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Pharmacognostical Evaluation of Seed of *Butea monosperma* Kuntze

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Abstract – *Butea monosperma* Kuntze, commonly known as 'Palash', is employed in various indigenous systems of medicine against several diseases and almost every part of the plant has diversified medicinal properties. The seeds are used as anthelmintic, aperient, digesti and to treat piles, skin diseases and abdominal troubles. They also have the property of reducing 'Kapha' and 'Vata' (in Ayurveda). In the present paper a detailed pharmacognostical evaluation of seeds has been undertaken. The study includes macro- and micro-scopical details, fluorescence powder study and HPTLC fingerprinting. The seed is characterized by finely ridged seed coat and palisade-like malpighian cells, discontinuous transparent linea lucida in upper half of malpighian layer and simple & oblong hilum. The study also concludes that the seed samples procured from different places have similar morphological and physico-chemical characteristics. These observations are also supported by similar TLC profiles. The estimation of heavy metals (to detect permissible toxic limits), and fatty acid composition have been carried out. An attempt has also been made to see the ecological and edaphic variations, if any.

Keywords : *Butea monosperma,* fatty acids, pharmacognosy, seeds, standardization

Introduction

Butea monosperma Kuntze (*B. frondosa* Roxb.) (Fabaceae) is a medicinally important deciduous, woody forage, environment friendly legume (Williams *et al.,* 1996), distributed throughout India. It is well known in indigenous systems of medicine as Dhak, Palash and Tesu from time immemorial for its miraculous curative effects (Anonymous, 1948; Chopra *et al.*, 1956).

Seeds have long been used in traditional medicines as anthelmintic (Lal *et al.*, 1976), considered to be hot, dry, digestive, aperient, useful in urinary discharges, piles, skin diseases, tumours, abdominal troubles and have the property of reducing 'Kapha'[1] and 'Vata'[2] (Kirtikar & Basu, 1933). It is also recommended for herpes, dermatitis, opthalmy, epilepsy, arthritis, diabetes and useful in flatulence and constipation. (Kirtikar & Basu 1933). Externally seeds when pounded with lemon juice and applied to skin, act as rubefacient and when made in paste are used as a remedy for ringworm and herpes (Anonymous, 1948). The powdered seeds are quite effective against hymenolepiasis (Dikshit *et al.*, 1970), dilirium (Rao, 1981) and also possess insecticidal property (Kirtikar & Basu, 1933; Jain *et al.*, 1980).

The seeds are also creditable for curing obesity and used as an antifertility agent (Choudhary *et al.,* 1980; Bhargava, 1986). The chemical constituents palasonin and butein present in the seed are responsible for their anthelmintic and contraceptive activities (Kaleysa *et al.*, 1967, 1968; Bhargava, 1986). Due to their hepatoprotective and anthelmintic behaviours, seeds find great usage in various indigenous Ayurvedic galenicals viz. 'Janamghutti', 'Krimighatini Bati', 'Krimikuthar Ras', 'Cruminll's Syrup', 'Krimiher Quath', 'Abhaya Lavan', 'Pippali Rasayana' etc. (Kapoor & Mitra, 1979; Agarwal *et al.,* 1994). The seeds of this plant are also used by different tribal communities in India, for example, seeds with jaggery are being used for abdominal pain by the tribals of Ranchi and Hajaribagh [Bihar] (Tarafdar, 1983), as anthelmintic by settlers of Navgarh, Lovari and Majhgain areas of Varanasi, (U. P.) (Singh and Maheshwari, 1983). The tribals of Mayurbhanj [Orissa]

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[1]"Kapha", in Ayurveda has a much wider connotation than "Phlegm" of the Humoral Theory with which it was often been equated. This concept, it appears, includes what can now be interpreted as the lypholytic system controlling irreversible degenerative changes in the organism.

