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Pharmacognostical standardization, formulation and evaluation of tablets incorporated with stem bark of *Butea monosperma* for anti cancer activity

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ABSTRACT

The plant *Butea monosperma* (Palas) popularly known as 'dhak' or 'palas' and commonly known as 'Flame of forest' belongs to family Fabaceae. This herb is indigenous to India. The plant is traditionally reported to possess astringent, bitter, alterative, aphrodisiac, anthelmintic, antibacterial and anti-asthmatic, anthelmintic, anti-conceptive, anti-convulsive, anti-diabetic, anti-diarrhoeal, anti-estrogenic, anti-fertility, anti-microbial, anti-fungal, anti-bacterial, anti-stress, chemopreventive, hepatoprotective. The present research investigation was envisaged on pharmacognostical standardization, formulation and evaluation of tablets incorporated with methanolic extract of stem bark *Butea monosperma* for anti cancer activity. Conventional release tablets of methanolic extract of *Butea monosperma* stem bark (MEBMSB) were formulated using Microcrystalline Cellulose as a filler and PVP-k30 as a binder by direct compression method. Finally from this research investigation it can be concluded that methanolic extract of *Butea monosperma* stem bark has moderate anti-cancer activity when compare to standards and be suggested as a better substitute for the synthetic anti cancer drugs.

Keywords: *Butea monosperma,* anti cancer activity, MTT assay, EAC cells, DAC cells, HepG2 cell lines.

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INTRODUCTION

The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different health problems. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. In India it is estimated that there are 2 to 2.5 million cancer patients at any given point of time with about 0.7 million new cases coming every year and nearly half die every year. It has long been realized that cancers of the head and neck in both sexes and of the uterine cervix in women are the most common malignancies seen in the country [1,2].

Butea monosperma (Dhak, Palas, Flame of forest, Modugu) It is an erect, medium-sized, deciduous tree with a crooked trunk and irregular branches.

Figure 1: *Butea monosperma* plant

It grows slowly and attains a height of about 5 to 8 m and diameter of about 20 to 40 cm when, it matures at the age of about 50 years or so. Its wood is greenish white in colour, the bark is ash colour. It is common throughout the greater part of India and Burma, up to 1000 m, and higher in the outer Himalaya and hill of South India up to 1300 m [3,4].

Figure 2: Seeds, flowers of *Butea monosperma*

Figure 3: Bark, leaves of *Butea monosperma*

MATERIALS AND METHODS

Plan of work

Butea monosperma (Lam.) is a commonly used herb in Ayurvedic medicine. It belongs to the family Fabaceae, and is grown wildly in many parts of India. The present study is aimed to establish characterization of pharmacognostical standards of stem bark of *Butea monosperma* L. Screening of *in vitro* anti-cancer activity by anti-mitotic activity on Chana. Trypan blue exclusion method on DLA & EAC cell lines, MTT assay on HepG2 cell lines, formulation and evaluation of tablets.

Collection of plant & authentication

The stem bark of the plant (*Butea monosperma* L., Fabaceae) was collected from surroundings of Khammam Dist., and Nalgonda dist., Telangana state. The plant material was identified and authenticated by Mr. N. Siddulu, M.Sc. (Lecturer in Botany), Nagarjuna Government College, (Affiliated to Osmania University) Nalgonda. 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 Pharmacognostic studies of stem bark of *Butea monosperma* L. was carried separately.

Minor extraction

Successive solvent extraction

The stem bark of the plant was collected, dried in shade and powdered. It is then subjected to successive solvent extraction separately in the increasing order of their polarity solvents respectively, petroleum ether, chloroform, ethyl acetate, methanol, water.

Major extraction

700 gm of stem bark power of *Butea monosperma* were taken and extracted with methanol at 600 ^{0}C for. The extract obtained was concentrated with the help of rotary vacuum evaporator.

Thin layer chromatography (TLC) [5, 6]

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of chemical compounds. It is the most basic method of confirming the presence of a phytochemical compound. All the successive extracts (stem bark of *Butea monosperma*) were subjected to TLC and their profiles were noted.

