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EVALUATION OF *IN VITRO* ANTI CANCER ACTIVITY OF HYDROALCOHOLIC FLOWER EXTRACT OF *BUTEA MONOSPERMA* VAR. LUTEA

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ABSTRACT: *Butea monosperma* Var. Lutea a native of India, is commonly known as "Palash" and popularly known as "Flame of Forest". Traditionally it has been found that flowers have antimicrobial, wound healing, antifungal, antidiarrhoeal, hypoglycemic, hepatoprotective, antioxidant, anthelmintic, anti-convulsive, antistress, anti diabetic, anti-inflammatory activity. In the present study crude hydroalcoholic flower extract was examined for anticancer activity. To determine *in vitro* anticancer activity, different concentrations of crude extract were tested on MCF-7 breast cancer cell line by 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The extract showed a significant antiproliferative activity and a dose dependant effect. Minimum inhibition 16.8% was shown by extract at concentration 62.5µg/ml and maximum inhibition (46.89%) observed at 500µg/ml. The flower extract showed activity in potential range for further investigation on cancer cells.

INTRODUCTION: Cancer, the one of leading cause of death worldwide, is a group of more than 100 diseases that can affect any part of the body, characterized by uncontrolled cellular growth. It is multifactorial, multifaceted and multi-mechanistic disease requiring a multidimensional approach for its control, treatment and prevention[1]. It is the third leading cause of death worldwide following cardiovascular and infectious diseases [2]. The major cause of cancer is smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation[2].

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Although chemotherapy is now being used as a standard treatment method [3], search for anticancer agents from natural product has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activities a number of plants have been explore. The utility of these plants is increasing day by day. Naturally obtained compounds are considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is also reduced.[4]

Butea monosperma under the family Fabaceae grows throughout the Indian subcontinent, especially in Indo-Gangetic plains. This tree grows upto 50 ft high, with clusters of flowers. It loses its leaves as the flowers develops in the month of January-march[5]. The flowers are 2 cm to 4 cm in diameter, these tend to be densely crowded on leafless branches. Flowers are large, rigid racemes

15 cm long with 3 flowers together form the tumid node of the dark olive green velvety rhachis. The leaves are trifoliate. The plant parts used are bark, leaf, flower, seed and gum [6]. It is mainly useful as antihelmentic, appetizer, aphrodisiac, laxative etc[7]. Moreover , it is used for ethno-veterinary medicine and as traditional medicine for many ailments in the various parts of India and South asia.[8-11] Traditionally it has been found that flowers have antimicrobial[12], wound healing[13], antifungal[14], antidiarrhoeal[15],hypoglycemic[16],hepatoprotective[17], anti-convulsive [18], antistress [19], anti diabetic, antioxidant and anti-inflammatory activity [20]. Moreover, they also have the property of 'Kapha" and 'Vata' (Ayurveda) [21].

MATERIAL AND METHOD:

Plant collection and identification:

Flowers were collected in the month of January at morning from MIDC area, Butibori. The species for the proposed study was identified and authenticated as *Butea monosperma Var. lutea* belonging to family Fabaceae at Department of Botany, RTM Nagpur University, Nagpur. The herbarium is kept in the department.

Extraction

The flowers' petals were dried in shade and powdered, and 100gm of dried powder was subjected to continuous hot soxhlet extraction with water and alcohol (ethanol 90%) at temperature range of 55^0C to 65^0C. The solvent was removed under reduced pressure and controlled temperature by using rotary vacuum evaporator. Phytochemical screening of the extract revealed the presence of tannins, flavonoids, alkaloids, sterols and terpenes.

Cell line.

Breast cancer MCF-7 cell lines [22] was obtained from NCCS, Pune, India. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) 50µg/ml gentamicin sulphate supplemented with 10% heat inactivated Fetal Bovine Serum (FBS), in a humidified atmosphere (incubator) of 50µg/ml CO_2 at 37^0C. The media were changed frequently.

Reagents:

DMEM, FBS and 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St.Louis, MO, USA).

