International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 3, 2019, pp.-1521-1527.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

PHARMACOGNOSTICALLY STANDARDISED EXTRACT OF *BUTEA MONOSPERMA* EXHIBITED ANTIMITOTIC AND ANTICANCER ACTIVITY ON HepG2 CELL LINES

SANDHYA S.*[1] AND VENKATESWARA RAO[2] AND P. VENKANNA[3]

[1]Department of Pharmacognosy, St. James College of Pharmaceutical Sciences, Kerala University of Health Sciences, Chalakudy, Thrissur, 680307, Kerala, India
[2]Department of Pharmacognosy, School of Pharmacy, Anurag Group of Institutions, Ghatkesar, 501301, Jawaharlal Nehru Technological University, Kakinada, 533003
[3]Department of Pharmacognosy, MAK college of Pharmacy, Chilkoor, 500075, Jawaharlal Nehru Technological University, Kakinada, 533003, India
*Corresponding Author: Email - sanpharm@gmail.com

Received: March 06, 2019; Revised: March 26, 2019; Accepted: March 27, 2019; Published: March 30, 2019

Abstract- Butea *monosperma* (Fabaceae) is popularly known as 'palas' or '*Flame* of forest' is traditionally reported to possess aphrodisiac, antidiabetic, antidiarrhoeal, antiestrogenic, antifertility, antistress, chemo preventive, *etc*. The current work was envisaged on development of a suitable dosage form by incorporating methanolic extract *B.monosperma* for anti-cancer activity. In microscopic studies it had revealed the presence of abundant xylem fibres and xylem vessels. The methanolic extract of stem bark showed the presence of phytoconstituents like flavonoids, tannins and glycosides. The stem bark extracts was screened for the in vitro anti-cancer activity with different methods like anti mitotic activity, MTT assay and Trypan blue exclusion methods. The methanolic extract of stem bark showed moderate anti-cancer activity compared to standard drugs. Further the extract was formulated into a tablet and it was evaluated. The tablets passed all the evaluation parameters as per standard book of reference.

Keywords- *Butea monosperma, EAC and DLA Cell Lines, HepG2, Chickpea, Mitotic, Tablets*

Citation: Sandhya S., *et al*., (2019) Pharmacognostically Standardised Extract of *Butea monosperma* Exhibited Antimitotic and Anticancer Activity on HepG2 Cell Lines. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 3, pp.-1521-1527.

Introduction

Cancer is a major problem in all developed and undeveloped countries, in which un controlled cell growth occurs, almost 90% of cancer-related deaths are due to tumour spreading – a process called metastasis. One-third of all cancers could be cured through early detection and proper treatment, 40% of most cancer cases are preventable by refraining from smoking, having a healthy diet, being more physically active and through the prevention of infections that may lead to cancer [1]. *Butea monosperma* (Lam.), family Fabaceae is an herb in Ayurvedic medicine which is widely grown in many parts of India. *Butea monosperma* has effective natural origin that has a tremendous future for research. This is amoderate sizeddeciduous tree which is widely distributed throughout India, known as 'dhak' or 'palas', commonly known as 'Flame of forest' [Fig-1-3]. It finds use both medicinally and commercially with each part of the plant having utility [2].

Fig-1 *Butea monosperma* plant image

The plant is traditionally reported to possess astringent, bitter, aphrodisiac, anthelmintic, antibacterial, ant dysenteric, antiulcer, antitumor, and antiasthma tic properties. Flowers yields a brilliant yellow coloring matter due to presence chalcones [Fig-2]. In the present work an attempt has been made to explore the pharmacognostical standards, anti-cancer activity followed by formulation and evaluation of tablets incorporated with the extract of *B.monosperma*.

Fig-2 Seeds, flowers of *Butea monosperma*

Fig-3 Bark, leaves of *Butea monosperma*

Materials and methods

Collection and authentication

The stem bark of the plant (*Butea monosperma* L.,Fabaceae) was collected from surroundings of Khammam Dist., and Nalgonda dist., Andhra Pradesh, India in the month of January and February.

Mr. N. Siddulu, Faculty of Botany, Nagarjuna Government College, Osmania University, Nalgonda identified and authenticated the plant. A herbarium of the plant specimen was prepared and submitted in the Department of Pharmacognosy under the voucher no: NCOP-NLG/Ph'cog/2012-13/045.

Pharmacognostic study

The pharmcognostic studies of stem bark of *Butea monosperma* L. was carried as per standard methods. Morphological studies like colour, odour, taste, size, shape and special features, like texture, touch, etc. were carried based on the morphological and sensory profiles of whole drug. Microscopic studies were performed as per standard procedures mentioned below and microphotographs were taken for identification cell and tissue arrangements. Quantitative microscopy like measurement of length and width of phloem fibres and diameter of starch grains in the powder of *B. monosperma* were performed as per standard book of references [3-5].

Proximate analysis

Determination of ash values like total ash, acid-insoluble ash, water soluble ash and sulphated ash; determination of extractive values like determination of alcohol-soluble extractives value, determination of alcohol-soluble extractives value and determination of water-soluble extractives were performed[3-5].