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

Fluorescence analysis [7-9]

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

Procedure

2gm of powdered drug sample was taken in a beaker and dissolved in 5ml of methanol. The sample was transferred to a watch glass and observed under a UV cabinet and the findings of various colours were reported. Similar procedure and observations were reported with different chemicals such as 50% sulphuric acid, 5% sodium hydroxide, concentrated hydrochloric acid, 50% nitric acid, 1N methanolic sodium hydroxide, 1N potassium hydroxide, 5% potassium hydroxide, 5% ferric chloride, concentrated sulphuric acid, ammonia & 50% nitric acid.

Preliminary phytochemical analysis [10-12]

The successive extracts of stem bark of *B. monosperma L.* were subjected to preliminary photochemical analysis.

In-vitro cytotoxic studies [13-18]

Anti-mitotic screening on chana

Anti-mitotic screening of stem bark of *B. monosperma* was determined using *in vitro* method as cited in literature as basis and developed a procedure to determine the anti-mitotic cell divisions.

Procedure

The anti-mitotic screening was tested on pulses (chana). 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.

Try pan blue exclusion method on DLA & EAC cell lines

The Tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1×10^6 cells in 0.1 ml) 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 37 ^{0}C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on haemocytometer. Dead cells taken up the blue colour of trypan blue while live cells do not take up by dye. The numbers of stained and unstained cells were counted separately.

$$\% \text{ Dead cells} = \frac{Number\ of\ dead\ cells}{Total\ number\ of\ cells} \times 100$$

MTT assay

HepG2 cell lines (Hepatocellular carcinoma) were purchased from National Centre for Cell Science (NCCS), Pune. These cells were grown in 25cm^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 37 ^{0}C 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.

Procedure

The adherent cells were trypsinized according to protocol and were resuspended in fresh medium. Cell suspension was mixed thoroughly by pippetting several times to get a uniform single cell suspension. Different dilutions of test samples (bark extract of *B. monosperma*) were made in sterile media with final DMSO concentration in the well to be less than 1%. 100µl of cell suspension (10^4 cells/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. The plate was then incubated at 37 ^{0}C for 72 hours in CO_2 incubator. 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 was recorded on the ELISA reader at 562nm wavelength. The absorbance of the test was compared with that of DMSO control to get the %inhibition.

% of Viability =

$$\frac{\text{Absorbance of sample} - \text{Absorbance of blank (media only)}}{\text{Absorbance of control} - \text{Absorbance of blank (media only)}} \times 100$$

Formulation and evaluation of tablets [19-21]

Formulation

The term "Direct compression" is defined as the process by which tablets are compressed directly from plant extract with suitable excipients. No pretreatment of the extract blend by wet or dry granulation procedure was required, direct compression excipients mainly included diluents, binders and disintegrants. Generally these are common materials that have been modified during the chemical manufacturing process, in such a way to improve compressibility and flow ability of the material. The physicochemical properties of the ingredients such as particle size, flow ability and moisture are critical in direct compression tableting. The success of direct compression formulation is highly dependent on functional behaviour of excipients.

General method

- Screen each powder ingredient in the mixture to remove any lumps or aggregates.
- Weigh the proper amount of each ingredient to prepare a total number of tablets.
- Blend the pre-weighed powder to make a homogenous powder mixture.
- Compress the powder mixture using the single punch press machine available in the lab.

Procedure for manufacturing of *Butea monosperma* tablets

Take the required quantity of the dried methanolic extract of *Butea monosperma* and then grinded to get the powder form after screened extract powder and other excipients of required quantity. Then take the amount of ingredients required for each tablet weight and mix the all the ingredients to obtain uniform mixture, then go to punching of tables by direct compression with compression machine

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 stearate	6	6	6
Talc	4	4	4
Total weight	450	450	450

Preformulation studies

The preformulation studies like organoleptic properties flow properties, solubility and drug-excipients compatibility studies were performed and reported.

RESULTS AND DISCUSSION

a) Morphological 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, channelled and single quill shape and had a rough texture.

b) Microscopy of stem bark

The Transverse section and longitudinal section of stem bark study on microscopical characteristics of *Butea monosperma* provide useful information for its correct identity and help to differentiate from the closely related other species.

