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FORMULATION DEVELOPMENT AND EVALUATION OF HERBAL TABLET CONTAINING METHANOLIC EXTRACT OF *BUTEA FRONDOSA*

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ABSTRACT

Keywords:

Butea frondosa;
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tablet formulations; Aerosil

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Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. *Butea frondosa* belongs to family Fabaceae is a deciduous tree indigenous to India and widely used in different disease conditions. The present paper deals with formulation and evaluation of herbal tablets prepared from methanol extract of the selected plant. A solid pharmaceutical dosage formulation using a novel dry plant extract (stem barks) using various excipients viz., sprays dried lactose, starch 1500, Aerosil-200 and magnesium stearate by direct compression method. The micromeritic properties were determined for all the physical mixtures, The results of angle of repose, Carr's Index and Hausner ratio indicated that the powder mixtures possess good flow properties and good packing ability. The physical properties of tablet were determined and all the samples of the test product complied with the official requirements of uniformity of weight. The drug content was found to be close to 100% in all formulations. The absorption curve of *Butea frondosa* methanolic extract showed characteristic absorption maximum at 274 nm in 0.1N HCl. The drug obeyed Beer's law in the concentration range of 10mcg/ml to 200mcg/ml, and it was found to be linear with $r^2 = 0.999$, regression equation $Y = 0.017x + 0.003$. It was found that the release rate of drug increased as the percentage of starch 1500 was increased from 10 mg to 30mg. As the concentration of starch 1500 increased the release rate increased from 45.25% to 98.69% (BFT4) in 6 hours by increasing the concentration of starch 1500. The drug interaction FT-IR studies indicated that there was no chemical interaction between the drug and the polymers used in tablet formulations. The optimized formulation BFT4 of the drug was subjected to accelerated stability studies and the results were reproducible, even on tablets that had been stored for about 3 months at 25°C/60% RH, 30°C/60% RH and 40°C/75% RH.

INTRODUCTION

Plants are always an exemplary source of drug. In fact, many of the currently available drugs were derived either directly or indirectly from the plants. The plant kingdom represents a rich source of organic compounds, many of which have been used for medicinal and other purposes ^[1]. Herbal medicine remains the major source of health care for the world's population. *Butea frondosa* belongs to family Fabaceae is a deciduous tree with a somewhat crooked trunk, up to 15m. in height and 1.6-2.0m (some times up to 3.80m) in girth ^[2]. The plant occurs and distribute commonly throughout the greater parts of India and Burma, up to an altitude of 3,000ft. and even higher in the outer Himalaya, Khandesh Akrani upto 3,700ft., hills of South India up to 4,000 ft., Ceylon ^[3].

The various parts used in the traditional medicine are gum, seeds, flowers, bark and leaves of the plant. The bark of *B. frondosa* is used as appetizer, aphrodisiac, laxative, anthelmintic, antidysentery, to cures ulcers and tumors ^[3-5]. The stem bark, leaf and flower of *Butea frondosa* is used as an antimicrobial and anti-inflammatory agent ^[6-9].

The reported phyto constituents present in the *Butea frondosa* are butin, butrin ^[10], flavonoid compounds ^[11-13], tannin ^[14], butolic acid, shellolic acid and jalaric acid (sesquiterpene) ^[15] and palasonin a monoterpene compound ^[16].

Before the extract subjected for the formulation preparation, the chloroform and methanol extracts of *Butea frondosa* stem barks were evaluated for their analgesic and anti-inflammatory activity studies by using different animal models like hot plate method, tail immersion method, acetic acid method, Carrageenan induced rat paw edema method, Complete Freund's Adjuvant (CFA) method and compared with the Standard drugs i.e. Morphine sulphate (5mg/kg), Diclofenac Sodium (5mg/kg) and Indomethacin 4mg/kg respectively. Among the two extracts the methanol extract at the dose of 200mg/kg body weight showed significant biological activities as compared to standard drug. The dose of the extract was selected for biological activity based on acute toxicity studies¹⁷. The experimental protocols were cleared by Institutional Animal Ethical Committee, Royal College of Pharmacy and Health Sciences, Berhampur (Vide No.10/2008/CPCSEA, dt.20.03.2008).

