

**Standardization and toxicological studies of *Butea frondosa* methanolic seeds extract****Mansoor Ahmad\*<sup>1</sup>, Hina Sharif<sup>1</sup>, S. Mahboob Alam<sup>2</sup>, Mehjabeen<sup>3</sup>, Noor Jahan<sup>4</sup>, Sikander Khan Sherwani<sup>5</sup>**<sup>1</sup>Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Karachi-75270, Pakistan<sup>2</sup>BMSI, Jinnah Postgraduate Medical Center, Karachi, Pakistan<sup>3</sup>Department of Pharmacology, Federal Urdu University for Arts, Science & Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan<sup>4</sup>Dow College of Pharmacy, Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan<sup>5</sup>Department of Microbiology, Federal Urdu University for Arts, Science & Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan

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**ABSTRACT**

The main objective of this study was to perform the standardization and toxicological studies of seeds of *Butea frondosa*. Standardization of seeds of *Butea frondosa* was performed included powder drug study, Identification of constituents, pH, loss on drying, thin layer chromatography, infrared spectrophotometry, gas chromatography, acid value, peroxide value and saponification value. Toxicological study on methanolic extract of seeds of *Butea frondosa* was performed on insects (*Tribolium castaneum*) and worms (*Lumbricus terrestris*). 1, 5, 10, 25, 50, 75 and 100 doses of crude methanolic extract of *B. frondosa* was used to investigate insecticidal and anti-helminthic activity. In standardization test microscopic powder drug studies revealed the presence of starch color pigments, oil globules and starch grains. The chemical constituents identified were Tannins, Alkaloids, Carbohydrates, Proteins and Sterols. pH of extract was slightly acidic as compare to powder. In florescence analysis powdered drug showed UV active property. Infrared spectrophotometric study of powder drug was resulted in peaks with different wave numbers. In gas chromatography major peaks were recorded at retention time 5.5 min. Crude methanolic extract of seeds of *B. frondosa* (25mg-100mg) showed significant insecticidal effect (50-100% mortality) as compare to control (0% mortality) but the effect was less significant as compare to Permethrin reference standard (100% mortality). Antihelminthic activity for seeds of *B. frondosa*

(1mg-100mg/2ml) showed 40-100% mortality and the effect was comparable with Albendazole which exhibited 50-100% mortality.

**Key words:** *B. frondosa*, standardization, anti-helminthic, insecticidal

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## Introduction

Majority of traditional medicines utilizes herbs. In order to formulate a medicine first step is standardization of raw material. The herbal drug standardization is essential for identification, safety, confirmation and authentication. It includes study of adulteration, foreign matters, organoleptics, macro and microscopic studies, chromatographic studies, pesticide residue, heavy metals, micro organisms etc. Determination of foreign matters is important for the chances of possible contamination by handling, transport and storage (Mujumdar *et al.*, 2006).

*Butea frondosa* commonly known as Palas or Dhak belongs to the family Fabaceae, grows throughout India except in very arid parts and is a medium sized deciduous tree. Major chemical constituents of seeds are fixed oil 18 %, water soluble albuminoid substances 19% and glucose 6 % (Patani, 2002). Seeds of *B. frondosa* contains fatty acids *n*-docosanoic, *n*-octacosanoic, and *n*-dotriacontanoic acids, sitosterol xyloside, zingiberene diglucoside (Alam *et al.*, 2010), 4-carbomthoxy-3,6-dioxo-5-hydro-1,2,4-triazine (Porwal *et al.*, 1989), human erythrocytes agglutinating lectin (Wongkham *et al.*, 1994), dihydromonospermoside (Chokchaisiri 2009), lectin molecular weight 141000 (Horejsi *et al.*, 1980), soft resins constitutes from four acid esters named jalaric ester-I, jalaric ester-II, laccijalaric ester-I and laccijalaric ester-II 8 (Singh *et al.*, 1974). *Butea frondosa* is anti ulcer (Banji *et al.*, 2011), antibacterial (Dhale *et al.*, 2010) and antimy cobacterial (Chokchaisiri 2009).