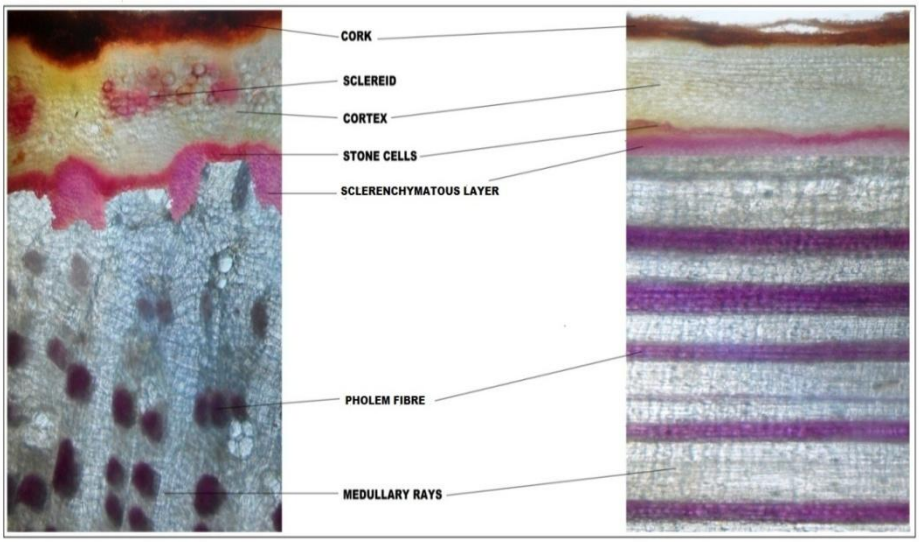

Figure 4: Transverse section (TS) and Longitudinal section (LS) of *Butea monosperma*

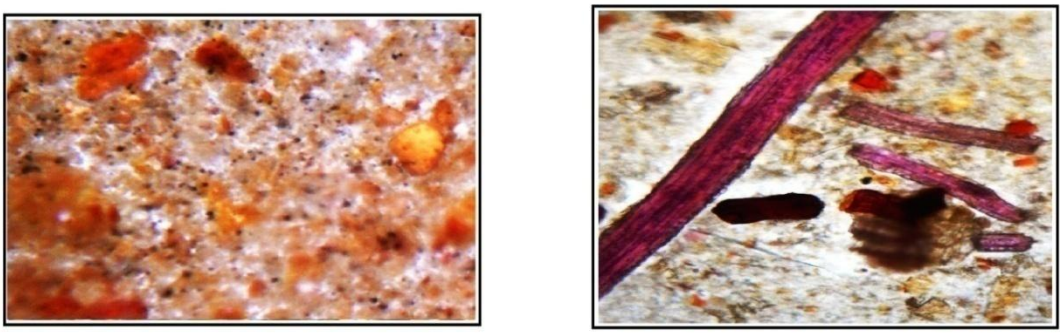

Figure 5: Starch grains and phloem fibres of *B. monosperma*

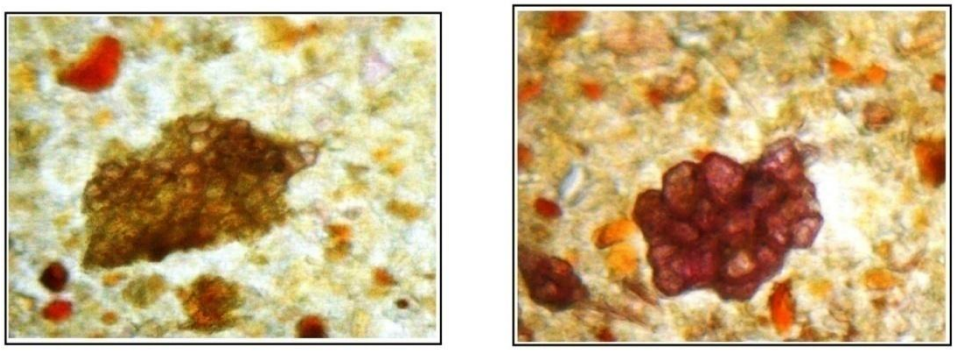

Figure 6: Cork and stone cells of *B. monosperma*

Table 2: Length and width of phloem fibers

Range	Length of fibre (µ)	Width of fibre (µ)
Highest range	2100 µ	180 µ
Lowest range	300µ	30µ
Average	948.5 µ	104.5 µ

