



# IN VITRO EVALUATION OF ANTICANCEROUS ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *B. MONOSPERMA* L. SWOLLEN RACHIS

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## ABSTRACT

Cancer is leading cause of the death worldwide after cardiovascular disease and the incident of cancer are increasing day by day. Plant derive medicine is alternative treatment as available anticancer drugs has severe side effects. In present investigation the inhibitory effect of different concentrations of methanolic extract of swollen rachis of *B. monosperma* L. on breast cancer cell lines was evaluated. Results of the study has been shown that the selected plant part inhibit the proliferation of cancer cells with LD50 value 42.3336 $\mu$ g/ml.

**Keywords:** Cancer, Proliferation, Cell lines MCF 7, Herbal medicine

## 1. Introduction

On this earth, every organism connected with another one directly or indirectly leads to the formation of association in them which may be harmful or beneficial. Due to the harmful association human being faced life threatening diseases. With such dreadful diseases some other diseases are there where human effort fails to detect exact cause and treatment. Now a day cancer is such disease of this category. In the current study, we focused on breast cancer as with ageing, older women posses more threat towards illness such as breast cancer, cardio toxicity and neurodegenerative diseases (de la Cruz *et al.*, 2014; Abouzahr, 2014; Alzheimer's association, 2014) and also understanding the role of medicinal plants available in the India, especially in Maharashtra which have been suggested by the herbal practitioner's especially in rural area and tried to find out the potent anticancer plant sources. It has been stated that, botanical extract are product of

multicompounds and they certainly affects each other, leading to additive, synergistic and /or antagonistic effects (Wagner, 2011; Pelkonen *et al.*, 2014). These have been further tested as complex fractions for breast cancer therapy under *in vitro* conditions to select the promising fractions out of all tested samples. To finalize any plant based remedies, it is suggested to discover the bioactive and toxic phytochemical available in them and further their mechanism of action needs to be deciphered (van Breemen, 2015).

In the present study, an attempt has been made to search for the potent plants and their parts with many active compounds capable of showing anticancer activity especially against breast cancer.

## 2. Experimental

Plant material was selected on the basis survey visits undertaken to herbal practitioners to record herbal anticancerous agents in the Gadchiroli region. After the selection of suggested plant part, the same was collected from the field.

**Plant source:** *B. monosperma* L.

**Common Name:** Palas

**Plant part:** Swollen rachis

### Classification

Kingdom: Plantae  
Division: Angiosperms  
Class : Dicotyledoneae  
Order : Fabales  
Family : Fabaceae  
Genus : *Butea*  
Species: *monosperma*



Fig. 1 – *Butea monosperma* plant part used

### 2.1 Preparation of Plant Extract

The fresh parts of plant was washed with tap water, chopped into smaller pieces and then kept in the shade for 15-20 days to dry and then crushed using pestle and mortar and further reduced to powder using electric blender and then stored in airtight closed bottles until required. The shade dried (10g) powder of each plant material were filled separately in the thimble and extracted with methanol using a Soxhlet extractor followed by evaporation.

### 2.2 Preliminary phytochemical screening:

Chemical tests were carried out using methanolic extract to identify various constituents using standard methods (Horborn, J. B.1998; Trease & Evans 1978).

### 2.3 Thin layer chromatography

Preparation of TLC plates and solvent system was done by the technique suggested by Harborne (1998). Derivatizing Reagent Preparation: (Wagner And Bladt, 1996).

### Detection of Phenols and phenolic acids

Solvent systems: n butanol-acetic acid- water (BAW, 4:1:5) top layer

: Acetic acid-chloroform (1:9)

Spray reagents : Folin Ciocalteu

: Vanillin-H<sub>2</sub>SO<sub>4</sub>

Detection: UV 254nm- dark absorbing spots

:Visible- After derivatization with

Folin Ciocalteu's reagent blue zones indicate presence of phenol & phenolic acid.

### 2.4 Quantification of Phenol

It was estimated from powder of the selected plant part with Folin-Ciocalteu reagent (Sadasivam and Manickam, 1996).