[2]"Vata", corresponds to some extent the "Pneuma" principle of the Humoral theory. In Ayurveda it denotes motor, sensory as well as higher central nervous system functions.

used these seeds for stomach disorders and as family planning agent (Mudgal and Pal, 1980).

Apart from aforesaid valuable medicinal properties, the seeds have gained economic importance by becoming a potential source of fatty oil, popularly known as 'Oodoga oil' or 'Kino tree oil' (Tummineath & Manjunath, 1929; Chandra *et al.*, 1977) which possess antimicrobial activities (Mehta *et al.*, 1983).

As there are no pharmacognostical details of this species on record, the present studies were undertaken to evolve pharmacognostical standards for the quality evaluation of the seed and to detect adulterations or substitutions in the commercial samples. Although, fatty acid composition of *Butea monosperma* seeds is on record (Anonymous, 1948), but in the present paper an attempt has also been made to explore the potential economic importance of seeds by not only estimating their composition but also comparing different samples procured/collected from various regions of India.

Materials and Methods

The seeds of *Butea monosperma* (about 500 g each) were procured from five different places in India viz. Allahabad (Uttar Pradesh-collected sample)-NBR 216590, Delhi (market sample)-NBR 221236, Junagarh (Gujrat-collected sample)-NBR 221234, Mumbai (Maharashtra-market sample)-NBR 221231 and Ranikhet (Uttaranchal-collected sample)-NBR 221235, and deposited in NBRI herbal drug museum depository. The collected samples were dried at 40-50°C. The transverse sections were made for microscopical details and histochemical evaluation of various contents present in seeds was studied. The behaviour of powdered seeds was also studied with different chemical reagents following the methods described by Chase and Pratt (1949) and Kokoski *et al.* (1958).

The percentage of physico-chemical values viz. moisture content, total ash, acid insoluble ash, water and alcohol soluble extractives was calculated according to methods described in Indian Pharmacopoeia (Anonymous, 1966). The percentage of protein (Lowry *et al.*, 1951), sugar/starch (Mont Gomery *et al.*, 1957) and tannin (Anonymous, 1984) were also analysed through Pharma Biotech 2000 Spectrophotometer.

For estimation of heavy metals, 1 g each of completely dried samples was digested with a mixture of concentrated nitric acid and perchloric acid (3:1) until a clear solution was obtained. After cooling, solutions were made upto desired volume (25 ml) with deionised water and analysed through an atomic absorption spectrophotometer (Perkin Elmer 5000). Hollow cathode lamps were used for detection of Pb, Cd, Cu, Zn, Co, Cr & Mn. The instrument was calibrated with standard solution using the concentration mode.

For HPTLC, 1 g powdered drug each from five different regions was refluxed separately for 5 min on water bath with 5 ml methanol and filtered. 25 µl filtrate of each test solution was applied on precoated silica gel 60-F 254 Merck TLC glass plates of 10×10 cm with the help of Camag Linomat IV applicator. The plate was developed to a distance of 8.7 cm at room temperature (33°C) in solvent system of toluene: ethyl acetate: methanol (85:15:0.5). It was sprayed with anisaldehyde sulphuric acid reagent, heated at 120°C for 10 min (Wagner *et al.*, 1984) and fingerprint profiles were obtained with the help of Desaga Video Documentation Unit.

For estimation of percentage of oil and GLC studies, the air dried powdered seeds were extracted with petroleum ether (40-60°C) in a Soxhlet apparatus, dried over anhydrous sodium sulphate and the solvent removed under vacuum at 40°C. Fatty acid composition of oil was determined through gas chromatography by a Hewlett Packard gas chromatograph (Model 5890 Series II) using a stainless steel column coated with 5% diethylene glycol succinate (DEGS) on chromo-sorb W (HP) (180 cm×3 mm) equipped with flame ionization detector attached to Wipro Computer 486. The column, injector and detector temperatures were maintained at 170°C, 230°C and 250°C, respectively, using nitrogen as a carrier gas at a flow rate of 30 ml/min.