Table 3: Diameter of starch grains

Range (µ)	Diameter of starch grains (µ)
Highest range	60 µ
Lowest range	15 µ
Average	33 µ

Table 4: Proximate analysis

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%

Table 5: Fluorescence analysis

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 + Conc HCl	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 +Dragendorff's 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	Light red
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 +Dil HCl	Brownish black	Greenish black	Bick red
Powder +Cold water	Dark brown	Greenish black	Brown
Powder +Hot water	Dark black	Greenish black	Brick red

Table 6: Preliminary phytochemical screening

Chemical constituents	Pet. ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Carbohydrates	Absent	Absent	Absent	Present	Absent
Proteins	Absent	Absent	Present	Present	Present
Flavonoids	Absent	Absent	Present	Present	Present
Tannins & phenolic compounds	Absent	Absent	Absent	Present	Present
Glycosides	Absent	Absent	Absent	Present	Present
Steroids	Absent	Absent	Absent	Absent	Absent
Alkaloids	Absent	Absent	Absent	Absent	Absent
Amino acids	Absent	Absent	Absent	Absent	Absent

Table 7: Thin layer chromatography of stem bark of *Butea monosperma*

Solvent system	Phytoconstituents	R_f values	Colour of spot
Ethylacetate:Formic acid:Glacial acetic acid:Water (10:1.1:1.1:2.6)	Flavonoids	Spot 1:0.69 Spot2:0.92	Blue fluorescence
Chloroform:Ethylacetate :Acetic acid (6:4:4)	Tannins	Spot 1:0.43 Spot 2:0.72	White fluorescence

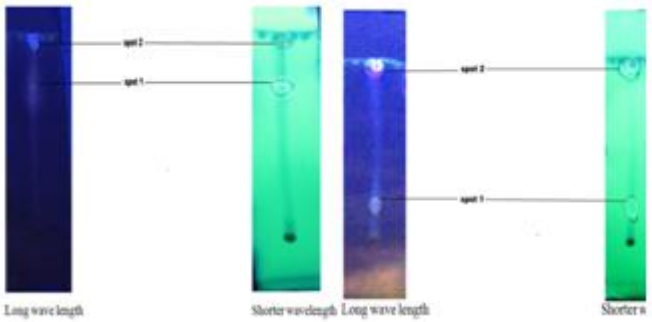

Figure 7: Chromatograms for flavonoids (UV long and short wave length)

Table 8: Average growth of seedlings

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						
MEBMSB	5mg/ml	-	-	-	0.17	0.43
MEBMSB	10mg/ml	-	-	-	0.12	0.35
MEBMSB	15mg/ml	-	-	-	-	-
MEBMSB	20mg/ml	-	-	-	-	-

