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LC-MS analysis of dye extracted from *Butea monosperma* (Lamk.) Taub. flowers

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ABSTRACT

In the present study dye was extracted from Butea monosperma (Lamk.) Taub. flowers using solvents like methanol and water. LC-MS analysis was carried out to identify major phytochemicals responsible for colour in Butea monosperma (Lamk.) Taub. flowers. The preparative HPTLC plate of methanolic dye was analysed. The three visible bands were further scraped and reconstituted in methanol for LC-MS analysis to obtain mass to charge ratio. Dye extracted form Butea monosperma (Lamk.) Taub. flowers exhibited several noticeable peaks and numerous small peaks. This showed butrin, isobutrin, butein and lanceoletin are some phytoconstituents which may be responsible for the yellow colour in Butea monosperma (Lamk.) Taub. flowers.

Keywords : Butea monosperma, LCMS, HPTLC, Flavonoids

I. INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Colour is one of the element of nature that made the human living more aesthetic and fascinating in the world. They are supposed to be associated with emotions, human qualities, seasons, festivals and passion in our life. In the past, at dawn of the civilization, people tried to ornament their surroundings similar to that of natural colours observed in the plant, soil, sky and other sources. This gave birth to the new science of colours from natural origin (Vankar, 2007). Colours in flowers are adaptations that attract insects and other animals that in turn pollinate and help the plants to reproduce. Flavonoids are one of the most important phytochemical in some plants and they can be easily recognised as flower pigment (Mishra *et al.*, 2012).

Butea monosperma (Lamk.) Taub. commonly known as Flame of forest, belongs to the family Fabaceae. It is said that this tree is a form of Agnidev, God of Fire. *Butea monosperma* (Lamk.) Taub. showed anti-inflammatory, anti-stress, anti-diarrhoeal and anti-cancer property. In the present study dye was extracted from *Butea monosperma* (Lamk.) Taub. flowers using various solvents. HPTLC and LC-MS analysis was carried out to identify major phytochemical responsible for colour in *Butea monosperma* (Lamk.) Taub. flowers.

II. METHODS AND MATERIAL

Material

Dried powder of *Butea monosperma* (Lamk.) Taub flowers.

Methods

1) Preparation of dye

The extract was prepared for dye using 2g of dried powder of *Butea monosperma* (Lamk.) Taub.

flowers in 20ml methanol and water(aqueous). The dye was extracted in various solvents under optimized condition of extraction, by heating it in water bath for 30 minutes (Samanta and Konar, 2011).

The extracts were filtered to obtain 40% clear extracts of coloured solution using Whatmann paper 42 and observations were noted. Methanolic and aqueous dye showed intense colour so further analysis of only these dye was carried out by studying absorption spectra by using UV-spectrophotometer at different wavelengths (400nm to 620nm) (Plummer, 2003).

2) HPTLC analysis and LC-MS analysis

Methanolic and aqueous dye extracted from *Butea monosperma* (Lamk.) Taub. flowers was further analysed by HPTLC. The samples were loaded on TLC plate and simultaneously mobile phase was saturated in the saturation chamber for 20 minutes. TLC plate was kept in the chamber and the mobile phase was allowed to run upto 3/4 length of TLC plate. Then the plate was removed and dried to observe and scan.

10µl of the extract of *Butea monosperma* (Lamk.) Taub. flowers was loaded on TLC plates 60F254 using Camag Linomat V and were developed using ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) as a solvent system for flavonoids separation. After drying the plates were observed under UV light at 254nm and 366nm and 520nm, using the UV cabinet and the separated spots were visualized. The plates were scanned by Camag TLC scanner at 520nm. The images were captured with Camag photo documentation at 254nm, 366nm and white light (without derivitisation).

The preparative HPTLC plate of methanolic dye was prepared for further analysis. The three visible bands were further scraped and reconstituted in methanol for LC-MS analysis to obtain mass to charge ratio. The equipment used in the LC-MS study was of AB SCIEX make with model API 2000 with Q1 tuning mode.

III. RESULTS AND DISCUSSION

The methanolic and aqueous dye extracted from Butea monosperma (Lamk.) Taub. flowers showed yellow dye as compared to other solvents. Methanolic and aqueous dye were further analysed to study maximum absorption using UV spectrophotometer. Maximum absorption of methanolic dye was observed at 520nm and aqueous dye was observed at 490nm as showed in (Figure 1).