Observations

A brief taxonomic description – Moderate sized deciduous tree, typically with a crooked trunk, upto 15 m high, commonly called *"flame of the forest"*. Leaves compound; petioles 7-15 cm long; stipules and stipels small, deciduous; leaflets 9-17 cm long, rigidly coriaceous, glabrescent above, silky tomentose, strongly veined beneath; terminal one rhomboid from a cuneate base, obtuse or emarginated, lateral obliquely ovate. Inflorescence raceme; inflorescence branches brown-velvety; flowers large, 4-6 cm long, bright yellowish red to orange red, with long pubescent, velvety, olive green peduncle, bracteate; bracts and bracteoles small, linear, velvety, orange green, deciduous; calyx campanulate, 5-partite, oblique, about 1 cm long, dark olive green, densely velvety outside, clothed with silky hair within; two upper teeth connate, large three lower ones unequal, the lowest being much shorter than the lateral ones; corolla 4-6 cm long, orange red, covered outside with silky white hairs, papilionaceous, standard 4-4.5 cm long, 1.7-2 cm broad, reflexed, veined in parallel fashion; wing petals incurred, adpressed to the keel about ½ of the length; keel incurred, semi orbicular, acute, veined; petals 2, connate; stamens

diadelphous (9+1), vexillary stamen free and shortest, others connate, anthers linear, yellow; ovary stipitate, silky, pubescent; style incurved, longer than the stamens; pod 9-17×4-6 cm grey-downy, narrowed suddenly into a stalk longer than the calyx. Seed 1 within the dehiscent apex of the pod (Duthie, 1960).

Description of Seed

Macroscopic (Plate I) – Seeds reddish brown, flat, reniform, 3.3-3.8×2.2-.5 cm, symmetrical, raphae equal to antiraphe, chalaza at the basal end, micropyle inconspicuous; seed coat reddish brown, thin waxy, finely ridged, with pleurogram; odour faint, taste slightly acrid to bitter with rubefacient properties; average weight of 100 seeds 99.45 g (range 81.9-116.0 g).

Microscopic (Plate I) – Single layered epidermis of testa interrupted by the balloon shaped cells; malpighian cells palisade like, thick-walled, red, measuring 20-31×10-18 μm, unlignified; lumen large but not uniform; linea lucida in upper half of malpighian layer discontinuous, transparent; osteosclereids irregular, nonlignified, highly thick-walled, columnar, compressed and superimposed; hour-glass cells not well-developed; mesophyll occuping major portion of the testa, upper and lower mesophyll cells small, isodiametric to elliptic, middle layers large, angular, condensed with small intercellular spaces; the inner epidermis reddish brown, distinct with small thick-walled elongated cells externally surrounded by thin culticle. (Figs. 1a, 1b; 2a, 2b; 3a, 3b, 4).

The pleurogram region shows an interruption of palisade with raised mesophyll, the top of which is occupied by 1-2 layers of osteosclereids. The hilum is simple and shortly oblong. The funicle often breaks off near its attachment to the hilum leaving usually no more than inconspicuous membrane round the edge of hilum, funicle shows a single layered epidermis of non-lignified elongated cells traversed by vascular supply of seed (Figs 2a-2b). Endosperm almost completely absorbed by developing embryo,which is straight having a radicle with well marked hypocotyl, epicotyl with a plumule and a pair of thick cotyledons (Fig. 3b).

The T.S. of cotyledon shows single layered, thick-walled epidermis having angular cells, followed by beaded parenchymatous cells containing starch grains and protein (Figs. 5a, 5b).

Powder

The whole seeds were dried, powdered and sieved through 60 mesh for microscopical and fluorescence studies. Powder is yellowish brown; acrid to bitter in taste with oily flavour and pleasant smell. On microscopical examination the powder shows small fragments of testa, broken and intact malpighian cells, osteosclereids, mesophyll cells-isolated or in groups, groups of vessels with annular to spiral thickening, cotyledonary parenchyma containing few starch grains, abundant protein granules, mucilage and oil globules (Fig. 6). Powder when treated with 50% H_2SO_4 and acetic acid emits reddish purple and yellow fluorescence under UV-254 nm respectively.