Fluorescence analysis

Powder of stem bark of *Butea monosperma* L. were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. Three parameters were taken into account i.e. observation under long U.V (365 nm), short U.V (256nm) and normal day light [6].

Extraction

Powdered stem bark weighing 500g was extracted with methanol using Soxhlet apparatus after defatting with petroleum ether. The extract thus obtained was concentrated using a vacuum rotary evaporator along with solvent recovery.

Preliminary phytochemical analysis

Further the extract was subjected to preliminary phytochemical analysis and thin layer chromatographic analysis [3-7].

TLC for flavonoids

Adsorbent: Pre-coated Silica Gel GF 254
Solvent system: Chloroform: Acetone: Formic acid (7.5:1.65:0.85)
Toluene: Ethyl acetate: Formic acid (3.6: 1.2: 2.5)
Toluene: Ethyl acetate (8: 2)
Chloroform: Ethyl acetate (4: 6)
Ethyl acetate: Formic acid: Glacial acetic acid: Water (10:1.1:1.1:2.6)
Visualization: UV Chamber

TLC for Tannins

Adsorbent: Pre-coated Silica Gel GF 254
Solvent system:Chloroform: Ethyl acetate: Acetic acid (6:4:4)
Visualization: Vanillin sulphuric acid.

***In vitro* cytotoxic studies**

Anti-mitotic screening on chickpea

In vitro antimitotic screening of stem bark of *Butea monosperma* L. was determined as per Daniel Zips *et al* 2005 with minor modifications [8,9]. The anti-mitotic screening was tested on pulses (chickpea). 10 pods were taken and are soaked in distilled water (control) till the seedlings grow. Duration of experiment is 5-8 days. Observe the everyday growth and note the average value for 10 pods. Similarly soak the seeds in standard methotrexate solution in water and test samples of various concentrations of 5mg/ml, 10mg/ml, 15mg/ml, and 20mg/ml dissolved in water and observe the growth of seedlings and compare it with the control group. Delay in the growth of seedlings in test samples determines the reduction or inhibition of mitotic cell division.

Trypan blue exclusion method on Daltons Lymphoma Ascites (DLA) and Erlisch Ascites Carcinoma (EAC) cell lines

The tumor bearing mice were taken and the tumor cells were aspirated form its peritoneal cavity. It was then washed three times with PBS or normal saline. Trypan blue exclusion method was used to determine the cell viability. 1×10^6 cells in 0.1 ml viable cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffer saline (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37ºC. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on hemocytometer. The numbers of stained and unstained cells were counted separately [10].

$$\%\ Dead\ cells = \frac{Numberofdeadcells}{Totalnumberofcells} \times 100$$

MTT assay on HepG2 cell lines

HepG2 cell lines (Hepatocellular carcinoma) were purchased from National Centre for Cell Science (NCCS) Pune. These cells were grown in 25 cm^2 tissue culture flasks containing suitable media. Cells were maintained by using Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1X Penicillin/Streptomycin at 37ºC in CO_2 incubator in an atmosphere of humidified 5%CO_2 and 95% air. The cells were maintained by routine sub culturing in 25cm^2 tissue culture flasks.as per the protocol followed the cells that were adherent in nature was tryptinised and then resuspended in the fresh medium. Cell suspension was mixed thoroughly by pipetting several times to get a uniform single cell suspension. Different dilutions of test samples (bark extract of *Butea monosperma*) were made in sterile media with final DMSO concentration in the well to be less than 1%. 100µl of cell suspension (10^4cells/ml) was transferred aseptically to each well of a 96 well plate, allowed to attach overnight, and to it 100µl of 1% media/ test sample was added and then the plates were incubated for 72hrs at 37ºC in CO_2. After 72 hours of incubation, 20µl of MTT was added to each well. The plate was again incubated for 2 hours. 80µl of lysis buffer was added to each well, the plate was wrapped in aluminum foil to prevent the oxidation of the dye and the plate was placed on a shaker overnight. The absorbance of the test recorded on ELISA reader at 562nm wavelength was compared with that of DMSO control to get the %inhibition [11,12].

% of Viability = [Absorbance of Sample – Absorbance of Blank (media only) / Absorbance of Control – Absorbance of Blank (media only)] × 100

Formulation and evaluation of tablets

Preparation of *Butea monosperma* tablets

250mg methanolic extract of *Butea monosperma* was prepared by direct compression method. Three ratios were tried and formula selected is given in [Table-1].

Table-1 *Composition of methanolic extracts of Butea monosperma Tablets*

Ingredients	F_1	F_2	F_3
Drug	250	250	250
PVP K30	60	70	80
MCC	130	120	110
Magnesium sterarate	6	6	6
Talc	4	4	4
Total weight	450	450	450

Preformulation studies

The preformulation studies like organoleptic properties, flow properties and solubility were performed [13,14]. Flow properties evaluated were angle of repose, bulk density, tapped density and compressibility index.

Evaluation of tablets

Colour, taste and odour, shape, pH, thickness, hardness test, friability test, weight variation test, disintegration tests were performed as per the standard procedures mentioned [13-18].

