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## Preliminary Screening of Anti-Microbial, Anti-Oxidant and Anti-Cancer Potential of *Butea monosperma* Flower Extracts

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#### ABSTRACT

Cancer an aggressive killer, even though there is a lot of development in cures and preventative therapies occur at global level still there is a need to develop effective, and affordable anticancer drugs. Butea monosperma, sacred tree, rich with various phyto constituents used as antimicrobial, anti-fertility, anticonvulsive, anti-helminthic, anti-diarrhoeal, wound healing, hepatoprotection, anti-hypertenstive, antitumor, anti-diabetic, and anti-inflammatory. In the present study, ethyl acetate, chloroform and methanol extracts of Butea monosperma flowers were screened for anti-microbial (agar well diffusion method), anti-oxidant (DPPH and TPC assay) and anti-cancer (MTT assay) activities. Among the three extracts, Ethyl acetate extract showed highest anti-bacterial (Bacillus subtilis, Staphylococcus aureus, E. Coli and Pseudomonas aeruginosa), anti fungal (Aspergillus niger), anti-oxidant (DPPH with IC50-29.64), total phenolic content (90.35mg GAE/g) and cyto-toxicity against MCF-7 cell lines (IC50-24.150µg/mL), while methanol extract exhibited highest anti-fungal activity (Candida albicans) and cyto-toxicity against A-549 cell lines (IC50-15.288µg/mL). Whereas the chloroform extract relatively exhibited less activity compared to the other two extracts. These results clearly indicate that Butea monosperma flowers possess valuable phytochemicals to treat various diseases.

Key words: Anti-cancer; Anti-microbial; Anti-oxidant; Butea monosperma.

#### **INTRODUCTION**

*Butea monosperma* is a flowering plant belonging to *Fabaceae* family, popularly known as "Flame of the forest" due to the presence of its clusters of orange - red (flame) coloured flowers. It is native to India with 33 species (fageria, 2015), has been in use in ayurveda, siddha, unani, folk and sowa – rigpa in the treatment of various diseases. It is medium sized deciduous tree, consists of trifoliate leaves, grey coloured bark, clusters of orange – red flowers and green coloured pods covered with velvet like hair on the surface. The flowers are rich sources of phytochemicals with medicinal properties and eco-friendly dyes used in the colouring of fabrics. It secretes commercially important gum known as *Butea* gum or Bengal kino (fageria, 2015).

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Butea monosperma

The whole plant material is useful in traditional medicinal system (IMPD). Leaf extracts exhibited anti-filarial (Gupta, 2012) anti-inflammatory and anti-oxidant (Borkar, 2010) properties. Flower extracts showed dopaminergic, free radical scavenging activities (Rasheed, 2010 & Velis, 2008). Seed extracts exhibited hormone balancing (Tiwari, 2017), anti-fertility, anti-helmenthic (Tiwari, 2017 & Iqbal, 2006) and anti-hyperglycemic, anti-hyper-lipidemic properties (Bavarva, 2008). Bark extracts exhibited anti-diarrhoeal, wound healing, anti-stress (Gavimath, 2009 & Sharma & Shukla, 2011), and osteogenic, osteoprotective, anti-inflammatory, effects on hormone level and anti-ulcer properties (Bhargavan, 2008, William & Krishna Mohan, 2007 & Panda, 2009). Fruit extracts showed anti-helmenthic activity (Mendhe, 2011).

Cancer is the uncontrolled growth of abnormal cells, second leading cause of deaths after cardio vascular diseases. It is estimated that increased cancer deaths at global level, from 7.1 million in 2002 to 11.5 million in 2030 (Mathers & Loncar, 2006). Present treatments for cancer; chemotherapy, radiotherapy and chemically derived drugs, which leading to cardiotoxicity, renal toxicity and myelotoxicity (Aviles et al., 1993, Manil et al., 1995, & Macdonald, 1999). Hence, there is a need to develop alternative therapies against cancer; plants are rich sources of secondary metabolites, showing anticancer activities, which attract the scientific community to develop new drugs. Considerable research work was done on some medicinal plants and they are classified based

on their pharmacological effect: anti-mitotics (vinca alkaloids (vincristine and vinblastine), podophyllotoxins (etoposide and teniposide), and taxanes (paclitaxel, docetaxel), topoisomerase inhibiters [Topo I (topotecan and irinotecan), Topo II (ellipticine and podophyllotoxins), ROS inducers (EGCG2 and thymoquinone), angiogenesis inhibitors (flavopiridol), histone deacetylases (HDAC) inhibitors (sulforaphane and pomiferin), and mitotic disruptors (roscovitine) (Henry, 2005 & Zaid et al., 2012). Hence in view of the important role of plants in cancer treatment and therapeutic development and based on the potential medicinal properties of Butea, the present investigation has been planned to screen the anti-microbial, anti-oxidant and anti-cancer potential of Butea monosperma flower extracts.