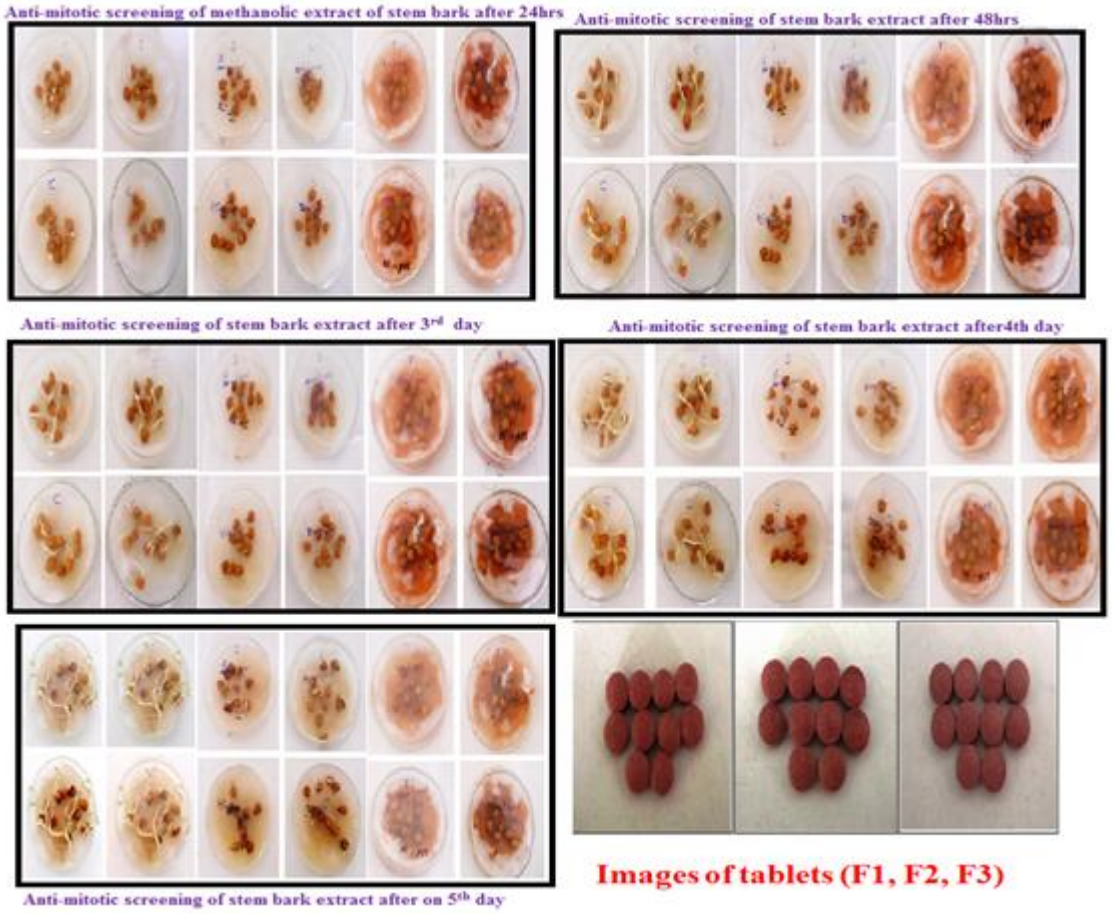

Figure 8: Anti-mitotic screenings of methanolic extract of *B. monosperma* stem bark

Based on experiment, we found that there was delay in the growth of seedlings in test samples when compared to control and standard. Hence, it can be said that the test samples have the good anti-mitotic activity.

Table 9: Effect methanolic extract of stem bark on EAC cells

Drug concentration (µg/ml)	Percentage cytotoxicity of EAC cells
10	0%
20	9%
50	18%
100	32%
200	60%
IC50 (µg/ml)	160

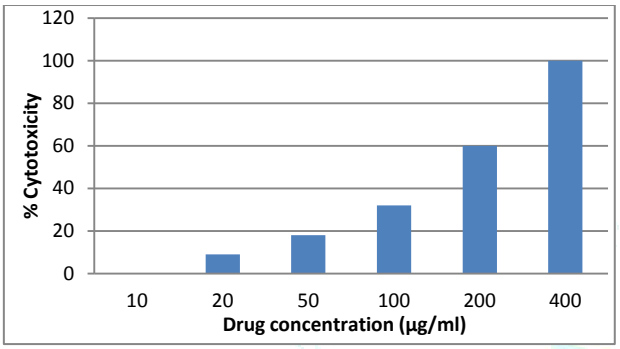

Figure 9: Effect methanolic extract of stem bark on EAC cells

Table 10: Effect methanolic extract of stem bark on DAC cells

Drug concentration (µg/ml)	Percentage cytotoxicity on DAC cells
10	2
20	10
50	18
100	28
200	54
400	82
IC50 (µg/ml)	185