Results

Pharmacognostical studies

Macroscopical studies

The macroscopic studies revealed that the stem bark had a light brown colour, sour too salty odour, and salty taste on prolonged contact with mouth. The bark was found in different shapes like curved, channeled and single quill with a rough texture [Fig-3].

Microscopy of stem bark

Transverse section and longitudinal section of stem bark

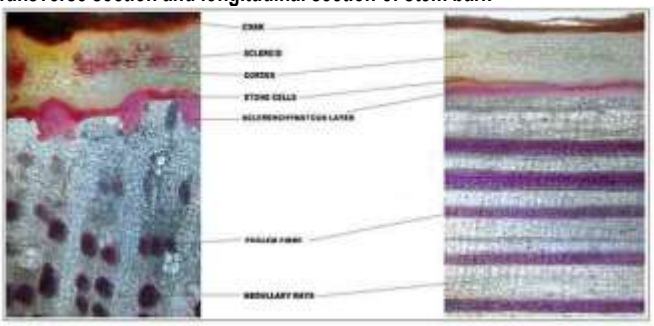

Fig-4 Transverse section and Longitudinal section (LS) of *Butea monosperma*

Periderm

Corkwas made of few layers of rectangular cells in which the outer layer was thin walled and the inner layer with thick lignified walls.

Cortexconsists of several layers of parenchyma in with scattered sclereids either isolated or in groups. Each sclereid was more or less rectangular and pitted with thickened inner and radial walls [Fig-4].

Sclernchymatous band or stone cell layer:A continuous band of sclereids was seen in between the primary cortex and secondary phloem. The sclereids were lignified, pitted with thickened inner and radial walls.

Secondary phloem: This region comprises of phloem parenchyma, phloem fibres, and medullary rays. Phloem parenchyma consists of number of layers with thin walled cells. Phloem fibres occurred mostly single and isolated, rarely in groups 2 to 3, embedded in parenchyma. The fibres were almost circular and lignified with stratification.

Medullary rays: Biseriate to multiseriate medullary rays were observed tapering towards the bottom and wider towards the cortex region.

Powder microscopy of stem bark

The powder microscopy of stem bark of *Butea monosperma* showed characters like Starch grains, phloem fibres, cork cells and stone cells.

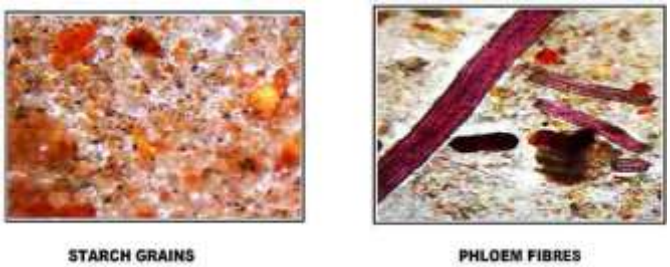

Fig-5 Starch grains and phloem fibres of stem bark of *B.monosperma*

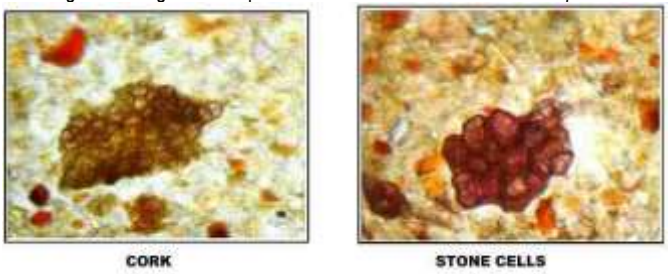

Fig-6 cork and stone cells of stem bark of *B.monosperma*

Starch grains: Abundant starch grains with simple and, round rarely compound was seen scattered throughout the powder [Fig-5].

Phloem fibres: They were numerous either entire or in fragments, occurring lengthwise and in groups [Fig-5].

Cork: Typically, thin walled cork cells with polygonal shape were seen scattered in the powder [Fig-6].

Stone cells: Occurred in three different forms – linear, conical and rectangular all pitted including their walls, often in groups and in U-shaped structure with reddish brown colour [Fig-6].

Microscopic measurements

The length and width of phloem fibres and diameter of starch grains were performed. The length and width of phloem fibres were found to be in the range of 2100 µ -948.5 µ -300 µ and 180 µ -104.4 µ -30 µ respectively. The diameter of the starch grains were found to be in the range of 60 µ -33 µ -12 µ.

Proximate analysis

Proximate analysis of *Butea monosperma* stem bark were determined and the results are tabulated in [Table-2].

Table-2 *Proximate values Butea monosperma stem bark*

Parameter	Result %w/w
Total ash value	10.5
Acid insoluble ash	0.3
Water soluble ash	1.865
Sulphated ash	17.2
Alcohol soluble extractive value	6.02
Water soluble extractive value	12.5

Determination of Fluorescence analysis

Powder of stem bark was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. The findings are tabulated in the [Table-3].

Preliminary phytochemical screening

The preliminary phytochemical studies revealed that the methanolic extract of *Butea monosperma* stem bark contained chemical constituents like flavonoids, proteins, cardiac glycosides, tannins and carbohydrates.