Phytochemical Studies

The percentages of total ash, acid insoluble ash, alcohol and water soluble extractives, tannins, proteins, sugars and starch were determined and their results are tabulated in Table 1. The heavy metals concentration (Pb, Cu, Zn, Cd, Mn, Cr, and Co) were determined and the results are presented in Table 2.

The characteristic HPTLC finger print profiles developed may be used as markers for quality evaluation and

Table 1. Physico-chemical parameters of *Butea monosperma* seeds

Parameters	Locations				
	Allahabad (U.P.)	Delhi	Junagarh (Gujrat)	Mumbai (Maharashtra)	Ranikhet (Uttranchal)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1. Moisture*	3.53 ± 0.55	3.56 ± 0.32	3.53 ± 0.15	3.90 ± 0.20	3.30 ± 0.20
2. Total ash*	7.46 ± 0.51	6.23 ± 0.07	5.91 ± 0.09	7.35 ± 0.31	7.54 ± 0.10
3. Acid insoluble ash*	0.24 ± 0.01	0.22 ± 0.02	0.10 ± 0.04	0.40 ± 0.05	0.39 ± 0.03
4. Alcohol soluble extractive**	21.03 ± 0.84	28.60 ± 0.93	22.33 ± 4.39	24.70 ± 0.24	16.85 ± 0.49
5. Water soluble extractive**	25.50 ± 0.87	39.50 ± 1.38	48.75 ± 1.78	37.35 ± 0.75	16.85 ± 0.49
6. Protein*	19.83 ± 3.00	21.36 ± 1.38	18.83 ± 3.43	22.66 ± 0.75	18.95 ± 1.32
7. Starch*	31.96 ± 5.62	31.30 ± 0.38	41.16 ± 3.49	32.72 ± 1.93	38.40 ± 4.49
8. Sugar*	8.51 ± 0.29	5.68 ± 0.17	8.20 ± 0.27	3.47 ± 0.83	7.17 ± 0.74
9. Tannin*	0.43 ± 0.31	0.48 ± 0.55	0.52 ± 0.76	0.51 ± 0.32	0.68 ± 0.52