Based on the analgesic and anti-inflammatory activities results, in the present study we design to formulate and evaluate the herbal tablet formulations containing methanol extract of *B. frondosa* stem barks.

The drug concentration used in the formulation was calculated on the basis of their drug tolerance study and effective dose on animal model. In tablet formulation the drug content per tablet was 100mg^[18-19].

MATERIALS AND METHODS

Plant material

The stem barks of *Butea frondosa* were collected from the forest of Similipal Biosphere Reserve, Mayurbhanj, Orissa in August 2006. The plant material was identified and authenticated taxonomically at the Central National Herbarium, Botanical Survey of India, Botanical Garden, Howrah-711103, West Bengal, India (Ref no-CNH/I-I(59)/2006/Tech II, dated- 27.10.2006). A voucher specimen of the collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

The collected stem barks were cleaned, dried under shade and powdered by a mechanical grinder. Hundred grams of the pulverized stem bark was extracted with petroleum ether, chloroform and methanol successively in a soxhlet apparatus. Petroleum ether was used in initial step of extraction for defatting the plant materials. The successive extracts were separately filtered and concentrated at reduced temperature on a rotary evaporator. The yield was found to be around 2.11; 4.38 and 18.08% (W/W) respectively. The biologically potent methanol extract was prepared for herbal tablet formulation.

Powder Characteristics:

Herbal powders are of wide range with varied physical properties and micromeritic properties. Powdered solids are heterogenous because they are composed of individual particles of widely differing sizes and shapes randomly interspersed with air spaces. It is more complicated in case of herbal powders to convert into tablet¹⁹.

Angle of repose

Flow properties of the physical mixtures of all the formulations were determined by calculating angle of repose by fixed height method. A funnel with 10 mm inner diameter of stem was fixed at a height of 2 cm. over the platform. About 10 gm of sample was slowly passed along the wall of the funnel till the tip of the pile formed and touches the stem of the funnel. A rough circle was drawn around the pile base and the radius of the powder cone was measured.

Angle of repose was calculated from the average radius using the following formula.

$$\theta = \tan^{-1} (h/r)$$

Where,

θ = Angle of repose

h = Height of the pile

r = Average radius of the powder cone

Bulk density

Bulk densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The volumes occupied by the sample were recorded. Bulk density was calculated

$$\text{Bulk density (g/ml)} = \frac{\text{weight of sample in gms}}{\text{volume occupied by the sample}}$$

Tapped density

Tapped densities of all types of granules were determined by pouring gently 25 gm of sample through a glass funnel into a 100 ml graduated cylinder. The cylinder was tapped from height of 2 inches until a constant volume was obtained. Volume occupied by the sample after tapping were recorded and tapped density was calculated.

$$\text{Tapped density (g/ml)} = \frac{\text{weight of sample in gms}}{\text{volume occupied by the sample}}$$

Compressibility

It is also one of the simple method to evaluate flow property of powder by comparing the bulk density and tapped density. A useful empirical guide is given by the Carr's compressibility.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio

It provides an indication of the degree of densification which could result from vibration of the feed hopper.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Lower Hausner ratio – Better flow ability, Higher Hausner ratio – Poor flow ability

Preparation of herbal tablets from *Butea frondosa* extract

Herbal tablets were prepared separately by direct compression process using different proportions of spray dried lactose and starch 1500 and denoted as BFT 1, BFT 2, BFT 3 and BFT 4. The composition of various formulations is given in Table 1. All the ingredients were passed through mesh no. 100 and mixed with 1% aerosil (Aerosil-200) and 1% of magnesium stearate. The micromeritic properties were determined for all the mixtures. The powder mixtures possess good flow properties and good packing ability. Thus, the mixtures were directly compressible. Tablets were compressed each of 300 mg weight on a 10-station Mini Press-I rotary tablet compression machine fitted with 8-mm flat-shaped punches. No manufacturing defects were observed in tablets like capping, lamination and chipping.