Thin layer chromatography (TLC)

The TLC of flavonoids and tannins showed different spots with different Rf values which are tabulated in [Table-4] and represented in [Fig-7] and [Fig-8].

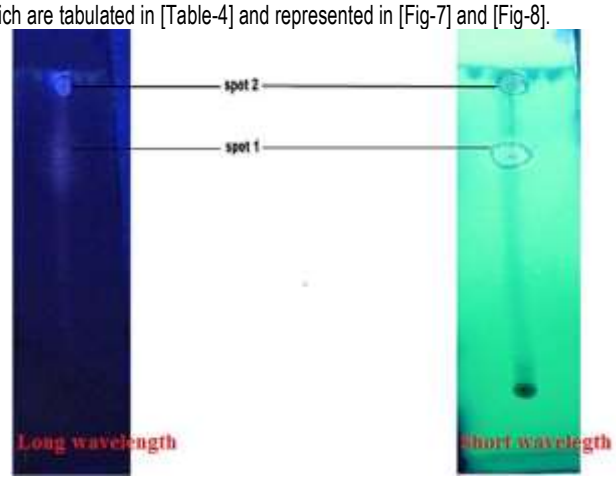

Fig-7 Chromatographic fingerprint of flavonoids

In vitro anti-cancer studies

Anti- mitotic screening

Based on experiment, we found that there was delay in the growth of seedlings in test samples when compared to control and standard. It was observed that the control treated seedlings started germination on the first day of treatment, the standard methotrexate treated seedling started germination only on the 3rd day, whereas the test extract treated seedlings started the germination only on the fourth day onwards [Fig-9-13].

Table-3 *Fluorescence analysis of powder of Butea monosperma stem bark*

Powder +Reagent	Long wavelength (365nm)	Short wavelength (256 nm)	Day light
Powder +Conc HNO_3	Black	Olive green	Brick red
Powder +Conc H_2SO_4	Black	Green	Brown
Powder +ConcHCl	Black	Dark green	Brown
Powder +Glacial acetic acid	Black	Green	Brown
Powder +Glacial acetic acid+ Conc HNO_3	Purple blackish	Greenish brown	Yellowish brown
Powder +10%NaOH	Black	Brownish green	Pale brown
Powder +10%NaOH +Conc HNO_3	Black	Greenish brown	Yellowish brown
Powder +Dragendroff reagent	Brown	Greyish brown	Brick red
Powder +Mayer's reagent	Brown	Green	Light brown
Powder +Hager's reagent	Brown	Yellowish black	Yellowish brown
Powder +Wagner's reagent	Dark brown	Pale green	Brick red
Powder +Benedict's reagent	Black	Greenish black	Brownish red
Powder +$CHCl_3$	Light brown	Greenish black	Dark brown
Powder +5%$CUSO_4$	Brownish black	Greenish black	Light brown
Powder +1% Ninhydrin	Dark brown	Blackish green	Brick red
Powder +5%KOH	Black	Greenish brown	Pale brown
Powder +Petroleum ether	Dark brown	Greenish black	Buff
Powder +5% $FeCl_3$	Black	Green	Brownish red
Powder +Methanol	Pale brown	Greenish brown	Brownish red
Powder +Dil H_2SO_4	Bluish green	Greenish brown	Light red
Powder +Dil HNO_3	Light black	Greenish black	Pale brown
Powder +DilHCl	Brownish black	Greenish black	Bick red
Powder +Cold water	Dark brown	Greenish black	Brown
Powder +Hot water	Dark black	Greenish black	Brick red

Table-4 *Thin layer chromatography extract of stem bark of Butea monosperma*

Solvent system	Phyto constituents	Rf values	Colour of spot
Ethylacetate:Formic acid:Glacial acetic acid:Water (10:1.1:1.1:2.6)	Flavonoids	Spot 1:0.69, Spot 2:0.92	Blue fluorescence
Chloroform :Ethylacetate :Acetic acid (6:4:4)	Tannins	Spot 1:0.43, Spot 2:0.72	White fluorescence

Table-5 *Anti-mitotic screenings of methanolic extract of Butea monosperma stem bark*

	Average growth of seedlings (mm)					
	Concentrations	1st day	2nd day	3rd day	4th day	5th day
Control		0.58	3.04	4.76	6.07	6.43
Standard						
Methotrexate	5mg/ml	-	-	0.63	0.94	1.18
Methotrexate	10mg/ml	-	-	0.46	0.61	0.65
Methotrexate	15 mg/ml	-	-	0.35	0.57	0.62
Methotrexate	20 mg/ml	-	-	0.22	0.31	0.44
Test sample						
MSB (Methanolic extract of *Butea monosperma*)	5mg/ml	-	-	-	0.17	0.43
MSB	10mg/ml	-	-	-	0.12	0.35
MSB	15mg/ml	-	-	-	-	-
MSB	20mg/ml	-	-	-	-	-

Fig-8 Chromatographic finger printing of tannins

It was to be noted that the average length of the seedlings in the test treated were less when compared to the standard and control form which it can be concluded that the methanolic extract of *B.monosperma* was found to possess potent antimitotic activity [Table-5]. The activity was observed to be in a dose dependent manner.