*Average of 3 readings.
**Average of 6 readings

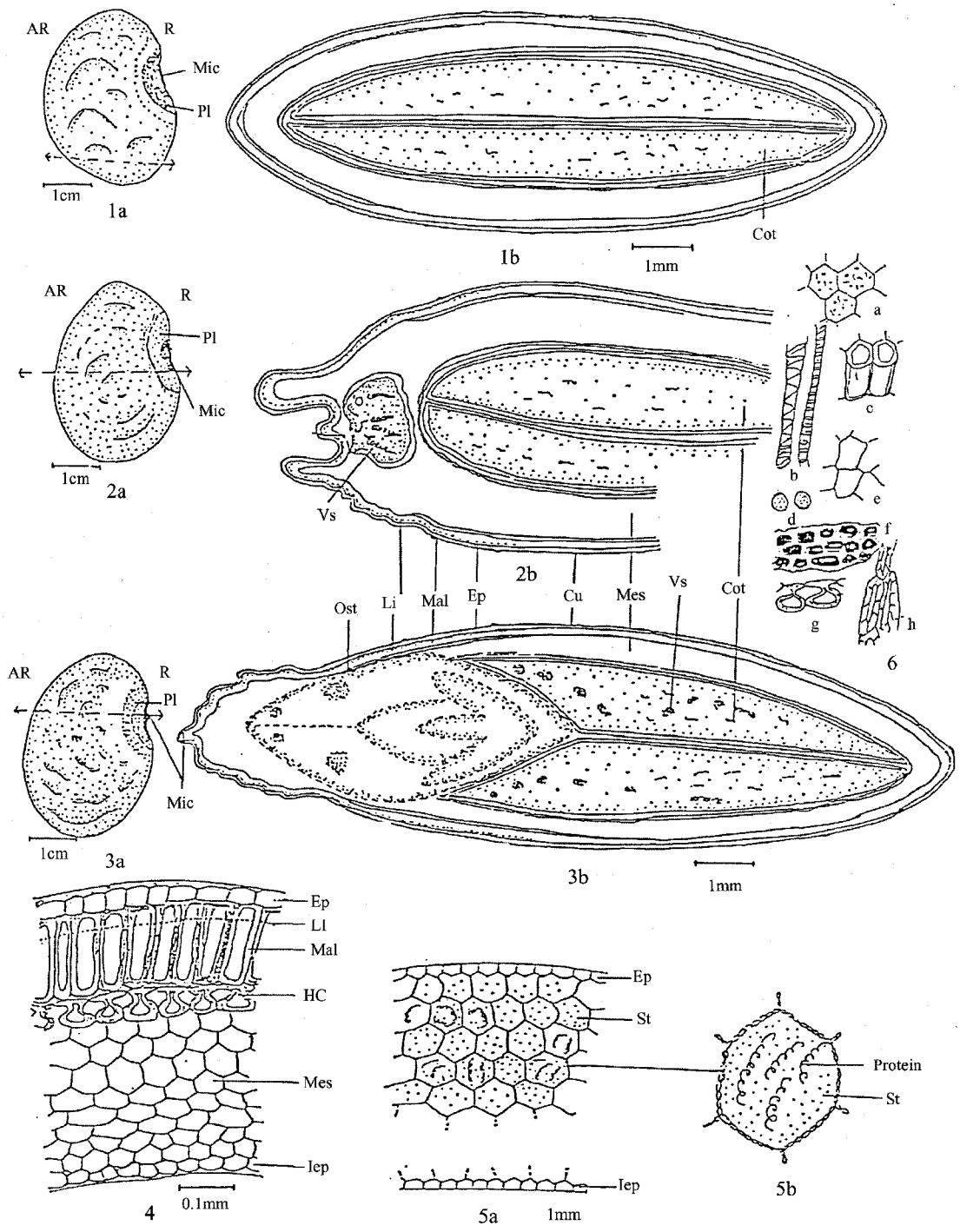

Fig. 1. (a) Diagrammatic representation of macroscopic details of seed. (b). Diagrammatic representation of section passing through distal end of the seed.

Fig. 2. (a) Diagrammatic representation of seed showing the position of section through pleurogram region. (b) Diagrammatic representation of section of the seed passing through pleurogram region.

Fig. 3. (a) Diagrammatic representation of seed showing section passing through micropylar region. (b) Diagrammatic representation of seed the section passing through micropylar region showing different cell layers of seed.

Fig. 4. Cellular structure of seed (testa and tegman).

Fig. 5. (a) T.S. of cotyledon of seed. (b) Single magnified cell of cotyledon showing protein and starch grains.

Fig. 6. (a-h) Microscopical details of powder. a = Patches of parenchymatous cells with protein and starch grains. b = Vessels showing annular to spiral thickening. c = Malpighian cells. d = Oil globules. e = Parenchymatous cells. f = Fragment of testa. g = Hourglass cells. h = Osteoblast.