Evaluation of herbal tablet**Drug content uniformity test**

From each batch 20 tablets were taken, weighed and finely triturated. An adequate amount of this powder equivalent to 100 mg of the drug was accurately weighed and shaken with 150 ml of 0.1N HCl for 10 minutes. The mixture was diluted with 0.1N HCl to produce 200ml and filtered.

10 ml of the filtrate was diluted to 100 ml with distilled water and the absorbance was measured at respective maximum wave length. The drug content in the formulation was calculated using the standard curve.

Hardness and friability test

Hardness was determined by using a Monsanto tablet hardness tester (n = 6). The friability of the tablets was tested using Roche friabilator.

Weight variation test

The tablets were evaluated as per I.P., 1996 for weight variation (n = 20) using 1mg sensitivity balance.

***In vitro* drug release**

Drug release was assessed by dissolution test under the following conditions: n = 6(in triplicate), USP type II dissolution apparatus (Lab India, DISSO 2000) at 50 rpm in 900 ml of 0.1N HCl maintained at $37 \pm 0.5^{\circ}\text{C}$. The tablet was allowed to sink to the bottom of the flask before stirring. Special precaution was taken not to form air pockets on the surface of the tablet. Five milliliters of the sample was withdrawn by using a syringe filter at regular intervals and replaced with the same volume of pre warmed ($37 \pm 0.5^{\circ}\text{C}$) fresh dissolution medium. The drug content in each sample was analyzed after suitable dilution using UV spectrophotometer method at respective maximum wave length.

Drug Polymer Compatibility Studies

The interaction studies were carried out to ascertain any kind of chemical interaction of drug with the excipients used in the preparation of tablet formulations. Fourier-transform infrared (DRS) spectra were obtained by using an FT IR-Affinity-1 spectrophotometer (DRS-8000) SHIMADZU, Japan. The dried pure drug sample BFP was previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr powder was used as blank for background correction in FT-IR (DRS) studies. Forty five scans were obtained at a resolution of 4 cm^{-1} , from $4000\text{ to }300\text{ cm}^{-1}$.

Stability testing of optimized herbal tablet formulation

The optimized formulation of the drug was subjected to accelerated stability studies at specified conditions of temperature and relative humidity of $25^{\circ}\text{C}/60\%\text{ RH}$, $30^{\circ}\text{C}/60\%\text{ RH}$ and $40^{\circ}\text{C}/75\%\text{ RH}$ for 3 months.

Development of UV-VIS Spectrophotometric method for estimation of formulated Herbal Tablet

Scanning and determination of maximum wavelength (λ_{max})

In order to ascertain the wavelength of maximum absorption of the extract, different concentrations of the extract (10 $\mu\text{g/ml}$, 20 $\mu\text{g/ml}$ and 30 $\mu\text{g/ml}$) in 0.1 N HCl were scanned using spectrophotometer within the wavelength range of 400-200 nm against 0.1 N HCl as blank and the wavelength corresponding to maximum absorbance was noted.

Preparation of standard stock solution

Accurately weighed 100mg of extract was dissolved in 3ml of methanol in 100ml volumetric flask and volume was made up to the mark with 0.1 N HCl to give a clear solution of 1000 $\mu\text{g/ml}$ concentration.

Preparation of working standard solutions and construction of Calibration Curve

A series of different concentrations of extract were prepared from working stock solution. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6.....1.8, 1.9 and 2.0 ml solutions were pipetted out from the working stock solution and were transferred in to 10 ml volumetric flasks. 10,20,30,40 upto 200 $\mu\text{g/ml}$ solutions were obtained respectively on making up the solution to 10 ml with 0.1 N HCl.

The absorbances of all these solutions were measured against a blank at respective λ_{max} using a UV double beam spectrophotometer (UV/Vis-1700, Shimadzu, Japan). A standard plot of absorbance v/s concentration of extract gives the standard calibration curve of the extract. This curve was used to determine *In vitro* drug release and drug content of herbal tablets and the observation is given in Table 4.