Trypan blue exclusion method

Methanolic extract of *Butea monosperma* on EAC and DLA cell lines exhibited a moderately potent cytotoxicity with IC_{50} values as 165µg/ml and 185µg/ml. The results obtained are tabulated in [Table-6].

Table-6 *Effect methanolic extract of stem bark on EAC and DLA cell lines*

Drug concentration µg/ml	Percentage cytotoxicity on EAC cell lines	Percentage cytotoxicity on DLA cell lines
10	0	2
20	9	10
50	18	18
100	32	28
200	60	54
400	100	82
IC50 (µg/ml)	165	185

MTT assay results on HepG2 cell lines

Methanolic extract of *Butea monosperma* on HepG2 cell lines showed moderate cytotoxicity with IC_{50} value of 330µg/ml. The activity can be attributed to flavonoids and tannins present in the extract [Table-7].

Table-7 *Effect methanolic extract of stem bark on HepG2 cell lines*

Drug concentration µg/ml	Percentage of cytotoxicity
50	5.67±2.49
100	16.56±2.64
200	35.81±4.13
400	58.97±2.99
IC50	330

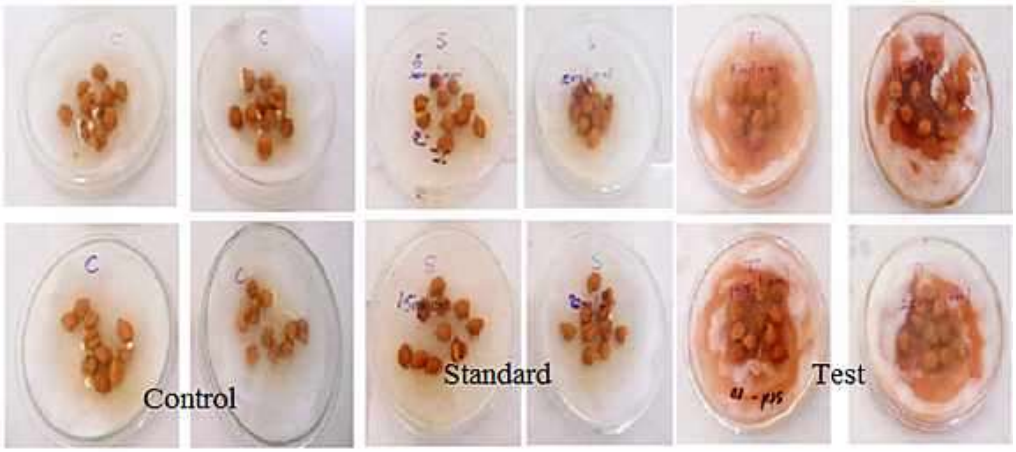

Fig-9 Anti-mitotic screening of methanolic extract of stem bark after 24hrs of experiment