Table 2. Estimation of heavy metals in *Butea monosperma* seeds (in ppm)

Heavy metals	Locations				
	Allahabad (U.P.)	Delhi	Junagarh (Gujrat)	Mumbai (Maharashtra)	Ranikhet (Uttaranchal)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
1. Pb	8.08±1.71	10.56 ± 1.29	14.29 ± 0.91	10.66 ± 1.49	27.03 ± 2.36
2. Cu	24.66±0.77	26.89 ± 1.02	22.38 ± 1.12	28.50 ± 0.81	21.58 ± 1.47
3. Zn	58.58±1.49	52.33 ± 2.06	52.89 ± 1.07	56.99 ± 1.93	54.66 ± 3.52
4. Cd	0.33±0.11	0.29 ± 0.00	0.28 ± 0.82	0.16 ± 0.11	0.25 ± 0.00
5. Mn	21.25±0.54	28.45 ± 0.97	20.20 ± 0.75	27.66 ± 1.31	25.16 ± 2.51
6. Cr.	3.25±1.27	3.28 ± 0.92	3.00 ± 1.81	3.0 ± 2.31	3.66 ± 1.94
7. Co	1.83±0.12	1.20 ± 1.12	1.82 ± 0.71	1.91 ± 0.31	1.75 ± 0.61

Table 3. Characteristic marker components obtained from HPTLC of Butea monosperma

Visible (after spraying)	
R_f. Values	Colour of component
0.07	Greyish green
0.26	Magenta
0.38	Dark greyish green
0.56	Light greyish green
0.61	Orange

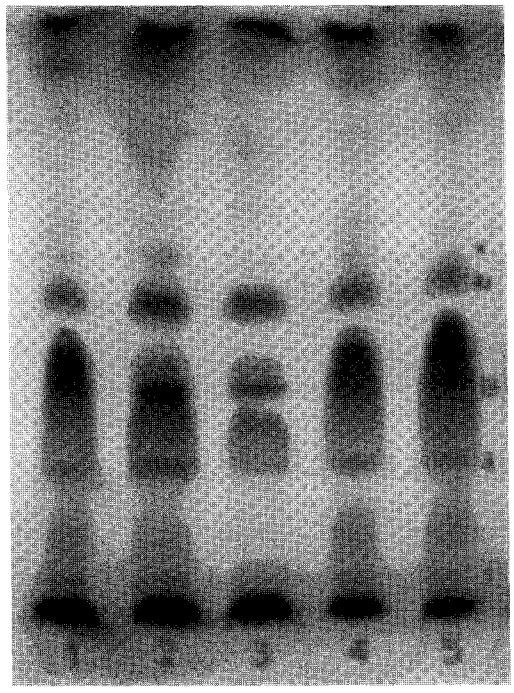

Fig. 7. HPTLC fingerprint profiles of different samples of *Butea monosperma* seeds procured from different regions.
1-Allahabad, collected sample; 2-Delhi, market sample; 3-Junagarh, collected sample; 4-Mumabi, market sample; 5-Ranikhet, collected sample.
i-Spot at R_f. 0.07, ii -Spot at R_f. 0.26, iii-Spot at R_f. 0.38, iv-Spot at R_f. 0.56, Spot at R_f. 0.61.

standardization of the raw drug. All the five samples show five common spots at R_fs. 0.07, 0.26, 0.38, 0.56 and 0.61 (Table 3 and Fig. 7).

The fatty oils from the seeds varying from 6.54- 16.64% were cream to greenish yellow in colour. The oil was saponified with 0.5N KOH in ethanol and acidulated to yield free fatty acids which were converted to methyl esters using 2% methanolic H_2SO_4 [both AR] (Phillips *et al.*, 1969) for GC analysis. The individual fatty acids were identified by comparing the retention time of the methyl esters so obtained with those of pure samples of fatty acid methyl esters. Peak areas were measured and the GC data reported is given in area percentage (Table 4).