### 2.5 Evaluation of anticancer activity on cell line by MTT assay:

There are two methods available to evaluate anticancerous activities like *in vivo* methods and *in vitro* as clinical trials direct on human are restricted. In present investigation evaluation of selected plant part extract using methanol as solvent was done by *in vitro* method. Five different concentrations of extract were prepared as 6.25, 12.5, 25, 50 and 100 µg/ml for the evaluation of anticancerous activity. Breast cancer (Code: MCF7) cell lines were treated with prepared concentrations of extract to determine their probable anticancer effects. MCF 7 (Breast cancer cells) was initially procured from National Centre for Cell Sciences (NCCS) Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen). MCF 7 cells were initially cultured in , 25 cm<sup>2</sup> tissue culture flask with DMEM (Sigma Aldrich, USA) supplemented with 10% FBS (Gibco), L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of Viability} = \frac{\text{Mean OD Samples}}{\text{Mean OD of Control}} \times 100$$

### 3. Results & Discussion

Results of the present investigation include Preliminary phytochemical screening, Thin layer chromatography, quantitative analysis and evaluation of antiproliferation activity with LD50 value.

The preliminary screening was performed for the presence of Alkloids, phenols, coumarins, , flavonoids, tannins, terpenoids, carbohydrates, sterols, oil and Fats and saponins

### 3.1 Preliminary phytochemical analysis

**Table 1 : Preliminary phytochemical analysis of selected anticancerous plant part.**

Plant Name	Alkaloids	Flavonoids	Terpenoids	Sterols	Tannins	Coumarins	Carbohydrates	Phenols	Oil & Fats	Saponin
<i>Butea monosperma</i> (Swollen rachis)	+	++	+	-	+++	-	+++	++++	++	-

Key: (++++)= indicate very high concentration, (+++)= indicate high, (++)=indicate Medium, (+)=indicate low, (-) = indicate absent

From the preliminary test of selected plant part it was found that *B. monosperma* contains many biological features due to presence of Phenolics, terpenoids, flavonoids, tannins, alkaloids, carbohydrate and oil-fats.

Worker Rmanjaneyulu K *et al.*, (2011) recorded *B. monosperma* leaf extract ethanolic and aqueous extract is rich in alkaloids, carbohydrate, Tannins and Phenolics compounds along with starch and flavonoids which in together reported to be efficient in

antibacterial activity. In another study Borkar *et al.*, (2010) reported that *B. monosperma* leaves in petroleum ether extract is rich in flavones and phytosterols; Hexane extract contain alkaloids and phytosterols; chloroform extract is rich in alkaloids, Tannins, and phenolics along with flavones and phytosterols; ethyl acetate contain carbohydrate, glycosides and lastly ethyl alcohol found to be containing carbohydrates and glycosides.

### 3.2 Thin Layer Chromatography (TLC)

**Table 2 : TLC analysis for Detection of Phenols in methanolic extract from selected medicinal plants**

Sr. No	Band Number	Colour of Band	Rf Value
1	1	Blue green	0.99
2	2	Blue green	0.31
3	3	Blue green	0.10

In all selected plants phenolic compounds were detected with blue green colour at different Rf values. In *B. monosperma* 3 bands were observed at 3 different Rf value as 0.09, 0.31, 0.10. On the basis of Rf value and band color, different secondary metabolites used for cancer treatment were successfully identified.

### 3.3 Quantification of Phenolics

Preliminary phytochemical and TLC analysis data suggest that this plant contain phenolics so the present study focused on recording the amount of phenol (mg/g) and the results found that the selected plant part contain 2.033 mg/g phenol.

**3.4 Evaluation of anticancer activity on cell line by MTT assay:****Table 3: MTT assay of different concentrations of *B. monosperma* plant extract on MCF-7 cell lines**

Sr. No	Sample Concentration (µg/ml)	Average OD at 540	Percentage Viability	LD % 50 Value
	Control	0.8596		
1	6.25	0.46725	79.26209	
2	12.5	0.3854	65.37744	
3	25	0.3168	53.74046	<b>42.3336µg/ml</b>
4	50	0.27745	47.06531	
5	100	0.2487	42.1883	

The extract of swollen part of rachis of this plant when evaluated for antiproliferation activity shows positive result at 42.33 µg/ml concentration shown in the table 3. When the results of the test analyzed it was shown that morphological changes occur in cells when the cells were exposed on different concentration of plant extract. Many cells without nucleus lysis were observed. Similarly, *B. monosperma* also found to be effective in controlling and cancer progress in mice especially prepared in alcoholic solvent when tested in vivo by the worker Banu Rekha J and Jayakar B, (2011).

**4. Conclusion**

Preliminary analysis shows the presence of important primary and secondary metabolites which opens clues to work on individual metabolites for anticancerous activity. Thin layer chromatography and quantitative analysis shows the presence of different secondary metabolites at different concentrations. Antiproliferation activity of all selected plants shows that plant parts contain important compounds which are able to slow down the proliferation of cancer cells. This study also provides the baseline for researchers to study individual compounds by Gas chromatography and Mass spectroscopy and

one can evaluate individual compounds in vitro and in vivo study.

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