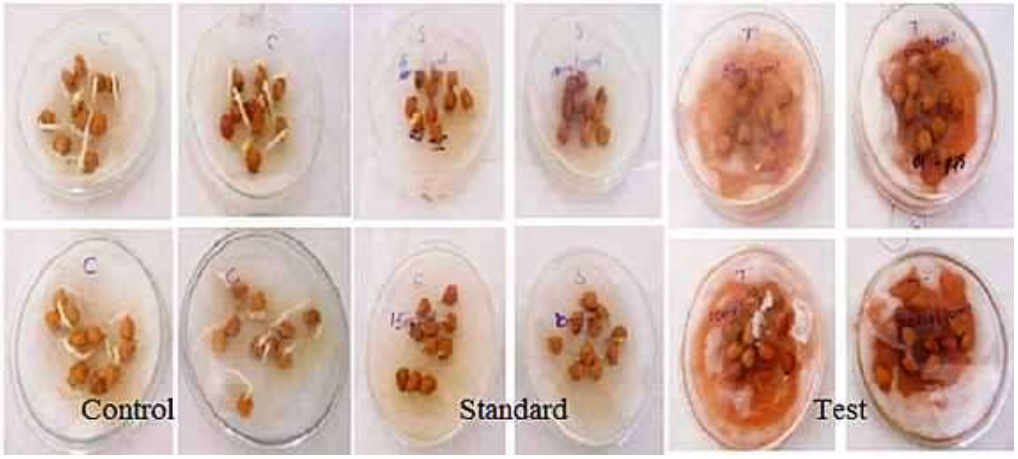

Fig-10 Anti-mitotic screening of stem bark extract after 48hrs of experiment

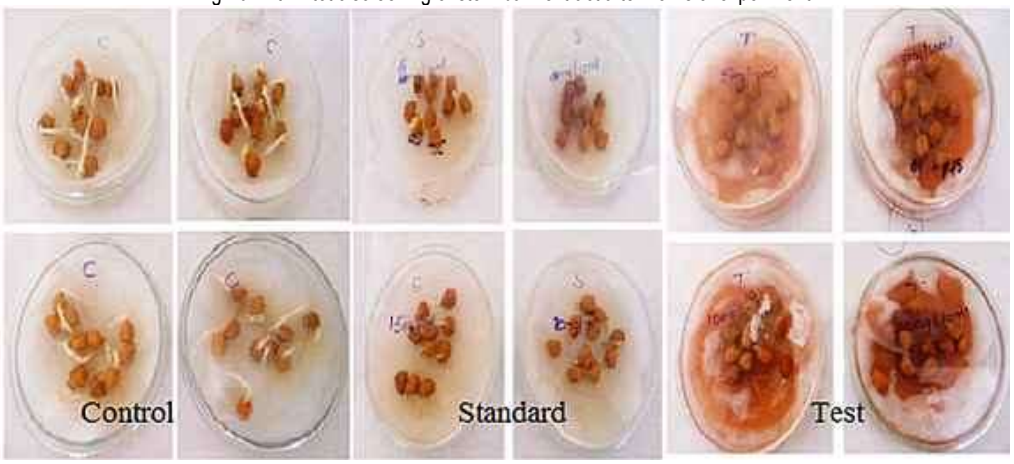

Fig-11 Anti-mitotic screening of stem barks extract after 72hrs of experiment

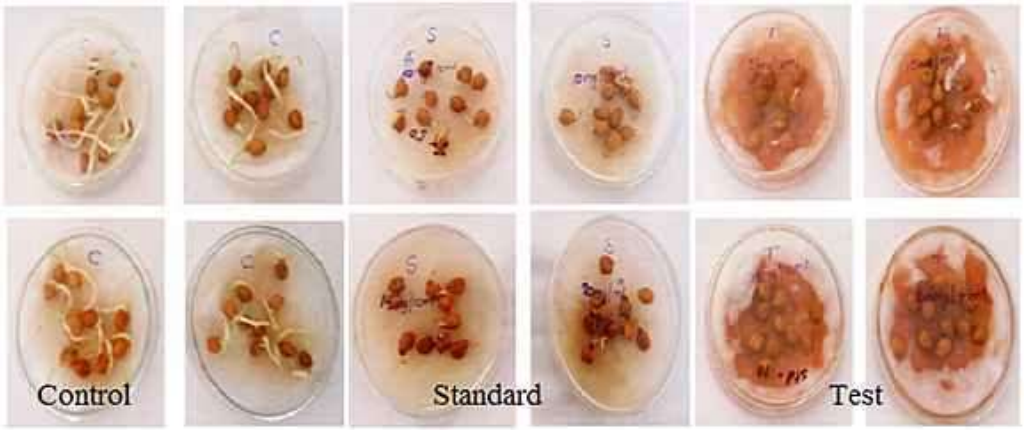

Fig-12 Anti-mitotic screening of stembark extract after 4th day of experiment

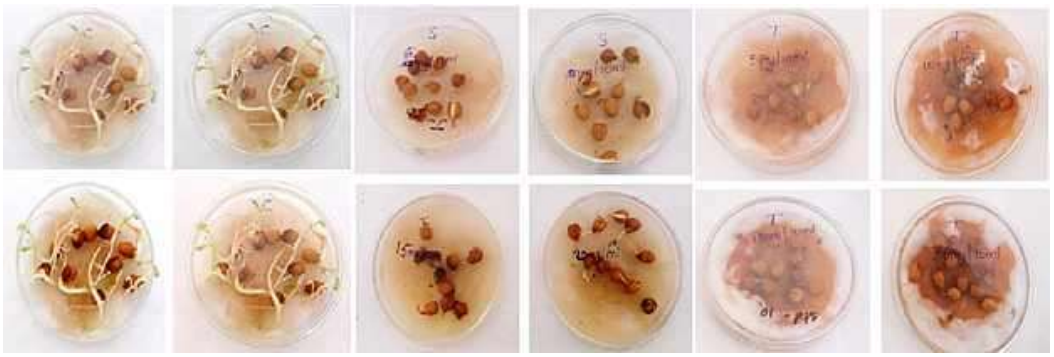

Fig-13 Anti-mitotic screening of stem bark extract after on 5th day of experiment

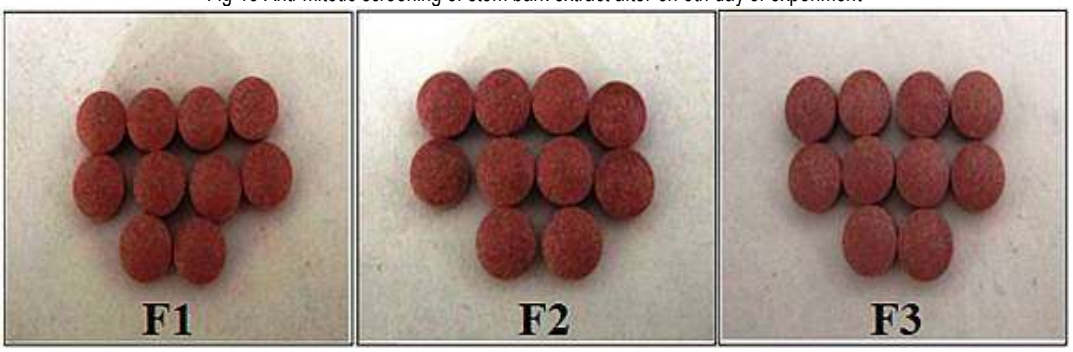

Fig-14 Images of tablets (F1, F2, F3)

Formulation and evaluation

The tablets punched in three different ratios were found to be elegant. Among the three ratio F3 was found to be better when compared to F1 and F2.