Discussions and Conclusion

From the ongoing studies it has been revealed that *Butea monosperma* seeds procured from different locations in India (Allahabad, Delhi, Junagarh, Mumbai and Ranikhet) showed similar morphological characteristics and some physico-chemical values viz. moisture and tannin percentage. These observations were also supported by similar HPTLC profile with five common spots (except slight variation in sample number 3) at R_fs. 0.07, 0.26, 0.38, 0.56 and 0.61. Thus, this HPTLC finger print profile may be utilized in general as an identifying marker for quality assurance of the *Butea monosperma* seeds. However, slight variations were observed in ash percentage and water and alcohol soluble extractives. For instance, the total and acid insoluble ash of the samples ranged from 5.9-7.56 and 0.096-0.40%, respectively, with the Junagarh sample having the lowest percentages of ash (both total and acid insoluble) and there is two to three fold increase in the alcohol and water soluble extractives of all the samples as compare to the Ranikhet sample. (Table 1). Similarly, the Mumbai sample had highest average percentage of protein (22.26%) while it was minimum in

Table 4. Oil percentage and fatty acid composition of *Butea monosperma* seeds

		Allahabad (C)	Delhi (M)	Junagarh (C)	Mumbai (M)	Ranikhet (C)
Fatty oil		6.54	16.64	16.37	6.83	10.48
Fatty acids						
Palmitic acid	(16:0)	22.964	19.327	45.592	18.889	30.560
Stearic acid	(18:0)	–	15.564	8.509	20.344	1.995
Oleic acid	(18:1)	36.762	30.625	13.342	38.647	33.370
Linoleic acid	(18:2)	16.405	20.860	–	10.270	7.696
Linolenic acid	(18:3)	3.309	4.443	2.888	5.684	4.281
Arachadic acid	(20:0)	3.904	–	13.115	–	7.304
Behenic acid	(22:0)	–	–	–	5.990	–
Capric acid	(10:0)	–	Insignificant	–	–	Insignificant
Lauric acid	(12:0)	Insignificant	–	Insignificant	–	–
Myristic acid	(14:0)	Insignificant	–	Insignificant	Insignificant	Insignificant

C = Collected samples.
M = Market sample.

Junagarh sample (18.33%). These variations may be attributed to some edaphic or ecological factors. Besides, there existed remarkable differences in the stearic acid and sugar percentage of collected and marketed drugs. It was observed that the amount of stearic acid was higher and the sugar content was lower in marketed drugs as compared to the collected ones. These variations may be due to the storage of marketed drugs.

The concentration of heavy metals was also estimated and it was found that the majority of the samples had higher range of lead concentration than that of permissible WHO limit of <10 ppm (Anonymous, 1992) except the Allahabad sample (8.08 ppm). This may be explained due to environmental pollution or high concentration of lead salts in soil.

Analytical data on seed oil, which is basically oleic type, showed considerable variation in oil percentage ranging from 6.54 to 16.64% (Table 4). The climatic variations may influence fatty acid composition, too. The amount of saturated fatty acids in seed oils of different locations ranged from 43.22 to 46.21% among which palmitic acid was found to be prominent (18.88-30.56%) followed by stearic acid having wide range of variability from absence of it in Allahabad sample to appreciable percentage of 20.34% in Mumbai sample. In addition, the long chain fatty acids as behenic acid (in Mumbai) and arachidic acid (in Ranikhet, Allahabad and Junagarh) are also present with traces of capric acid, lauric acid and myristic acid. Apart from saturated fatty acids, oil contained significant amount of unsaturated fatty acids too (45.3 to 56.4%) which are preferred more than saturated ones for edible purposes. They are mainly oleic acid (30-38%), linoleic acid (7.69-20.86%) and linolenic acid (2.88-5.68%). It is interesting to note that Junagarh sample showed an exceptionally different behaviour by having very high amount of saturated fatty acids (81.58%) as compared to unsaturated fatty acids (16%) with complete absence of linoleic acid. According to Muralidharudu *et al.* (1998) the quality of oil may be assessed by its stability index i.e. ratio of oleic to linoleic. Thus, Ranikhet sample seems to be more stable by having stability index of 4.3 as compared to other samples and it can be stored for the longer period too. Similarly, these oils have enough collective content of oleic linoleic acids (upto 53.16%) and such oils can be exploited for edible purposes, provided they do not exhibit any toxicity.

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