Control tube contained only 2 dead cells

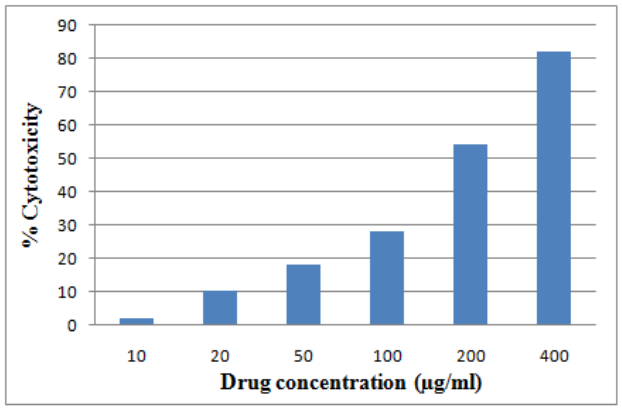

Figure 10: Effect methanolic extract of stem bark on DAC cells

Table 11: 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

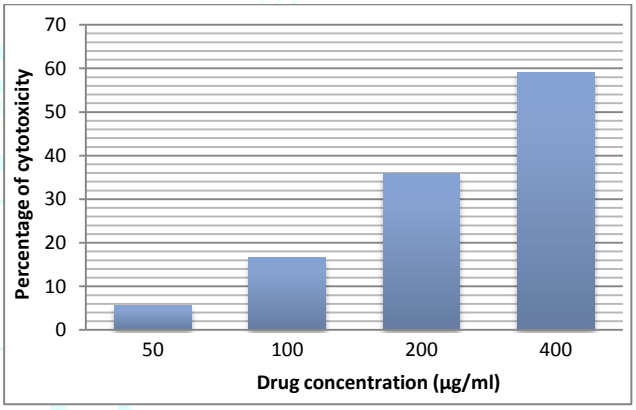

Figure 11: Effect methanolic extract of stem bark on HepG2 cell lines

Table 12: 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

Images of tablets (F1, F2, F3)

Figure 12: Formulation of tablets

Table 13: Evaluation 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

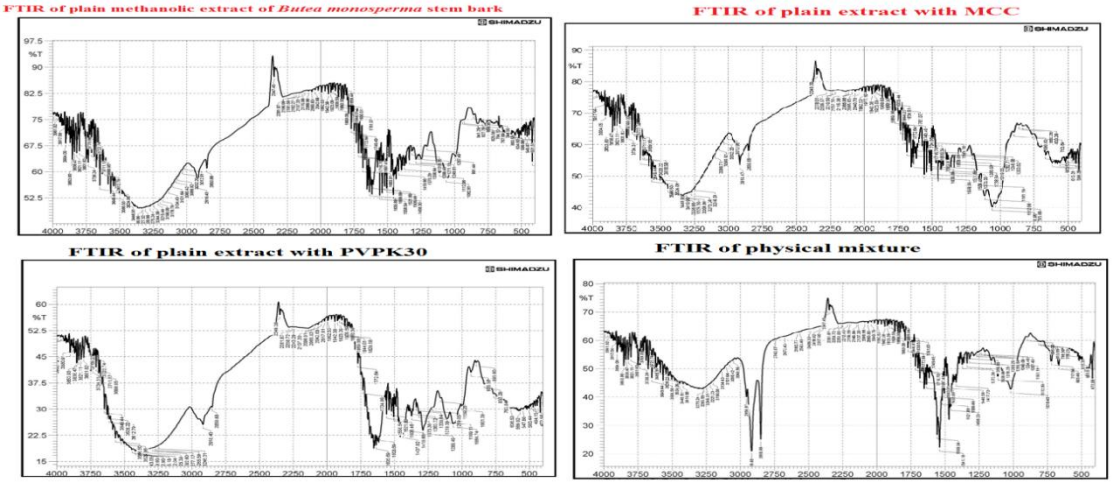

Figure 13: FTIR no interactions with excipients MCC, PVPK30, magnesium stearate, talc

From the above FTIR results, it had been found that all samples had showed same frequencies and vibrations. This indicates that the extract had shown no interactions with excipients MCC, PVPK30, Magnesium stearate and talc.

DISCUSSION

Pharmacognostic study

The quantitative determination of pharmacognostic parameters is useful for setting standards for crude drugs. Identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO,1998), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. 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. The macroscopic studies revealed that the stem bark had a light brown colour, sour to 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.