Preformulation studies

The preformulation studies were conducted for the three ratios and it was observed that F3 was found to be the optimised formulation as the results obtained were complying with the standard limits [Fig-14]. The parameters conducted for flow properties like bulk density, tapped density, hausner's ration, %compressibility and angle of repose showed F3 to be the optimised formulation [Table-8]. F3 was found to the optimized formulation among the three formulations (F1, F2, F3), as it shows good hardness and friability is within the limits when compared to the F1 & F2 formulations. The disintegration time was found within the limits for F1, F2 and F3. But the friability was found to be elevated in F1 & F2 and hence F3 was confined to be optimized formulation. The other evaluation parameters like weight variation, hardness, thickness, disintegration time and friability were conducted and it was observed that the results obtained for F3 was complying with standard values and hence F3 was confirmed to be the optimised formula [Table-9].

Table-8 *Flow properties of powder*

Formulations	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner ratio	% Compressibility	Angle of Repose
F1	0.57	0.76	1.33	25	29.72
F2	0.58	0.74	1.27	21.6	28.7
F3	0.57	0.68	1.19	16.17	27.82

Flow properties like bulk density, tapped density, hausner ratio, compressibility, angle of repose to be determined for formulations F1, F2, F3 among the three formulation mixtures F3 showed good flow properties and hence F3 was selected as the optimised formulation.

Table-9 *Standardization of tablets*

Ingredients	F1	F2	F3
Weight variation	450±1.6	450±1.2	450±1.366
Hardness	1.9±0.62	2.7±0.69	3.8±0.81
Thickness	5.4±0.08	5.45±0.083	5.4±0.05
Disintegration time	15.4±0.7	20.5±0.547	25.5±0.836
Friability	5.4	1.3	0.87
pH	5.1	5.1	5.1

Discussion

Macroscopic studies play an important role for primary identification of drugs by our sensory organs. Microscopic studies or a structural detail helps for the secondary identification of drugs. A macroscopic and microscopic study helps for the standardization and identification of different constituents of drugs and also adulterants present in powdered drug [5]. The macroscopic studies revealed that the stem bark had a light brown colour, sour too salty odour, and salty taste on prolonged contact with mouth. The bark was found in different shapes like curved, channeled and single quill shape and had a rough texture. The transverse & longitudinal section of stem bark showed cork, sclereids, cortex, phloem fibres, and medullary rays, in the powdered microscopy observed abundant starch grains, phloem fibres and stone cells. The measurement of fibres, starch grains helps to differentiate the species and adulterants. Measurements set a limit and range for identification of authenticity and proximate analysis determined the limits of extraneous matter that can be present in the plant specimen. Ash values used to determine quality and purity of drug. Ash values denote the concentration of inorganic salts present whereas the acid insoluble ash denotes the amount of dirt and sand present in the plant powder sample. From the results of ash values it was found that acid insoluble ash value is less than total ash value. Extractive values play a vital role for the evaluation of the crude drugs which gives an idea about the nature of the chemical constituents present in plant. Presence of the adulterants and faulty processing as well as poor quality of the drug can be broadly determined by alcohol and water soluble extractives. In this plant water extractive value was more when compared with the alcoholic extractive value. Fluorescent studies of the stem bark with various reagents showed wide range of colour changes at day light, UV-chamber (256nm and 365nm). These colour changes reflect the nature of the chemical components present in the plant parts when exposed to the respective chemical reagent. The soxhlet extraction of stem bark revealed the presence of various constituents like flavonoids, proteins, cardiac glycosides, tannins and carbohydrates. The TLC analysis of stem bark showed spots for flavonoids in two different solvent systems and the Rf values were obtained and tabulated. Cancers arise owing to the accumulation of mutations in critical genes that alter normal programmes of cell proliferation, differentiation and death [19]. The processes involved in cell division must be controlled closely so that all the systems and organs work properly. When an uncontrolled multiplication of cells take place the results can become disastrous. [20]. Mitotic screening is the growth of the seedlings of pulses by repeated mitotic and meiotic divisions which are accompanied with various enzymatic reactions [8].