## MATERIALS AND METHODS Collection of plant material

*Butea monosperma* flowers were collected from Yadadri Bhuvanagiri District, Telangana State in the Month of April, 2013, identified and authenticated by Dr. A. Vijaya bhasker Reddy, Dept. of Botany, Osmania University, Hyderabad. A voucher specimen no. OUB-1128 is deposited in the herbarium. The Flowers were shade dried, powdered and extracted with methanol, chloroform and ethyl acetate solvents by the cold extraction method. **Solvent extraction and Rotary vaporization** Powdered flower material weighing one kg was extracted with methanol, chloroform and ethyl acetate separately and filtered. Later, the filtrates were concentrated by using rotary

evaporator and the extracts were stored for further use.

## 1. Anti-microbial studies

## A. Anti-bacterial activity

Methanol, chloroform and ethyl acetate extracts of flowers were tested for anti bacterial activity against gram positive strains; Bacillus subtilis and Staphylococcus aureus and gram negative strains; E. coli and Pseudomonas aeruginosa, in Luria - Bertani agar medium plates containing five wells on each plate by agar well diffusion method (Valgas, 2007). Norfloxacin for gram positive (Bacillus subtilis and Staphylococcus aureus), while Ciprofloxacin for gram negative strains (E. coli and Pseudomonas aeruginosa) were used as standard drugs to observe the susceptibility of bacterial strains. The test organisms were inoculated on the surface of the medium aseptically. Concentrations of 10µl, 25µl, 50µl, 75µl and 100µl three different solvent extracts were placed separately in the wells of plates and labelled and kept for incubation at 37°C for 12-16hrs. Later, the zone of inhibition was measured in mm.

## **B.** Anti-fungal activity

Methanol, chloroform and ethyl acetate flower extracts were screened for anti fungal activity against Aspergillus niger and Candida albicans by agar well diffusion test (Magaldi, 2004) in six sterile Petri plates containing potato dextrose agar medium (PDA) with 5 wells on each plate and divided into two sets. The first set of plates was inoculated with 20µl of Aspergillus niger while the second set was inoculated with 20µl of Candida albicans under sterile conditions and allowed to stand for five minutes. Three solvent extracts; 10µl, 25µl, 50µl, 75µl and 100µl concentrations respectively were placed in the appropriate wells, kept for incubation at 25°C for 72 hrs and later the zone of inhibition was measured in mm. Nystatin is used as standard drug.

## 2. Anti-oxidant activity

## a. DPPH assay

The anti-oxidant activity of flower extracts was determined through sequestration capacity of free radical DPPH (2, 2 -diphenyl-1-

picrylhydrazyl) (Williams, 1995). An amount of 0.1 ml of DPPH radical in ethanol containing 5 ml of extracts in the concentration of 5, 10, 25, 50, 75 and 100  $\mu$ g/ml were incubated at room temperature for 30 min, absorbance of the extracts were recorded at a wavelength of 517nm using the spectrophotometer and the required concentration of the extract to capture 50% of the free radical DPPH (IC50) was calculated.

# b. Determination of total phenolic compounds

The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteu colorimetric method (Singleton, 1999). The samples of extracts were diluted appropriately, mixed with the Folin-Ciocalteu reagent in tubes. After 6 minutes, 7.5%  $Na_2CO_3$  solution was added and kept for incubation in the dark at room temperature for 2 hours. Later, the absorbance was recorded at 765 nm in a spectrophotometer and compared to Gallic acid. The results were expressed in grams of Gallic acid per kilogram of dry sample. The absorbance was recorded and expressed as mg Gallic Acid Equivalents.