Microscopic measurements help for the identification of drugs belongs to the same family and species. The transverse & longitudinal section of stem bark showed cork, sclereid, cortex, sclereid fiber, phloem fibers, 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. Total ash value, acid insoluble ash value, water soluble ash and sulphated ash were found to be 10.5%w/w, 0.3%w/w, 1.865% w/w, 17.2%w/w respectively. 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 a crude drug and also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. Alcohol and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. In this plant water extractive value (12.5%w/v) was more when compared with the alcoholic extractive value (6.02%w/v).

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. Hence, this parameter is very important technique for the proper identification of the plant species.

The successive solvent extraction of stem bark was done according to increasing polarity of solvent. The preliminary phytochemical screening is used to know the presence of various chemical constituents present in the plant extracts. Then the extracts were subjected to the preliminary tests in which the methanolic extract had shown the presence of

various constituents like flavonoids, Proteins, cardiac glycosides, Tannins and Carbohydrates. Hence the methanol was selected as the suitable solvent for the major extraction. The information obtained from preliminary phytochemical screening will be useful in finding out the genuity of the drug. The presence of various primary and secondary metabolites can be confirmed by Thin Layer Chromatography (TLC). The TLC analysis of stem bark showed spots for flavonoids in two different solvent systems and the R_f values were obtained and tabulated.

Pharmacological screening

Cancer is a disease it is 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. There are more than 100 types of cancers which affects any part of the body. Tobacco is the single largest preventable cause of cancer in the world. 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.

Reported pharmacological properties *Butea monosperma* include anthelmintic, anti-conceptive, anti-convulsive, anti-diabetic, anti-diarrhoeal, anti-estrogenic and anti-fertility, anti-microbial, anti-fungal, anti-bacterial, antistress, chemopreventive activity on flowers, haemaggultinating, hepatoprotective, radical scavenging, thyroid inhibitory, anti-peroxidative, hypoglycemic effects and wound healing activities. It is powerful astringent and is given in many forms of chronic diarrhoea. Seeds have anthelmintic property especially for roundworms and tapeworms. Such herbal medicines may provide potential effect as of compared to the conventional available synthetic drugs, with less or no side effects.

Anti-mitotic screening on chana

Mitotic screening is the growth of the seedlings of pulses by repeated mitotic and meiotic divisions which are accompanied with various enzymatic reactions. 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 active constituents present in methanolic 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.

Cytotoxic activity by trpan blue exclusion method

The drug at toxic concentration damages the cell that makes pores on the cell membrane through which trypan enters, which are stained blue and which can be therefore distinguished from the viable cells. Since the viable cells are excluded from this staining the method is also described as trypan blue exclusion method. A pronounced moderate cytotoxic activity was observed for methanolic extract of stem bark of *Butea monosperma.* The promising activity may be attributed to the active constituents content present in the extract they showed maximum activity 100% on EAC cell lines at 400µg/ml, IC50 value is 165 µg and showed maximum activity 82% on DAC cell lines at 400µg/ml. IC50 value is 185 µg. Finally it can conclude from the above experiment test sample showed moderate cytotoxicity on DAC & EAC cell lines.

MTT assay on HepG2 cell lines

HepG2 is a perpetual cell line which was derived from the liver tissue of a 15-year-old Caucasian American male with a well-differentiated hepatocellular carcinoma. These cells are epithelial in morphology, have a model chromosome number of 55, and are not tumorigenic in nude mice. The cells secrete a variety of major plasma proteins, e.g., albumin, transferrin, and the acute-phase proteins fibrinogen, alpha 2-macroglobulin, alpha 1-antitrypsin, transferrin, and plasminogen. They have been grown successfully in large-scale cultivation systems. Hepatitis B virus surface antigens have not been detected. HepG2 will respond to stimulation with human growth hormone. HepG2 cells are a suitable *in vitro* model system for the study of polarized human hepatocytes. To determine cell viability the colorimetric MTT assay to be used it showed maximum activity 58.97 5% on HepG2 cell lines at 400µg and the IC50 value is 330µg.