RESULTS

Table 1: Composition of various tablet formulations containing *Butea frondosa* methanolic extract

Formulation Code	Drug (mg)	Spray Dried Lactose (mg)	Starch 1500 (mg)	Mg. Stearate (mg)	Aerosil (mg)	Total Wt. (mg)
BFT 1	100	184	10	3	3	300
BFT 2	100	179	15	3	3	300
BFT 3	100	174	20	3	3	300
BFT 4	100	164	30	3	3	300

BFT = *Butea frondosa* methanolic extract tablet formulation

Table 2: Micromeritic parameters of physical mixtures containing *Butea frondosa* methanolic extract

Formulations	Bulk density (gm/ml)	Tapped density (gm/ml)	% Compressibility	Hausner ratio	Angle of repose
BFT 1	0.48	0.57	15.78	1.18	25.27
BFT 2	0.46	0.55	16.36	1.19	26.24
BFT 3	0.48	0.59	18.64	1.22	28.23
BFT 4	0.45	0.58	22.41	1.28	30.12

BFT = *Butea frondosa* methanolic extract tablet formulation

Table 3: Physical properties of compressed tablets of *Butea frondosa* methanolic extract

Formulations	BFT 1	BFT 2	BFT 3	BFT 4
Drug content (mg/tab)	100.8±1.2	100.2±0.5	100.1±0.5	100.3±0.9
Weight variation (%)	±5.2	±4.6	±4.4	±4.8
Hardness(kg/cm ²)	5.4±0.6	5.3±0.8	5.4±0.3	5.2±0.6
Friability (%)	0.83	0.75	0.85	0.76

BFT = *Butea frondosa* methanolic extract tablet formulation

Table 4: Calibration of *Butea frondosa* methanolic extract in 0.1 N HCl at λ_{max} of 274nm

Sl. No.	Conc.(mcg/ml)	Absorbance at 274 nm
1	10	0.022
2	20	0.05
3	30	0.08
4	40	0.092
5	50	0.114
6	60	0.132
7	70	0.157
8	80	0.176
9	90	0.198
10	100	0.222
11	110	0.241
12	120	0.264
13	130	0.29
14	140	0.312

15	150	0.339
16	160	0.365
17	170	0.382
18	180	0.401
19	190	0.424
20	200	0.444

Table 5: In-vitro dissolution profile of herbal tablet containing *Butea frondosa* methanolic extract in 0.1 N HCl

Mean cumulative percent \pm S.D* drug released					
Time (hr)	BFP	BFT 1	BFT 2	BFT 3	BFT 4
1	19.22 \pm 2.23	10.13 \pm 1.99	18.19 \pm 3.81	26.13 \pm 3.93	30.43 \pm 4.25
2	38.24 \pm 2.65	19.38 \pm 3.12	30.13 \pm 3.78	43.25 \pm 2.81	50.32 \pm 3.15
3	57.31 \pm 2.87	25.26 \pm 3.45	42.21 \pm 2.98	57.12 \pm 3.32	70.09 \pm 2.65
4	69.32 \pm 3.90	30.13 \pm 3.32	51.16 \pm 2.67	70.13 \pm 3.42	83.59 \pm 3.82
5	74.31 \pm 3.98	38.15 \pm 3.21	60.3 \pm 2.54	75.34 \pm 3.71	95.35 \pm 2.21
6	82.38 \pm 3.76	45.25 \pm 2.87	69.31 \pm 3.34	80.45 \pm 1.85	98.69 \pm 4.26

* SD values (n=6), BFP= *Butea frondosa* pure methanolic extract.

Table 6: Stability data of the optimized formulation BFT- 4

Time	% Drug content at different storage conditions		
	25 ⁰ C/60% RH	30 ⁰ C/60% RH	40 ⁰ C/75% RH
1 month	99.52	99.19	99.50
2 months	99.38	98.56	98.32
3 months	99.47	98.25	96.09