## 3. Anti-cancer activity

The in vitro anti-cancer activity of test compounds was determined by MTT assay (Tim, 1983). RPMI - 1640, MTT [3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. Flask of 25 and 75  $\text{cm}^2$  and micro titre plates with 96 wells were purchased from eppendorf India. The Cancer cell lines; A-549 (Human lung cancer) and MCF-7 (Human breast cancer) were purchased from NCCS, Pune and were **RPMI-1640** maintained in medium supplemented with 10 % FBS besides the antibiotics, penicillin/streptomycin (0.5 mL<sup>-1</sup>), in atmosphere of 5% CO  $_2/95\%$  air at 37  $^0$  C. For MTT assay, each test extract sample was weighed separately and dissolved in DMSO. The cell lines were treated with a series of extract concentrations from 10 to 100 µg/ml.

#### Cell viability by MTT assay

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and tryphan blue assay was performed to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X  $10^3$  cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 ° C. After incubation, old media was replaced with fresh media in the amount of 100 µl with different concentrations of test compound in respective wells in 96 plates. After 48 hrs., drug solution was discarded and added fresh media with MTT solution (0.5 mg / mL<sup>-1)</sup> to each well and plates were incubated at 37 °C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density was measured at 570 nm on a microplate reader. The percentage of growth inhibition was calculated using the following formula and concentration of test drug needed

to inhibit cell growth by 50 % (IC50) values is generated from the dose-response curves for each cell line using with origin software.

% Inhibition=100 (Control-Treatment)/Control

## RESULTS

## 1. Anti-microbial activity

## A. Anti-bacterial activity

The anti-bacterial activity of the standard drug Norfloxacin against Gram positive; B. subtilis and S. aureus, and the standard drug Ciprofloxacin against Gram negative, E.coli and P. aeruginosa bacteria, along with the Butea monosperma flower extracts; methanol, chloroform and ethyl acetate is given as Supplementary Information Table-1 and Fig.-1). Among three extracts, the ethyl acetate extract exhibited the highest anti-bacterial activity against both the Gram +ve and Gram ve strains, while methanol extract showed moderate anti-bacterial activity against both the Gram +ve and Gram -ve strains, and whereas the chloroform extract did not show any activity against both the Gram +ve and Gram -ve strains.

| Zone of Inhibition in mm |                    |                |          |     |             |       |     |  |    |               |     |    |   |    |  |      |
|--------------------------|--------------------|----------------|----------|-----|-------------|-------|-----|--|----|---------------|-----|----|---|----|--|------|
| Extract                  |                    |                | Gram -ve |     |             |       |     |  |    |               |     |    |   |    |  |      |
| ( µg)                    | Norflo-            | <b>B.</b> s    | ubtili   | 5   | <i>S. a</i> | ureus | 1   | -  |    | E.coli P. aer |     | -  |   |    |  | nosa |
|                          | xacin <sup>s</sup> | E <sup>M</sup> | EC       | EEA | EM          | EC    | EEA | $\begin{array}{c c} \mathbf{xacin}^{\mathrm{s}} & \\ \hline \mathbf{E}^{\mathrm{M}} & \mathbf{E}^{\mathrm{C}} & \mathbf{E}^{\mathrm{EA}} \\ \end{array}$ | EM | EC            | EEA |    |   |    |  |      |
| 10                       | 9                  | 1              | 0        | 12  | 0           | 0     | 12  | 10   | 0  | 0             | 10  | 2  | 0 | 12 |  |      |
| 25                       | 10                 | 2              | 0        | 14  | 4           | 0     | 13  | 11   | 1  | 0             | 11  | 5  | 0 | 13 |  |      |
| 50                       | 11                 | 4              | 0        | 14  | 6           | 0     | 14  | 13   | 2  | 0             | 12  | 8  | 0 | 14 |  |      |
| 75                       | 14                 | 5              | 0        | 16  | 8           | 0     | 16  | 15   | 3  | 0             | 13  | 12 | 0 | 15 |  |      |
| 100                      | 16                 | 6              | 0        | 16  | 10          | 0     | 16  | 16   | 4  | 0             | 14  | 14 | 0 | 16 |  |      |

 Table 1: Comparative potential (zone of inhibition in mm) of standard drugs vis-à-vis Butea monosperma flower extracts against gram +ve and gram –ve bacteria

 $(E^{M} = Methanol extract; E^{C} = Chloroform extract; E^{EA} = Ethyl acetate extract; s = standard$ 