In-vitro assay for Cytotoxicity activity (MTT assay):

The cytotoxicity of sample on MCF-7 was determined by the MTT assay[23]. Cells (1×10^4/well) were plated in 100µl of medium/well in 96 well plates (Coster Corning, Rochester, NY). After 24 hrs when confluent growth growth was observed, the medium was removed and the drug at various concentrations dissolved in maintenance medium (DMEM containing 2.5% FBS) containing 0.1% DMSO was added to each of the well. The plates were incubated for 48h. After incubation, the medium was removed, and 50µl of freshly prepared MTT (2mg/ml in PBS) was added to each of the well and incubated for 4h at 37^0C. The Formazan crystals formed were solubilized in 50µl DMSO. The absorbance was measured at 540 nm using microtitre plate reader (Elisa reader, Bio-Tek XL-800) and percentage viability was calculated. Measurements were performed and the concentration required for inhibition (IC50) was determined graphically. The effect of the sample on the proliferation of MCF-7 was expressed as the %cell viability & cell death using the following formulas:

% Cell death= (Control OD – Sample OD)/ Control OD × 100.

RESULTS AND DISCUSSION:

In-vitro Anticancer Activity:

From MTT assay, after treatment with various concentration of extract, the parameters like cell viability, cell death were compared with untreated (control) cells. The results for cell growth inhibition by the extract against MCF-7 cell lines for various concentrations are shown in **Table 1.** As the concentration increases, there is an increase in the cell growth inhibition.

Plants are storehouse of "pre-synthesized" molecules that act as lead structures, which can be optimized for new drug development. In practice large number of chemotherapeutic agents that are currently available can be traced back to their plant source. Some of the plant derived compounds gained importance in anticancer therapy includes

pacitaxel, vincristine, podophyllotoxin, camptothecine etc. Although there are some new approaches to drug discovery, like combinatorial chemistry and computer based molecular modeling and design but none of them can replace the importance of natural products in drug discovery and development. 8-9 (28-29)

Literature data proved that flavonoid and triterpenes are biologically active against different strains of bacteria as well as many human cancer cell lines [24]. Flavonoids may alter hormone production and inhibits aromatase to prevent development of cancer cells [25].

The present study showed *in-vitro* anticancer activity of flower extract of *Butea monosperma* on human breast cancer cell line(MCF-7) at increasing concentrations. Inhibitory concentration (IC50) was found to be 683.80µg/ml.

TABLE 1: *IN-VITRO* ANTICANCER EFFECT OF *BUTEA MONOSPERMA* EXTRACT ON MCF-7 CELL LINE

Sr.No	Concentration (µg/ml)	% Cell death
1	500	46.89
2	250	30.42
3	125	18.62
4	62.5	16.08
5	Cell Control	0

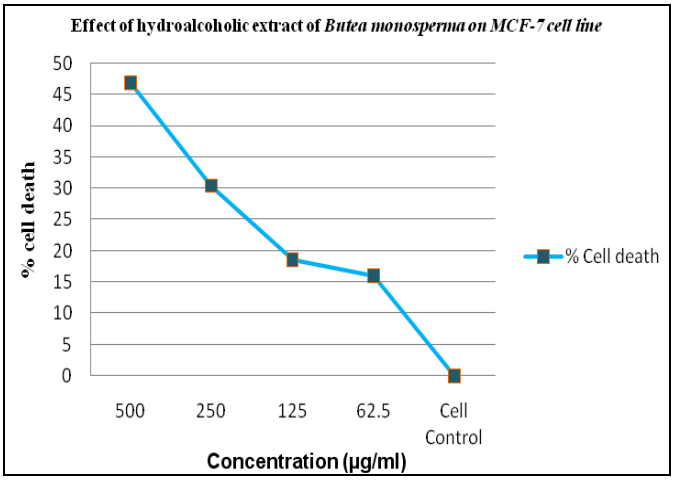

FIG. 1: EFFECT OF HYDROALCOHOLIC EXTRACT OF *BUTEA MONOSPERMA* ON MCF-7 CELL LINE

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