The purpose of this study is to standardize the physical, chemical and toxicological activities of methanolic extract of seeds of *Butea frondosa*. Physical and chemical research included the powder drug study, Identification of constituents, pH, loss on drying, infrared spectrophotometry,

gas chromatography, acid value, peroxide value and saponification value. While toxicological studies included insecticidal and anti-helmintic activities.

## **Material and Methods**

### **Collection and identification**

The plant material was collected from local market of Karachi, Pakistan. The plants were identified and deposited (Voucher specimen number; BFH-02-7) in Department of Pharmacognosy, Research institute of Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

### **Powdered drug preparation**

All dried plant materials were cleaned and cut into small pieces separately. Then each sample was ground in a grinder separately to get powder material and was sieved with the help of cotton cloth. For each sample separate piece of cloth was used just to avoid contamination.

### **Plant extraction**

The plant material (seeds of *B. frondosa*) was chopped into small pieces. The chopped material was macerated with methanol for 15 days (2 times) at room temperature. The methanol extract was then filtered through a Whatmann filter paper No. 42 (125mm). The extracts were concentrated using a rotary evaporator under reduced pressure with the water bath set at 40°C to yield a residue (Ahmad *et al.*, 2012).

### **Phytochemical screening**

#### **Identification by color reaction**

Simple qualitative methods were used to identify the presence of tannins, saponins, alkaloids, proteins, terpenes etc (Sofowara 1993, Trease, 1989).

**Powdered drug study**

Small quantity of powder was placed on a glass slide and one or two drops of iodine or glycerin or chloral hydrate solution was added. Later slide was observed under microscope and microscopic characters of each powder drug were studied. The presence of different tissues in the powder drug were identified and sketched with the help of pencil and distinct characters of the powder were recorded (Sofowara 1993, Trease, 1989).

**pH determination**

pH of powdered drug and methanolic extract of *Butea frondosa* was recorded in water treated different techniques of purification which was plain water, distilled water, double distilled water and sterile water. All readings were recorded at 20-25°C (British Pharmacopeia, 2004).

**Loss on drying**

Loss in drying was performed by placing 1 gm of powdered drug of *Butea frondosa* in a weighing pan previously dried at 105°C and then cooled in desiccators. Dry the powdered drug to constant mass or for 1 hour at 105°C (British Pharmacopeia, 2004).

**Acid value**

Dissolve 10.00 g of the substance to be examined, or the quantity prescribed, (m g), in 50 ml of a mixture of equal volumes of ethanol (96 per cent) and ether, previously neutralized with 0.1 M potassium hydroxide, using 0.5 ml of phenolphthalein solution as indicator. When the substance to be examined has dissolved, titrate with 0.1 M potassium hydroxide until the pink color persists for at least 15 s is produced (British Pharmacopeia, 2004).

**Peroxide value**

Place 5.00 g of the substance to be examined in a 250 ml conical flask fitted with a ground-glass stopper. Add 30 ml of a mixture of 2 volumes of chloroform and 3 volumes of glacial acetic acid. Shake to dissolve the substance and add 0.5 ml of saturated potassium iodide solution. Shake for exactly 1 min then add 30 ml of water. Titrate with 0.01 M sodium thiosulphate, adding the titrant slowly with continuous vigorous shaking, until the yellow colour is almost discharged. Add 5 ml

of starch solution and continue the titration, shaking vigorously, until the colour is discharged (n1 ml of 0.01 M sodium thiosulphate). Carry out a blank test under the same conditions (n2 ml of 0.01 M sodium thiosulphate) (British Pharmacopeia, 2004).