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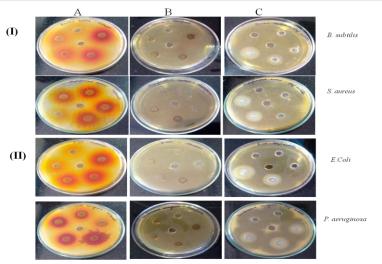


Fig. 1: Anti-bacterial activity of (I) Gram positive strains *B. subtilis* and *S. aureus* and (II) Gram negative strains *E. Coli* and *P. aeruginosa* (A=Methanol extract; B=Chloroform extract; C=Ethyl Acetate extract)

#### A. Anti-fungal activity

The anti-fungal activity (zone of inhibition) of methanol, chloroform and ethyl acetate extracts of *Butea monosperma* flowers against *A. niger* and *C. albicans* is depicted (Table-2 and Fig.-2). Ethyl acetate exhibited a maximum activity of 8mm at 100  $\mu$ g followed by 5mm at 75  $\mu$ g, while both the methanol and chloroform extracts showed similar activity of 6mm and 2mm respectively at a concentration of 100  $\mu$ g and 75  $\mu$ g against *A. niger*, whereas the other concentrations did not show any

activity against *A. niger*. The anti-fungal activity (zone of inhibition) of methanol, chloroform and ethyl acetate extracts of *Butea monosperma* flowers against *C. albicans* is depicted (Table-2 and Fig.-2). The methanol and chloroform extracts showed similar activity of 6mm and 2mm at concentrations of 100  $\mu$ g and 75  $\mu$ g respectively against *C. albicans*, whereas the other concentrations of all the three extracts did not show any activity against *C. albicans*.

 Table 2: Comparative potential (Zone of Inhibition in mm) of standard drugs vis-à-vis Butea monosperma flower extracts against A. niger andC. albicans fungal strains

|         | Zone of Inhibition in mm |         |                |                            |             |         |                            |  |  |  |  |
|---------|--------------------------|---------|----------------|----------------------------|-------------|---------|----------------------------|--|--|--|--|
| Extract | Standard                 |         | A. niger       |                            | C. albicans |         |                            |  |  |  |  |
| ( µg)   | (Nystatin)               | $E^{M}$ | E <sup>C</sup> | $\mathrm{E}^{\mathrm{EA}}$ | $E^M$       | $E^{C}$ | $\mathrm{E}^{\mathrm{EA}}$ |  |  |  |  |
| 10      | 12                       | 0       | 0              | 0                          | 0           | 0       | 0                          |  |  |  |  |
| 25      | 13                       | 0       | 0              | 0                          | 0           | 0       | 0                          |  |  |  |  |
| 50      | 15                       | 0       | 0              | 0                          | 0           | 0       | 0                          |  |  |  |  |
| 75      | 18                       | 2       | 2              | 5                          | 2           | 1       | 0                          |  |  |  |  |
| 100     | 20                       | 6       | 6              | 8                          | 6           | 5       | 0                          |  |  |  |  |

 $(E^{M} = Methanol extract; E^{C} = Chloroform extract and E^{EA} = Ethyl acetate extract)$ 

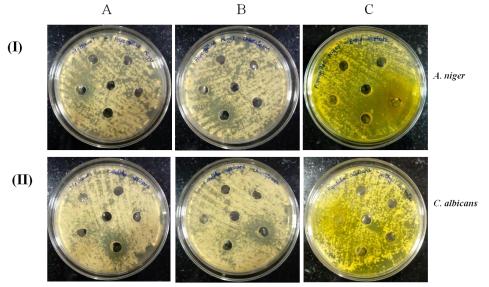


Fig. 2: Anti-fungal activity of (i) *A. niger* and (ii) *C. albicans.* (A=Methanol extract; B=Chloroform extract; C=Ethyl Acetate extract)

## 2. Anti-oxidant activity

## a. DPPH Assay

The concentration required to capture 50% of the free radical DPPH (IC50) was calculated. The extracts with lower IC50 value exhibits higher anti-oxidant activity. In this present study, ascorbic acid was used as standard antioxidant with IC50 value 27.20. The antioxidant activity of methanol, chloroform and ethyl acetate extracts and standard ascorbic acid against the DPPH free radical is given in the table-3 respectively. The anti-oxidant potential of the tested extracts is as follows: Ethyl acetate extract (IC50 – 29.64) > Methanol extract (IC50 – 85) > Chloroform extract (IC50 – 166.489).