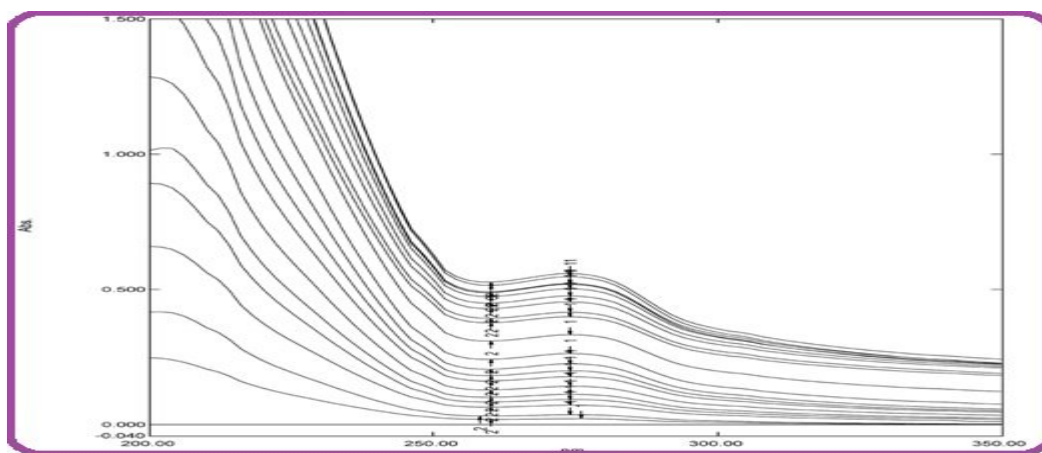


Fig 1: Overlay spectra of *Butea frondosa* Methanolic extract in 0.1N HCl at λ_{max} of 274nm