### **Saponification value**

Dissolve 35 to 40 g of potassium hydroxide in 20 ml of water and add sufficient ethanol (96%) to produce 1000 ml. Allow to stand overnight and pour off the clear liquid. Weigh 2 g of the substance into a 200 ml flask, add 25.0 ml of the ethanolic solution of potassium hydroxide and boil under a reflux condenser for 1 hour, rotating the contents frequently. While the solution is still hot, titrate the excess of alkali with 0.5M hydrochloric acid, using 1 ml of phenolphthalein solution as indicator. Repeat the procedure without the substance being examined. Calculate the saponification value from the expression  $28.05v/w$ , where  $v$  is the difference in ml, between the titrations and  $w$  is the weight in g, of substance taken (British Pharmacopeia, 2004).

### **Infrared spectrophotometry**

Infrared spectrophotometers are used for recording spectra in the region of  $4000-650\text{ cm}^{-1}$  ( $2.5-15.4\text{ }\mu\text{m}$ ) or in some cases down to  $200\text{ cm}^{-1}$  ( $50\text{ }\mu\text{m}$ ). Spectrophotometers for recording spectra consist of a suitable light source, monochromator or interferometer and detector. Fourier transform spectrophotometers use polychromatic radiation and calculate the spectrum in the frequency domain from the original data by Fourier transformation. Spectrophotometers fitted with an optical system capable of producing monochromatic radiation in the measurement region may also be used. Normally the spectrum is given as a function of transmittance, the quotient of the intensity of the transmitted radiation and the incident radiation. It may also be given in absorbance. The absorbance ( $A$ ) is defined as the logarithm to base 10 of the reciprocal of the transmittance ( $T$ ):

$$A = \log_{10} (I/T) = \log_{10} (I_0/I)$$

$T = I / I_0$ ,  $I_0$  = intensity of incident radiation,  $I$  = intensity of transmitted radiation (British Pharmacopeia, 2004).

### **Gas chromatography**

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives which are volatilized under the temperatures employed. Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution and the reference solution as prescribed. The solutions must be free from solid particles (British Pharmacopeia, 2004).

### **Toxicological studies**

#### **Insecticidal activity test**

Freedom from insect infestation and contamination has become an important consideration in storage of grain and to maintain a high quality product. This is also an issue to herbal medicine, therefore, for the determination of pest in herbal medicine the classical method of analysis is used during experiments (Collins, 1998; Tabassum, 1997; Abbott, 1925).

#### **Anti-helmintic activity test**

*Lumbricus terrestris*, 10 each were placed in a Petri dish containing either aqueous extract (1, 5, 10, 25, 50, 75 and 100 mg/2ml.) The worms were observed for their spontaneous motility (paralysis) and evoked responses to pin prick. The paralytic score was recorded at different time intervals. Immediately after inhibition of response to pin prick, the worms were placed in fresh water and observed for recovery. Duration required for the final recovery or death was noted. Mean paralytic score were with Albendazole as reference standard (Ahmad *et al.*, 2013).

## RESULTS

### Phytochemical screening

The phytochemical screening of the crude extract of *B. frondosa* showed the presence of flavonoids terpenoids, tannins, alkaloids, carbohydrate, protein and sterols. In powdered drug study presence of starch color pigments, oil globules and starch grains have been recorded (Table 1a). The pH of powdered drug was found to be neutral ranges from 5.78-7 where as methanolic extract of *B. frondosa* showed slight acidic pH ranges 5.25-6.69 (Table 1b) . The drying of powdered drug was carried out in an oven within the temperature range 105°C and recorded as 6.5%. The acid value of crude methanolic extract of *B. frondosa* seed was found to be 206.2. Crude methanolic extract of *Butea frondosa* seed resulted in mean peroxide value as 11.8. The mean saponification value of methanolic extract of *Butea frondosa* was 181.9±5.94 (Table 1a).