| Extract                     | Dose (µg/ml)                     | Dose Response                         | % inhibition  | IC <sub>50</sub> |
|-----------------------------|----------------------------------|---------------------------------------|---|------------------|
| Ascorbic acid<br>(Standard) | 5<br>10<br>25<br>50<br>75<br>100 |                                       | 53.17<br>28.88<br>26.78<br>16.8<br>18.26<br>8.06        | 27.20            |
| Methanol                    | 5<br>10<br>25<br>50<br>75<br>100 | · · · · · · · · · · · · · · · · · · · | 58.921<br>58.11<br>57.347<br>53.959<br>51.908<br>47.566 | 85               |
| Chloroform                  | 5<br>10<br>25<br>50<br>75<br>100 |                                       | 81.154<br>79.866<br>75.62<br>71.278<br>66.603<br>63.311 | 166.49           |
| Ethyl Acetate               | 5<br>10<br>25<br>50<br>75<br>100 | • • •                                 | 80.35<br>63.44<br>46.40<br>37.26<br>19.00<br>8.93       | 29.64            |

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#### b. Total phenolic compounds

Poly phenols from plants have anti-oxidant nature; hence they are essential for protecting from diseases. TPC activity tested for knowing the antioxidant potential of the different solvent extracts of *Butea monosperma* flower. The total phenolic compounds of methanol, chloroform and ethyl acetate extracts and standard Gallic Acid are as shown in the table-4 respectively. The Ethyl acetate extract showed the highest TPC than the methanol and chloroform extracts. The decreasing order of Total phenol compounds in the three extracts are as follows: Ethyl acetate (90.35mg GAE/g) > Methanol extract (82.98 mg GAE/g) > CHCl<sub>3</sub> extract (49.113 mg GAE/g).

| Extract                   | Dose<br>(µg/ml)                  | Dose Response | R <sup>2</sup>   | mg/ gm<br>sample<br>(GAE) |
|---------------------------|----------------------------------|---------------|--|---------------------------|
| Gallic Acid<br>(Standard) | 5<br>10<br>25<br>50<br>75<br>100 |               | 67.94<br>52.64<br>41.99<br>21.56<br>12.66<br>6.85        | 17.78                     |
| Methanol                  | 5<br>10<br>25<br>50<br>75<br>100 |               | 93.303<br>85.914<br>83.469<br>73.693<br>48.883<br>41.876 | 82.98                     |
| Chloroform                | 5<br>10<br>25<br>50<br>75<br>100 |               | 67.127<br>63.835<br>57.681<br>56.87<br>41.555<br>24.809  | 49.113                    |
| Ethyl Acetate             | 5<br>10<br>25<br>50<br>75<br>100 |               | 94.75<br>91.12<br>83.93<br>70.48<br>56.02<br>46.55       | 90.35                     |

#### Table 4: Total phenolic content activity of Standard and solvent extracts of *B. monosperma*

## 3. Anti-cancer activity

All the three extracts showed a dose dependent anti-cancer activity against human cancer cell

lines; A-549 and MCF-7. The IC50 values of methanol, chloroform and ethyl acetate extracts against A549 cell lines are shown in

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|--------------------|--|---|
| table-5, and MC    | F-7 cell lines are shown in                              | extract (IC50-34.912 µg/m                       |
| table-6. Whereas   | , the % of viability of the                              | extract (IC50-76.256µg/mL                       |
| A549 cell lines    | and MCF-7 cell lines are                                 | lines. Whereas, among the                       |
| depicted in fig3   | The cyto-toxic activity of all                           | ethyl acetate extract exhibite                  |
| the three extracts | was found to be more at the                              | toxic activity (IC50-24.15                      |
| lowest IC50 value  | es against both the cancer cell                          | by chloroform extract (I                        |
| e                  | three extracts, the methanol highest cyto-toxic activity | and methanol extract (I<br>against MCF-7 cells. |

(IC50-15.288 $\mu\text{g/mL})$  followed by ethyl acetate

ISSN: 2582 – 2845 nL) and chloroform L) against A549 cell e three extracts, the ted the highest cyto-50µg/mL) followed IC50-68.541µg/mL) IC50-71.827µg/mL) against MCF-7 cells.