Anti-mitosis reveals the inhibition of growth of seedlings in test samples. Hence, it can be said that the test samples have good anti-mitotic activity, when compared to standard drug methotrexate. This might be due to the presence of flavonoids and tannins present in the extract. It was observed that the test samples showed good anti-mitotic activity in dose dependent manner when compared to standard drug methotrexate and control as water. The drug at toxic concentration damages the cell that makes pores on the cell membrane through which trypan blue enters, and stain the dead cells, which can be distinguished from the viable cells. Since the viable cells are excluded from this staining the method is also described as trypan blue exclusion method [10]. A moderate cytotoxic activity was observed for methanolic extract of stem bark of *Butea monosperma*. The IC_{50} value was found to be 165µg and 185µg for EAC and DLA cell lines, respectively. HepG2 cells are epithelial and usually are not tumerogenic in nude mice. These cells considered to be suitable models for in vitro study of polarised human hepatocytes. These were the perpectual cell lines derived from liver tissue of Caucassian American male of 15years age suffering from well differentiated hepatocellular carcinoma. The MTT assay performed exhibited a maximum activity 58.97 5% on HepG2 cell lines at 400µg and the IC_{50} value obtained was330µg As the crude extract was dry powder, direct compression method was used for preparation of tablets. Among the three ratios selected F1,F2 and F3, it was observed that F3 showed satisfactory evaluation parameters and within the limits as set by the standard boos of reference for the pre formulation studies. Hence it was considered to be the optimised formulation. Tablets were formulated with methanolic extract of stem bark by direct compression method passed all the evaluation parameters and the values obtained were within the official limits.

Conclusion

The detailed pharmacognostical standardization performed will help in setting a suitable plant profile for the stem bark of *B. monosperma*. Since, the plant extract exhibited moderate toxicity, it can be suggested that the formulated tablet can be preferred for a prophylactic treatment or can be suggested for treatment of cancer either in the beginning stage of mild to severe form of cancer. Further studies are yet to be performed to identify the exact chemical entity responsible for the anti-cancer activity obtained.

Application of research: Study of plant profile for the stem bark of *B. monosperma*

Research Category: Anti-cancer drugs, dosage forms

Abbreviations:

EAC: Ehrlich ascites carcinoma, DLA: Dalton's Lymphoma Ascites
TLC: Thin Layer Chromatography
MTT: 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
TS: Transverse section, LS: Longitudinal section

Acknowledgement / Funding: Authors are thankful to St. James College of Pharmaceutical Sciences, Kerala University of Health Sciences, Chalakudy, Thrissur, 680307, Kerala, India. The authors express their deep sense of gratitude to Dr Vinod K.R., Principal, Sanjo College of Pharmaceutical Studies, Vellapara, Palakkad, 678702, Kerala. Authors are also thankful to Nalanda College of Pharmacy, Nalgonda, Telangana for supporting this work.

***Research Guide or Chairperson of research: Dr Sandhya S.**
University: Kerala University of Health Sciences, Chalakudy, Thrissur, 680307, Kerala
Research project name or number: PhD Thesis

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Khammam and Nalgonda, Andhra Pradesh

Plant name: *Butea monosperma* L

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

References

[1] Baruchin A.M., Sagi A., Yoffe B., Ronen M. (2001) *Burns*, 27(7), 781.
[2] PravinaWanjari, Deogade Meena, Daf Sunil (2016) *International Ayurvedic Medical Journal*,1(1),101-106
[3] Kokate C.K. (2008) *Practical Pharmacognosy*, 123-125.
[4] Kailasnath S.T., Rama Krishna K.N., Sai Siva Ramakrishna V., Gourinath A., Satyanarayana Reddy (2004) *Intermediate First year Botany*, 45-48.
[5] Madhavan V., Hema B., Guru Deva M.R., Yoganarsimhan (2009) *Indian Journal of Traditional Knowledge*, 8 (3), 326-333.
[6] Kokate C.K., Purohit A.P., Gokhale S.B. (2006) *Pharmacognosy*, 1.2-1.4.
[7] Wagner H., Bladt S. (2004) *Plant drug analysis. A thin layer chromatography atlas*, 196-197.
[8] Daniel Zips, Howard D Thames, Michael Baumann (2005) *In vivo*, 19, 1-8.
[9] Ch Wong., Kl Goh. (2006) *Biomedical Imaging and Intervention Journal,* 2(3), e7.
[10] Leon Lachman, Herbert A Liberman. (2009)*The theory and Practice of Industrial Pharmacy*, 293-345.
[11] Jain R., Jain S.K. (2010) *International Journal of Phytomedicine*, 2,186-196.
[12] Rahman N., Mahmood R., Rahman H., Haris M. (2014) *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(8), 173-9.
[13] Margret C., Jayakar B. (2010) *International Journal of Pharmaceutical Sciences Review and Research*, 3(1), 101-110.
[14] Rakesh Sharma (2010) *The Open nutraceuticals Journal*, 3, 129-140.
[15] Ghiware N.B., Gattani S.G., Chalikwar S.S. (2010) *International Journal of Pharmtech Research*, 2(1),171-176.
[16] Mishra U.S., Murthy P.N., Sahoo S.K., Sahu K.C. (2012) *International Journal of Pharmacy*, 2(1), 181-186.
[17] Gupta S., Gahlot K. (2012) *Asian journal of pharmaceutical and clinical Research*, 5(1), 148-153.
[18] Desai P.P., Patel N.M., Hardik Soni K., Bhatt S.B. (2012) *Asian Journal of Biochemical and Pharmaceutical Research Issue*,2 (2), 132-136.
[19] Helen D., Graham R.B. (2002) *Nature*, 417,949–954.
[20] Alireza S. (2016) *J Pharma Care Health Sys*, 3(2), 1-4.