Infrared spectrophotometric study of powder drug resulted in following wave numbers. 3288.99  $\text{cm}^{-1}$  (OH alcoholic), 3002.95  $\text{cm}^{-1}$  (aromatic C-H), 2962.09  $\text{cm}^{-1}$  (Aliphatic C-H), 2835.41  $\text{cm}^{-1}$  (OH acidic), 1629.97  $\text{cm}^{-1}$  (C=O), 1540.07  $\text{cm}^{-1}$  and 1474.69  $\text{cm}^{-1}$  (Benzene) and 1041.54  $\text{cm}^{-1}$  (C-O-C) (Figure 1 , Table 2 ).

In gas chromatography major peaks were recorded at retention time 5.5 min (peak area=801282 mV), 6.9 min (peak area=27039 mV), 7.46 min (peak area=28174 mV), 9.68 min (peak area=113160 mV), and 23.7 min (peak area=412923 mV) indicates the presence of stearic acid, oleic acid, linoleic acid, linolenic acid and lignoceric acid (Figure 2 , Table 2).

### Toxicological studies

#### Insecticidal activity

Insecticidal activity of *Butea frondosa* was determined on *Tribolium castaneum* at different concentrations of extract. Permethrin (copex) was used as standard drug (Table 3). The results of Insecticidal activity of crude extract of *B. frondosa* showed 50% and 70% mortality at the concentration of 25 and 50 mg/ respectively during 30 minutes. The results of drug treated insects when compared with standard Permethrin (Copex) showed that crude extract of *B. frondosa* has some insecticidal activity.

**Anti-helmintic activity**

Anti-helmintic activity of *Butea frondosa* was determined against standard drug Zentel (Albendazole). The crude extract of *B. frondosa* showed significant antihelminthic activity. Table 3 showed 0 % mortality in vehicle treated (control). while the crude extract of *B. frondosa* showed 40, 50, 60, 40, 70, 100 and 100% mortality at concentrations 1, 5, 10, 25, 50, 75 and 100 respectively. Standard drug Zentel (Albendazole) at these doses showed 50, 60, 70, 60, 90, 100 and 100% mortality (table 3). Results of antihelminthic activity of *B. frondosa* was comparable to Standard drug Zentel (Albendazole).

**Table 1a: Phytochemical Screening of *B. frondosa* extract and powder**

TEST	RESULTS
<b>CHEMICAL CONSTITUENTS</b>	
Tannins	Positive
Saponins	Negative
Alkaloids	Positive
Carbohydrates	Positive
Proteins	Positive
Sterols	Positive
<b>Physical characteristics of <i>b. frondosa</i></b>	
Acid value of <i>Butea frondosa</i>	206.2±2.57
Per oxide value	11.8±0.19
Saponification	181.9±5.94
LOD	6.5±0.52



**Table 1b: pH determination of powder and extract of *B. frondosa***

<b>pH (Powder)</b>	<b>Results</b>	<b>pH (Extract)</b>	<b>Results</b>
Plain water	6.7±0.14	Plain water	6.19±0.12
Distilled water	6.3±0.05	Distilled water	5.33±0.02
Double distilled water	6.19±0.03	Double distilled water	5.32±0.02
Sterile water	6.18±0.15	Sterile water	5.32±0.02

**Table 2: Spectral analysis of *B. frondosa* extract**

<b>INFRARED SPECTROPHOTOMETRY</b>		<b>GAS CHROMATOGRAPHY</b>		
<b>Absorption (cm<sup>-1</sup>)</b>	<b>Compounds</b>	<b>Retention time (min.)</b>	<b>Peak area (mV.)</b>	<b>Compound</b>
3002.95	Aromatic C-H	5.5	801282	Stearic acid
2962.09	Aliphatic C-H	6.9	27039	Oleic acid
2835.41	Acidic OH	7.46	28174	Linoleic acid
1629.97	C=O	9.68	113160	Linolenic acid
1540.07 & 1474.69	Benzene	23.7	412923	Lignoceric acid
1041.54	C-O-C	---	---	---