Table 5: Cyto-toxic activity of methanol, chloroform and ethyl acetate extracts of B. monospermaflowers against A549 human lung cancer cell lines

| against A549 human lung cancer cen intes |               |                     |       |         |               |             |                       |         |  |  |  |
|--|---------------|---------------------|-------|---------|---------------|-------------|-----------------------|---------|--|--|--|
| Extract                                  | Conc. (ug/ml) | Absorbance at 570nm |       | Average | Average-Blank | % Viability | IC <sub>50</sub> (ug) |         |  |  |  |
| Control                                  | -             | 1.854               | 1.856 | 1.854   | 1.854         | 1.849       | 100.00                | -       |  |  |  |
| Blank                                    | -             | 0.005               | 0.006 | 0.005   | 0.005         | 0           | 0                     | -       |  |  |  |
|  | 5             | 0.991               | 0.993 | 0.994   | 0.992         | 0.987       | 53.38                 |         |  |  |  |
|  | 10            | 0.962               | 0.963 | 0.965   | 0.963         | 0.958       | 51.81                 |         |  |  |  |
| Methanol                                 | 25            | 0.887               | 0.889 | 0.891   | 0.889         | 0.884       | 47.81                 | 15.29   |  |  |  |
| Methanor                                 | 50            | 0.726               | 0.728 | 0.729   | 0.727         | 0.722       | 39.05                 | 15.29   |  |  |  |
|  | 75            | 0.682               | 0.684 | 0.685   | 0.683         | 0.678       | 36.67                 |         |  |  |  |
|  | 100           | 0.602               | 0.604 | 0.605   | 0.603         | 0.598       | 32.34                 |         |  |  |  |
|  | 5             | 1.223               | 1.225 | 1.226   | 1.224         | 1.219       | 65.59                 | - 76.26 |  |  |  |
|  | 10            | 1.138               | 1.141 | 1.142   | 1.14          | 1.135       | 61.38                 |         |  |  |  |
| Chloroform                               | 25            | 1.099               | 1.101 | 1.103   | 1.101         | 1.096       | 59.27                 |         |  |  |  |
| Chioroforni                              | 50            | 0.984               | 0.985 | 0.987   | 0.985         | 0.98        | 53.00                 |         |  |  |  |
|  | 75            | 0.927               | 0.929 | 0.931   | 0.929         | 0.924       | 49.97                 |         |  |  |  |
|  | 100           | 0.868               | 0.869 | 0.871   | 0.869         | 0.864       | 46.73                 |         |  |  |  |
|  | 5             | 1.095               | 1.097 | 1.098   | 1.096         | 1.091       | 59.004                |         |  |  |  |
|  | 10            | 1.016               | 1.017 | 1.019   | 1.017         | 1.012       | 54.732                | 34.91   |  |  |  |
| Ethyl acatata                            | 25            | 0.969               | 0.971 | 0.973   | 0.971         | 0.966       | 52.244                |         |  |  |  |
| Ethyl-acetate                            | 50            | 0.825               | 0.827 | 0.828   | 0.826         | 0.821       | 44.402                |         |  |  |  |
|  | 75            | 0.746               | 0.748 | 0.749   | 0.747         | 0.742       | 40.129                |         |  |  |  |
|  | 100           | 0.681               | 0.683 | 0.684   | 0.682         | 0.677       | 36.614                |         |  |  |  |

Table 6: Cyto-toxic activity of methanol, chloroform and ethyl acetate extracts of B. monosperma flowers against MCF-7 human breast cancer cell lines