Formulation & evaluation

Tablets are solid dosage forms. They are a unit dose form, and they have the greatest capabilities of all oral dosage forms for greatest dose precision and the least content variability. Their cost also lowest of all oral dosage forms, and have most compact of all oral dosage forms. They may provide the greatest ease of swallowing that's way more patients acceptability. They are better suited to large scale production than other oral dosage forms. They have the best combined properties of chemical, mechanical and microbiological stability of all oral forms. As the crude extract was dry powdered, direct compression method was used for preparation of tablets. Extract was compress along with MCC (micro crystalline cellulose) as a diluent, PVPK30 (poly vinyl pyrrolidine), as a binder and magnesium stearate, talc used as glidents to promote flow properties.

Preformulation studies used study of flow properties powdered form of drug and excipients, FTIR spectroscopic studies to study the interaction studies between the drug and excipients, The frictional force between the particles can be measured by the angle of repose (Θ), Bulk density is very important in the size of containers needed for the handling, shipping, and storage of raw material and blend. It is also important in size blending equipment; the compressibility index (Carr's index) is a measure of the porosity of a granules/powder to be compressed. It is determined from the bulk and tapped densities. Flow properties were found to be satisfactory that is within the limits.

To achieve better formulation binder concentration increased gradually for three formulations from 50 mg to 80 mg for each tablet in each formulation. Final formulation contain PVPK30 80 mg was selected as an optimized formulation, the weight variation of the tablets can be measured by weighing each individual tablets and determining the percent difference from the intended amount. In hardness testing crushing strength of the tablets to be measured, it is to be a satisfactory method of determining the drug content uniformity; the tablets must be hard enough to withstand mechanical stress during packaging, shipment, and handling by the consumer. And thickness of each was recorded in kg/cm^2, in friability testing measuring percentage of weight loss during the mechanical agitation; in the disintegration parameter determine the time to be required to complete breakdown of a tablets.

According evaluation parameters F3 formulation showed satisfactory hardness (3.8±0.81), friability (0.87), and disintegration time of about 25 minutes, weight variation (450±1.366), and showed acidic pH (5.1). Finally it can be concluded that among the three formulations (F1, F2, F3), F3

showed satisfactory evaluation parameters and within the limits that's way selected F3 formulation to my study.

Pharmacognostic standardization of stem bark of *Butea monosperma* was performed as per the standard procedures and it is useful for setting standards for crude drugs, and pharmacognostic study of plant drug is more reliable for establishing identity and purity of crude drug. Methanolic extract of stem bark of *Butea monosperma* showed moderate cytotoxic effect in *in-vitro* models on different cell lines like DLA, EAC & HepG2 and the tablets formulated with this extract can be suggested for prophylactic treatment of cancer.

CONCLUSION

Based on literature review *Butea monosperma* Linn belongs to the family Fabaceae, stem bark of this plant was traditionally used in the treatment of cancer, it is commonly called as modhuga in Telugu. Different activities are already reported in different parts of plant. Cancer also reported on the flowers of plant. The macro and microscopical characters along with physicochemical and fluorescence characters of stem bark powder and sections of bark of *Butea monosperma* L., is used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guidelines. Preliminary photochemical screening of methanolic extract revealed the presence of flavonoids, proteins, cardiac glycosides, tannins and carbohydrates and finally presence of these active constituents confirmed by thin layer chromatography (TLC).

The methanolic extract of *Butea monosperma* stem bark was screened for *in-vitro* anti cancer activity. These studies were carried out by anti mitotic activity on chana, in which the stem bark exhibited a potent anti mitotic activity in a dose dependent manner. Trypan blue exclusion method on DLA & EAC cell lines showed moderate cytotoxic activity. MTT assay on HepG2 cell lines (Hepatocellular carcinoma), showed moderate cytotoxic activity. 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. Since the plant extract exhibited moderate toxicity, it can be suggested that the stem bark can be referred for a prophylactic treatment or can be suggested for treatment of cancer either in the beginning stage of diagnosis or else in the mild form of cancer. Further more research has to be conducted on identification, isolation and characterization of the specific phytochemical responsible for the investigated anti-cancer activity.

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CONFLICTS OF INTEREST

The author declares that there is no conflict of interest to disclose.

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Nil.

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