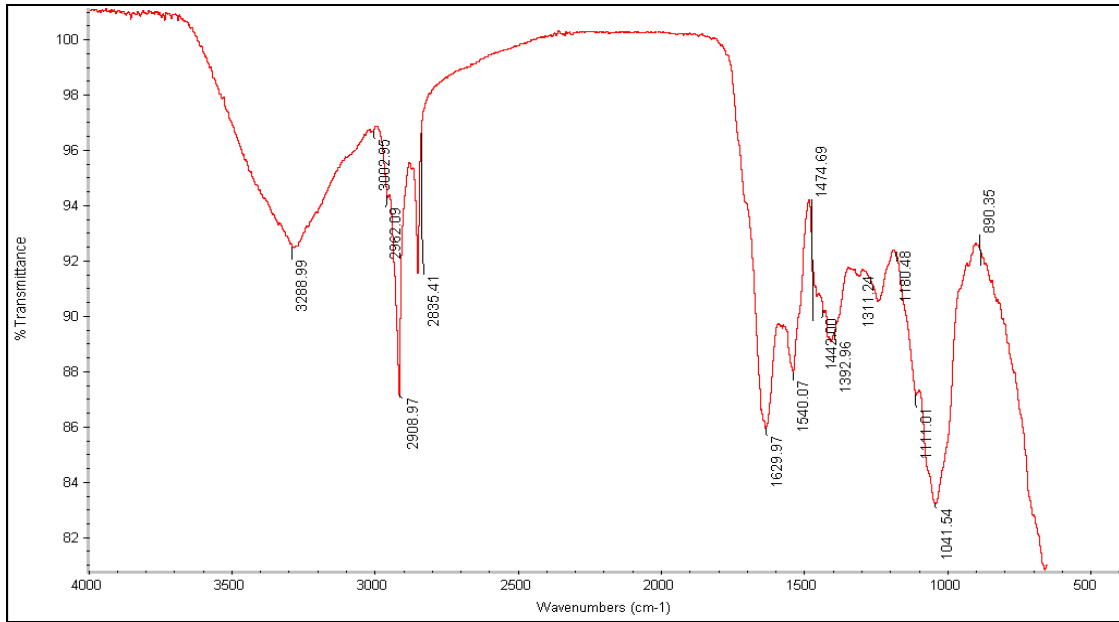


Figure 1: Infrared spectrum of *Butea frondosa*.