| Extact     | Conc. (ug/ml) | Absorbance at 570 nm |       | Average | Average-Blank | % Viability | IC <sub>50</sub> (ug) |        |
|------------|---------------|----------------------|-------|---------|---------------|-------------|-----------------------|--------|
| Control    | -             | 1.136                | 1.137 | 1.136   | 1.136         | 1.134       | 100                   | -      |
| Blank      | -             | 0.002                | 0.003 | 0.002   | 0.002         | 0           | 0                     | -      |
|            | 5             | 0.87                 | 0.871 | 0.872   | 0.871         | 0.869       | 76.631                | 71.827 |
|            | 10            | 0.729                | 0.731 | 0.733   | 0.731         | 0.729       | 64.285                |        |
| Methanol   | 25            | 0.685                | 0.686 | 0.687   | 0.686         | 0.684       | 60.317                |        |
| Wiethanoi  | 50            | 0.61                 | 0.612 | 0.613   | 0.611         | 0.609       | 53.703                |        |
|            | 75            | 0.563                | 0.564 | 0.566   | 0.564         | 0.562       | 49.559                |        |
|            | 100           | 0.494                | 0.496 | 0.497   | 0.495         | 0.493       | 43.474                |        |
| Chloroform | 5             | 0.858                | 0.859 | 0.861   | 0.859         | 0.857       | 75.573                |        |
|            | 10            | 0.716                | 0.717 | 0.719   | 0.717         | 0.715       | 63.051                |        |
|            | 25            | 0.674                | 0.675 | 0.677   | 0.675         | 0.673       | 59.347                |        |

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|---------------|-----|-------|-----------|--------------|----------|-----------|--------|--------|
|               | 50  | 0.602 | 0.603     | 0.605        | 0.603    | 0.601     | 52.998 |        |
|               | 75  | 0.55  | 0.552     | 0.553        | 0.551    | 0.549     | 48.412 |        |
|               | 100 | 0.485 | 0.486     | 0.488        | 0.486    | 0.484     | 42.68  | 68.541 |
|               | 5   | 0.659 | 0.66      | 0.662        | 0.66     | 0.658     | 58.024 |        |
|               | 10  | 0.6   | 0.601     | 0.603        | 0.601    | 0.599     | 52.821 |        |
| Ethyl acetate | 25  | 0.556 | 0.557     | 0.559        | 0.557    | 0.555     | 48.941 |        |
|               | 50  | 0.463 | 0.464     | 0.466        | 0.464    | 0.462     | 40.74  |        |
|               | 75  | 0.409 | 0.41      | 0.412        | 0.41     | 0.408     | 35.978 |        |
|               | 100 | 0.36  | 0.362     | 0.363        | 0.361    | 0.359     | 31.657 | 24.150 |

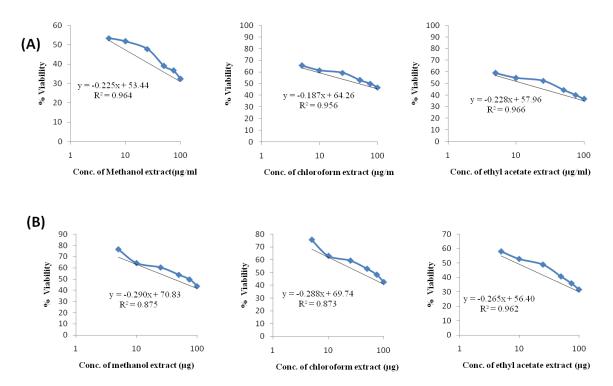


Fig. 3: Cyto-toxic activity of *B. monosperma* flower extracts against (A) A-549 human lung and (B) MCF-7 breast cancer cell lines

#### DISCUSSION

Butea monosperma is being used in Ayurveda since ages, and the medicinal importance of Butea was also mentioned in the ayurvedic literature Sushruta samhita. Dhanvantari nighantu, Raj nighantu and Sodala nighantu. The pharmaceutical properties of Butea plant parts also reported in many previous studies. Ethanolic and aqueous extract of Butea showed monosperma bark anti-bacterial activity against Bacillus aureus, Pseudomonas aerugenosa and E.coli (Lohitha, 2010). Petroleum ether and alcoholic extracts of Butea monosperma gum showed significant anti microbial activity against Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Copyright © Nov.-Dec., 2020; IJPAB

Salmonella typhinurium, Pseudomonas Escherichia coli, Candida aeuriogenosa, albicans *Saccharomyces* cerevisiae and (Gaurav, 2008). Flavonoids of Butea flowers exhibited anti-mycobacterial activity (Chokchaisiri et al., 2009). In the present investigation, all the three solvent extracts of Butea flowers showed significant pharmacological activities on dose dependent manner. Among the three extracts, Ethyl significant acetate extract showed anti-(Bacillus bacterial activity subtilis. *Staphylococcus* aureus, Е. Coli and Pseudomonas aeruginosa), similar to other studies; Artemisia indica, Medicago falcata and Tecoma stans (Javid et al., 2015), Justicia