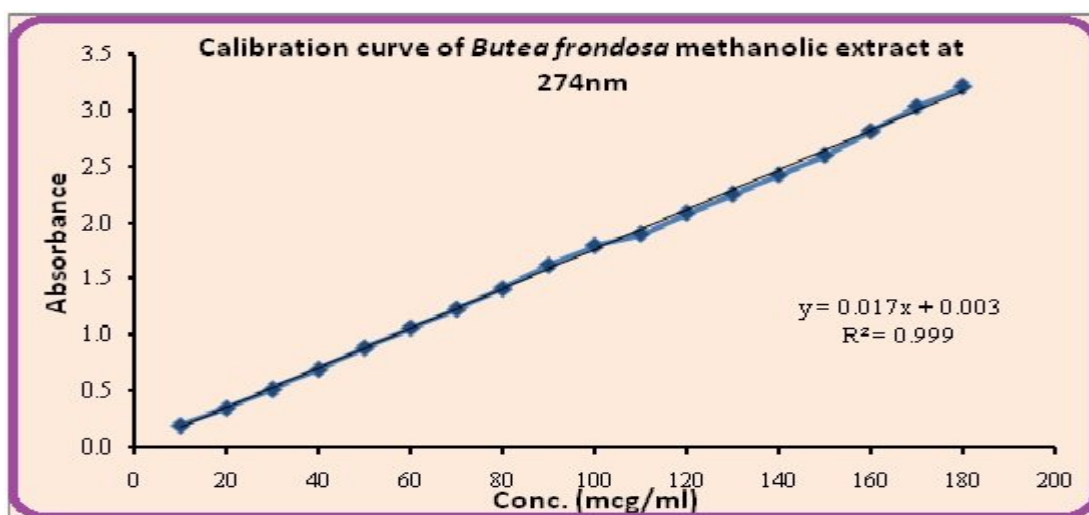


Fig 2: Calibration curve of *Butea frondosa* Methanolic extract at 274nm

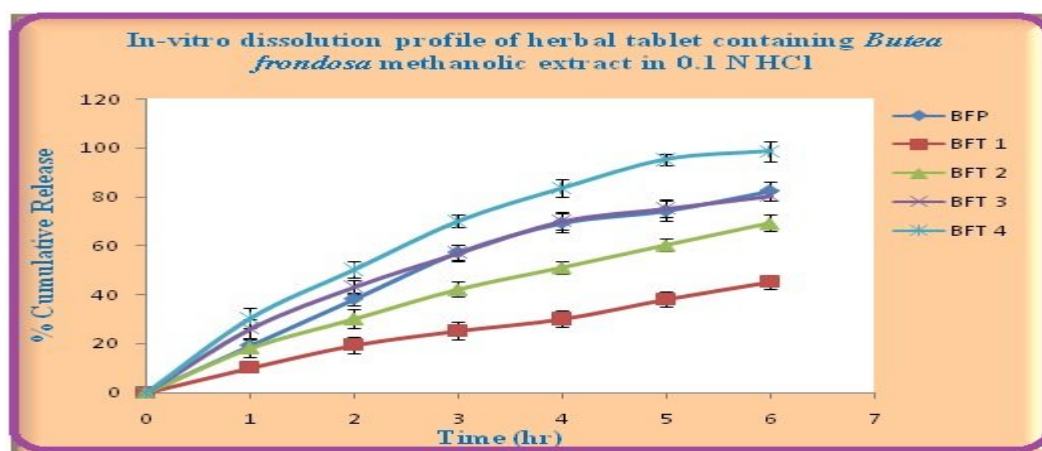


Fig 3: In-vitro dissolution profile of Butea frondosa extract from the formulations in 0.1 N HCl