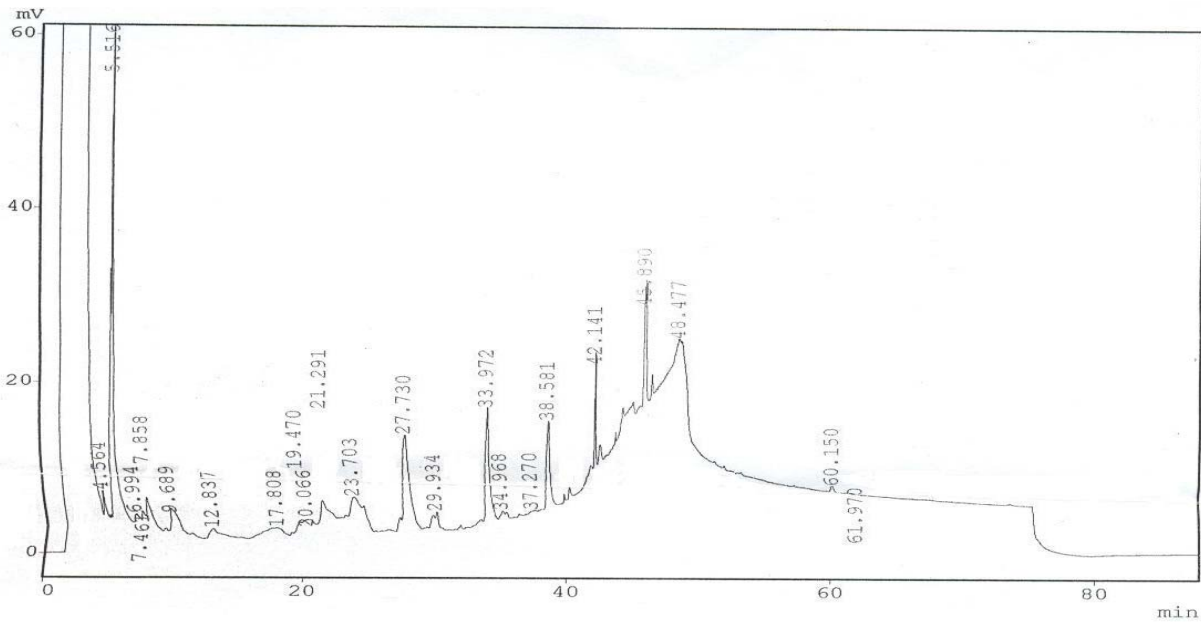


Figure 2: GC chromatogram of *Butea frondosa*.

**Table 3: Insecticidal and antihelminthic activity of *B. frondosa***

Dose	Insecticidal activity			antihelminthic activity		
	% Mortality			% Mortality		
	Control	Test		Control	Test	
1 mg	0	0		50	40	
5mg	0	0		60	50	
10mg	0	0		70	60	
25mg	0	50*		60	40	
50mg	0	70*		90**	70*	
75mg	0	100**		100**	100**	
100mg	0	100**		100**	100**	

Mean  $\pm$  S.E.M; N = 10; Significance with respect to control (\* = Significant results, \*\* = Highly significant results)

## DISCUSSION

Herbal medicines are globally manufactured by herbs available locally, animals and minerals, which are naturally present in sufficient quantities in that specific region. In order to formulate a medicine first step is standardization of raw material. The herbal drug standardization is crucial for identification and safety. It includes study of adulteration, foreign matters, organolaptics, macro and microscopic studies, chromatographic studies, pesticide residue, heavy metals, micro organisms etc. (Mujumdar *et al.*, 2006.)

Microscopic studies revealed the presence of starch color pigments, oil globules and starch grains in seeds of *B. frondosa*. Color reaction identification technique was used to determine the

presence of chemical constituents in the seeds of *B. frondosa*. pH of extract is slightly acidic as compare to powdered drug. The plant of *B. frondosa* extract contained saponin, alkaloid, tannins and glycosides (Londonkar and Ranirukmini 2010). These chemical constituents, except saponins were also identified in the seed extract (Table 1).

Toxicological studies were conducted on insects and worms Insecticidal activity of *Butea frondosa* was determined on *Tribolium castaneum* at 1, 5, 10, 25, 50, 75 and 100 mg/ 2ml concentrations. The results of Insecticidal activity of crude extract of *B. frondosa* showed 50 and 70% mortality at the dose of 25 and 50 mg respectively during 30 minutes. The crude extract of *B. frondosa* showed significant Anti-helmintic activity. The extract exhibited 100% mortality of worms at 100 mg/2ml concentration. *B. frondosa* seed extract showed the positive test for tannins (table 1) therefore, it can be suggested that the anti-helmintic and insecticidal response of the drug probably due to the presence of tannins. Paralysis and 100% mortality of the worms occurred because of inhibition of oxidative phosphorylation in them (Jitendra et al., 2011).

## CONCLUSION

Research on standardization of seeds of *Butea frondosa* make us to conclude the presence of starch color pigments, oil globules and starch grains. The chemical constituents identified were Tannins, Alkaloids, Carbohydrates, Proteins and Sterols. FTIR and GC spectra also provide identification and purity of extract. Crude methanolic extract of seeds of *B. frondosa* also exhibited significant insecticidal and Anti-helmintic activity.

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