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zelanica, Phyllanthus urinaria, Thevetia nerifolia, Acacia leucophloea (Dabur et al., 2007), lemongrass, oregano, rosemary and thyme (Dahiya & Purkayastha, 2012), Rubus fruticosus (Weli et al., 2020). Dabur et al., the anti fungal activity pointed of S. surattense against A. Fumigates (Dabur et al., 2004), while Acacia nilotica, Justicia zelanica, Lantana camara and Saraca asoca exhibited potential anti fungal activity (Dabur et al., 2007) Artemisia herba alba, Cotula cinerea, Asphodelus tenuifolius, and Euphorbia guyoniana showed potential anti fungal activity against Fusarium graminearum and Fusarium sporotrichioides (Salhi et al., 2017), similarly methanol extract of Butea flowers exhibited highest anti-fungal activity against Candida albicans. Yadava and Tiwari isolated flavone glycosides from flowers and seeds of Butea monosperma exhibited antiviral and anti-fungal properties respectively (Yadava & Tiwari, 2005), (Yadava & Tiwari, 2007). In the present investigation ethyl acetate extract showed significant anti oxidant activity and total phenol content compare to methanol and chloroform, while methanol extract of Butea monosperma leaves showed high anti oxidant activity than standard ascorbic acid (Badgujar et al., 2018), whereas chloroform and ethyl acetate extracts of Butea monosperma bark showed high antioxidant activity (Kaur, et al., 2018). Butein isolated from flowers has free radical scavenging activity (Sehrawat & Kumar, 2012). Torilis leptophylla exhibit antioxidant and radical scavenging activity because of its high total phenolic content (Saeed et al., 2012). Ethyl acetate extract of Ceratonia siliqua L. Leaves and A. hydaspica showed significant anti oxidant activity due to its significant poly phenol content (Afsar et al., 2018). Methanolic extract of Butea flowers showed anti proliferation against MCF-7 cell lines (Kamble et al., 2015), induces apoptosis, inhibit angiogenesis and metastasis (Karia et al., 2018). Chloroform and ethyl acetate extracts of Butea bark showed growth inhibition of MCF-7 cell lines (Kaur, et al., 2018). Chloroform extract of Butea

monosperma leaves has anti-proliferative activity against A-549 cell lines (Badgujar et al., 2018). While in the present study methanol extract of Butea monosperma flowers showed significant cyto-toxic effect against A-549 compare to ethyl acetate and chloroform, whereas in the case of MCF-7 ethyl acetate extract showed the highest cyto-toxic activity. Similarly, ethyl acetate extract of Potentilla chinensis exhibited anticancer activity through apoptosis induction, cell cycle arrest, DNA damage and inhibition of cell migration (Wan et al., 2016). Ou-Yang et al., (2019) pointed out that ethyl acetate extract of Nepenthes exhibited potential anti-proliferative activity against breast cancer cell lines MCF7 and SKBR3 through apoptosis, oxidative stress, and DNA damage. Methanol and ethyl acetate fractions of V. foetens significantly inhibit the cells MCF-7 and Caco-2 proliferation (Waheed et al., 2013). In the present investigation, among the three extracts, ethyl acetate extract showed highest anti-bacterial, anti fungal and anti-oxidant (DPPH) activity and also possess the cyto-toxic potential against MCF-7 cell lines, whereas, the methanol extract exhibited highest anti-fungal and cytotoxic potential against A-549 cell lines than the ethyl acetate and chloroform extracts indicating that ethyl acetate is effective solvent to extract the active components of Butea monosperma flowers.

#### CONCLUSION

The results indicate that *Butea monosperma* is a potential source of phyto-chemicals to fight against various diseases. The results of the present investigation scientifically conforms the use of different extracts of this plant as pharmaceuticals in ethno-medicinal ayurvedic medicine. However, testing against different diseases after the isolation and identification of compounds in pure form will further strengthen the use of *Butea monosperma* not only in ayurvedic medicine but also in other forms of medical treatments.

## **Competing interests**

The authors declare that they have no competing interests.

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Authors' contributions The authors; DMR, SP and NM planned and designed the experiments; while SP carried out the experiments by collecting the plant material, conducting different experiments and analyzed the results under the supervision of DMR and all the three authors jointly prepared and approved the manuscript.

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