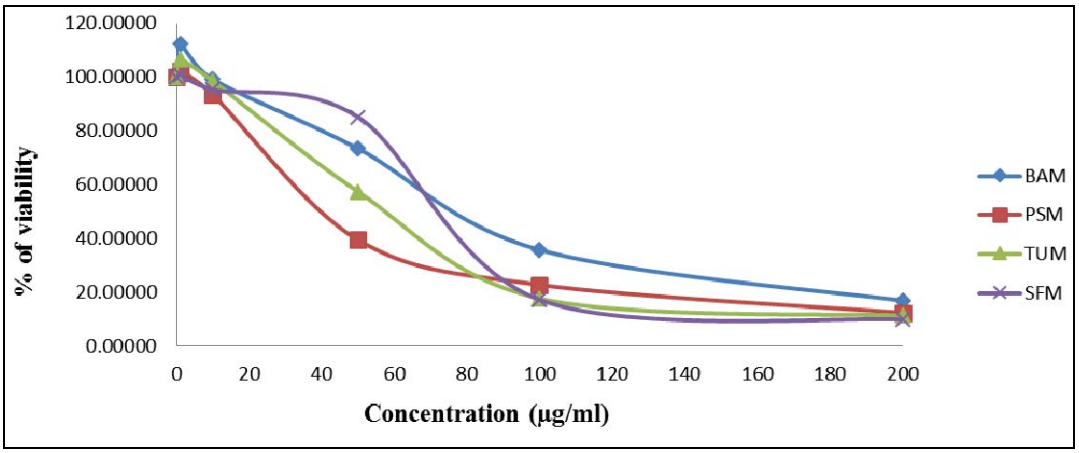

Fig 2: Graphical representation of Cytotoxicity of the test extracts (*B. axillaris*, *T. ulignosa*, *P. semierectus* and *S. fruticosa*) on MDA-MB cell line. All values are mean ± SD, n = triplicate experiment after 24h exposure

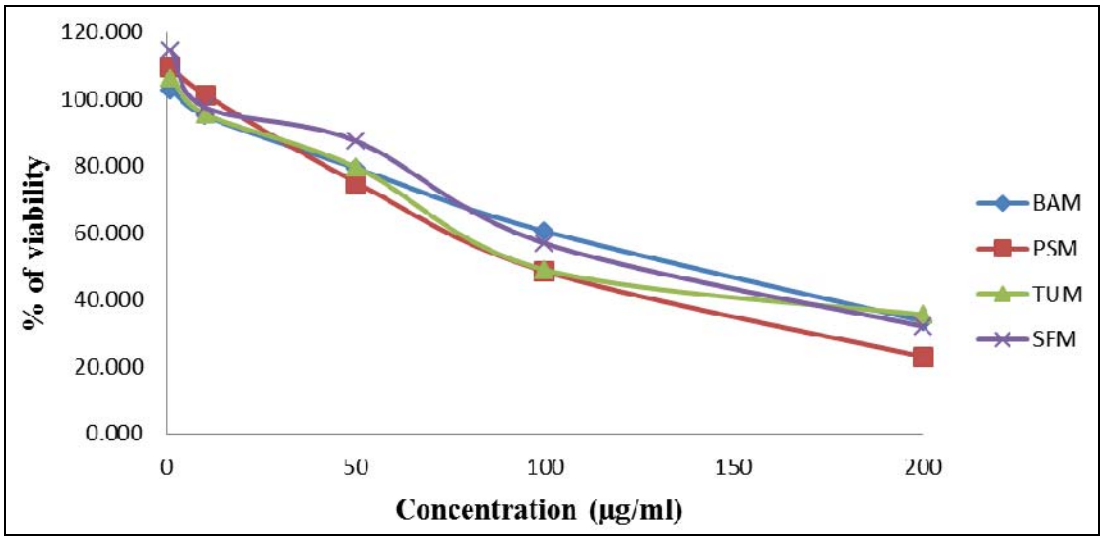

Fig 3: Graphical representation of Cytotoxicity of the test extracts (*B. axillaris*, *T. ulignosa*, *P. semierectus* and *S. fruticosa*) on HT-29 cell line. All values are mean ± SD, n = triplicate experiment after 24h exposure

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