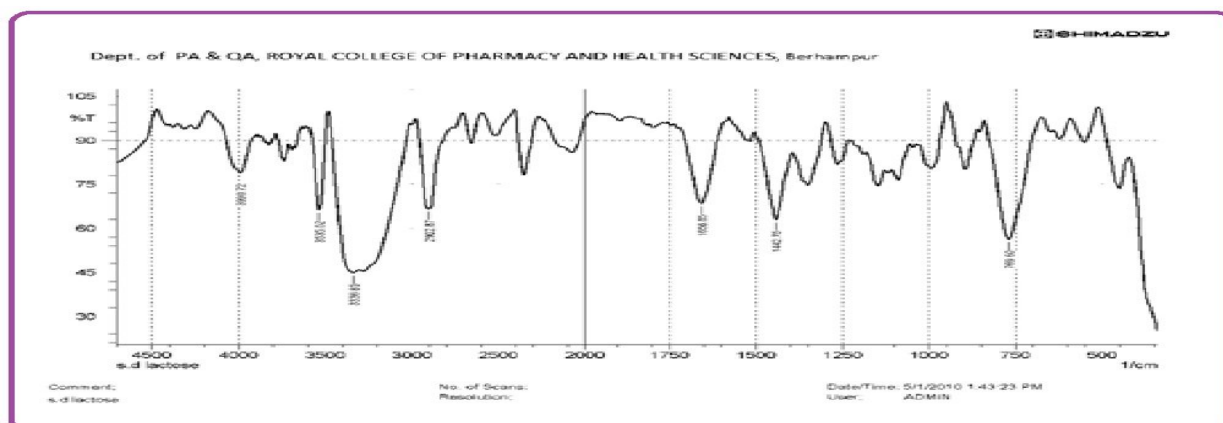


Fig 4: FT-IR Spectrum of S.D. lactose

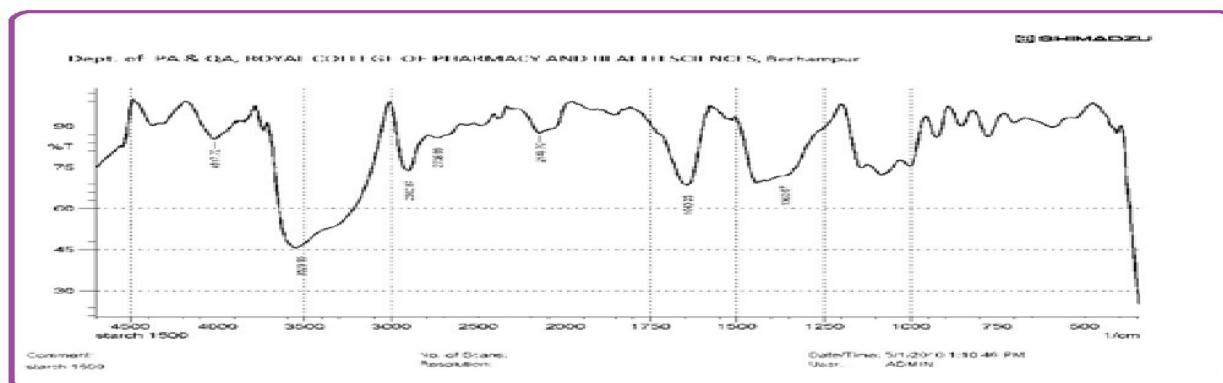


Fig 5: FT-IR Spectrum of Starch 1500

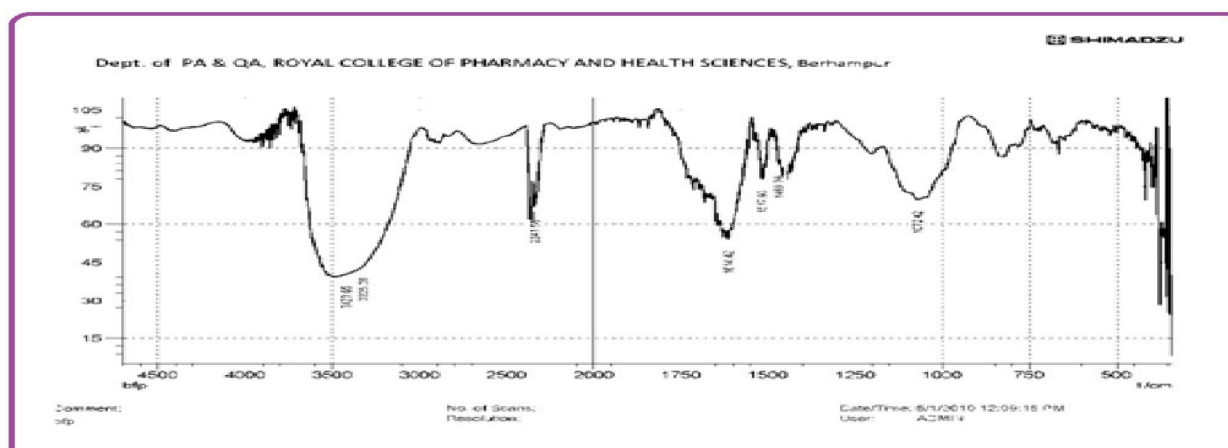


Fig 6: FT-IR Spectrum of BFP

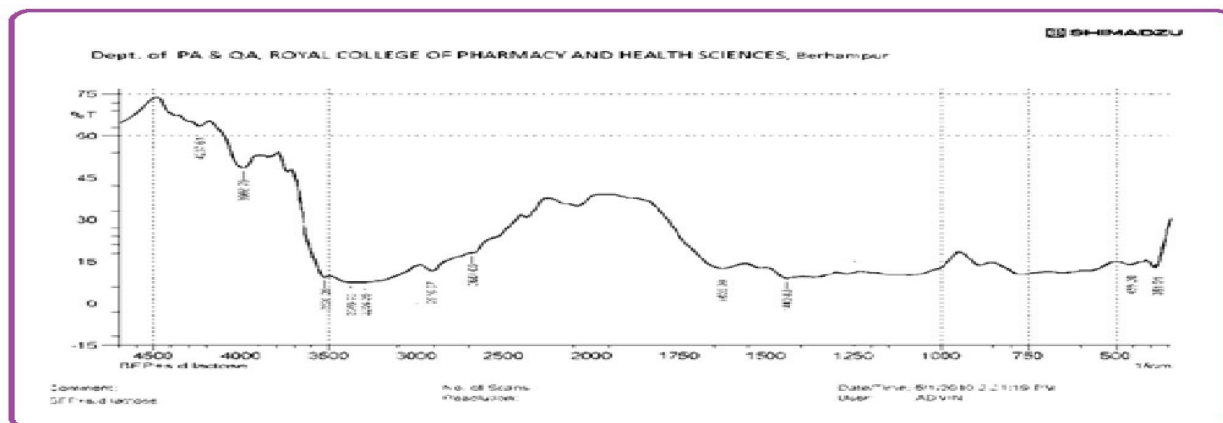


Fig 7: FT-IR Spectrum of BFP + S. D. lactose

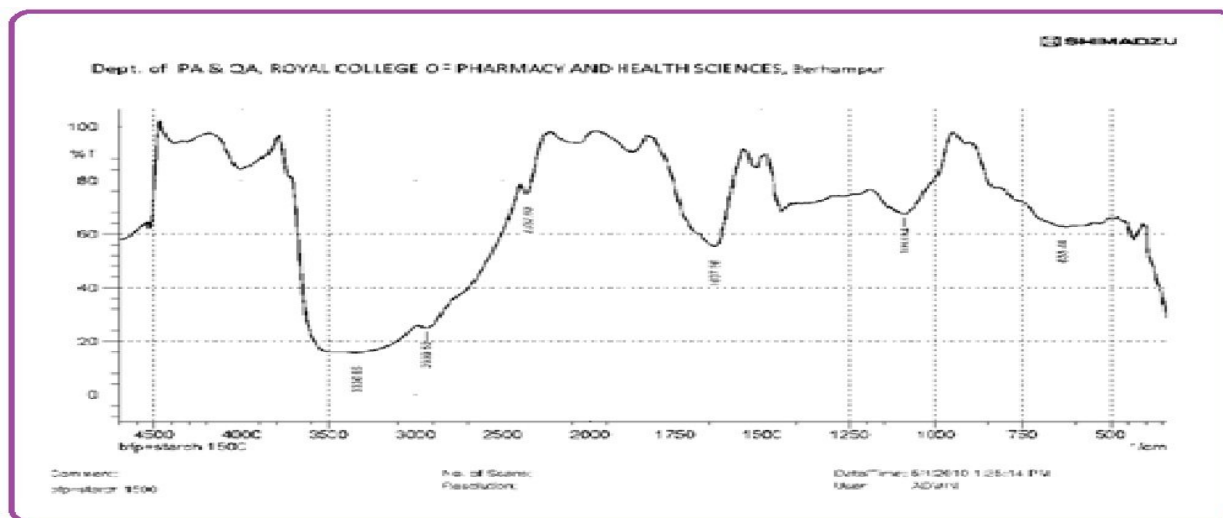


Fig 8: FT-IR Spectrum of BFP + Starch 1500

DISCUSSION

The various composition of the prepared herbal tablet formulations are shown in Table 1. The micromeritic properties were determined for all the physical mixtures of *Butea frondosa*. The results of angle of repose, Carr's Index and Hausner ratio indicated that the powder mixtures possess good flow properties and good packing ability. The physical property of tablet was determined and the results of the uniformity of weight, hardness, drug content and friability of the tablets are given in Tables 3. All the samples of the test product complied with the official requirements of uniformity of weight. The drug content was found to be close to 100% in all formulations. The low friability indicates that the herbal tablets are compact and hard. The

results are reproducible, even on tablets that had been stored for 3 months at 25 °C and 60% relative humidity. The absorption curve of *Butea frondosa* methanolic extract showed characteristic absorption maximum at 274 nm in 0.1N HCl. The drug obeyed Beer's law in the concentration range of 10mcg/ml to 200mcg/ml, and it was found to be linear with $r^2 = 0.999$, regression equation $Y = 0.017x + 0.003$. In-vitro dissolution studies were conducted on tablets of each of the formulations such as BFT1, BFT2, BFT3 and BFT4. The mean cumulative percent of drug released at different time intervals for each formulation is shown in Tables 5 and Fig. 3. It was found that the release rate of drug increased as the concentration of starch 1500 increased. In formulations containing dried methanolic extract of *Butea frondosa*, as the herbal drug was converted to tablet formulation, the release rate was reduced from 82.38% (BFP) to 45.25% (BFT1) in 6 hours. The release rate increased as the percentage of starch 1500 was increased from 10 mg to 30mg. As the concentration of starch 1500 increased the release rate increased from 45.25% to 98.69% (BFT4) in 6 hours by increasing the concentration of starch 1500. The FT-IR spectra of tablet formulations did not show the presence of any additional peaks for new functional groups. The major peaks of the drug remained unchanged in the mixtures. These results suggest absence of any chemical interaction between the drug (BFP) and the excipients used in tablet formulations. Hence, the drug was found to be compatible with all the excipients used. The optimized formulated herbal tablet BFT-4 was kept for stability studies at different temperature and relative humidity conditions to ascertain the stability of the drug. The results were reproducible, even on tablets that had been stored for about 3 months at 25°C/60% RH, 30°C/60% RH and 40°C/75% RH.

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