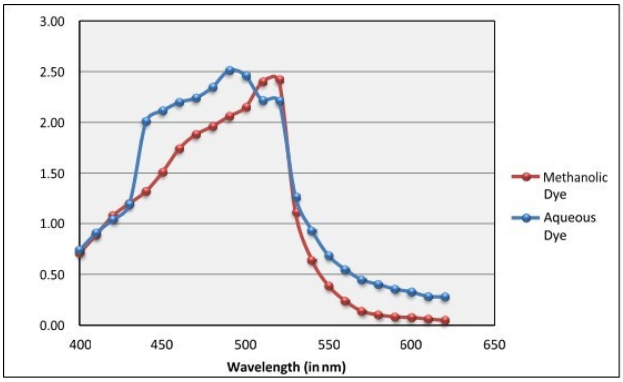

Figure 1. Absorption spectra of methanolic and aqueous dye extracted from Butea monosperma (Lamk.) Taub. flowers at different wavelengths

HPTLC analysis of 10µl methanolic dye extracted from Butea monosperma (Lamk.) Taub. flowers at 520nm showed 10 spots with Rf value 0.02, 0.14, 0.32, 0.42, 0.50, 0.75, 0.82, 0.84, 0.90, 0.91 (Table I). In 10µl aqueous dye of Butea monosperma (Lamk.) Taub. flowers at 520nm showed 10 spots 0.02, 0.06, 0.10, 0.31, 0.41, 0.75, 0.84, 0.86, 0.89, 0.91(Table I). As observed in Plate 1 in white light.

The methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. flowers with Rf value 0.32 and aqueous dye extracted from *Butea monosperma* (Lamk.) Taub. flowers with Rf value 0.31 at 520nm showed dark yellow visible band, which may corresponds to butrin. While Rf value

0.42 at 520nm in methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. flowers and 0.41 at 520nm in aqueous dye showed light yellow visible band, which may correspond to isobutrin and Rf value 0.91 at 520nm in methanolic and aqueous dye extracted from of *Butea monosperma* (Lamk.) Taub. flowers showed yellow visible band, which may corresponds to butein. This showed that flavonoids were the major phytochemical responsible for the colour in *Butea monosperma* (Lamk.) Taub. flowers. These bands of methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. were visible when separated from preparative HPTLC fingerprint, so these bands were scraped using scalpel and reconstituted in methanol for further analysis by LC-MS (Plate 2). Dye extracted from *Butea monosperma* (Lamk.) Taub. flowers exhibited several noticeable peaks and numerous small peaks.

In the present study the molecular mass 597.8 may correspond to butrin (596) in spot 1 with Rf 0.32, 597.5 may correspond to isobutrin (596.17) in spot 2 with Rf 0.42 and 273.5 may correspond to butein (272) in spot 3 with Rf 0.91 (high intensity peak). It was observed that the molecular mass 302.50 may correspond to lanceoletin (302) in spot 3 with Rf 0.91. This showed that butrin, isobutrin, butein and lanceoletin are some phytoconstituents which may be responsible for the yellow colour in *Butea monosperma* (Lamk.) Taub. flowers. Many other flavonoids may be in combination or pure forms are present in *Butea monosperma* (Lamk.) Taub. flowers.

Table 1. Hptlc Fingerprint Of Methanolic And Aqueous Dye Extracted From Butea Monosperma (Lamk.) Taub. Flowers

Peak No.	Methanolic dye	Aqueous dye
	Max Rf at 520nm	
1	0.02	0.02
2	0.14	0.06
3	**0.32**	0.10
4	**0.42**	**0.31**
5	0.50	**0.41**
6	0.75	0.75
7	0.82	0.84
8	0.84	0.86
9	0.90	0.89
10	**0.91**	**0.91**

Further investigation is necessary for standardising the other flavonoids and extraction of other compounds from Butea monosperma (Lamk.) Taub. flowers. In the earlier study it was observed that Butea monosperma (Lamk.) Taub. flowers contains phytoconstituents butrin, isobutrin and butein as suppressor of tumour cells (Rasheed et al., 2010). A flavone was isolated from the flowers of Butea monosperma (Lamk.) Taub. identified for the first time as lanceoletin (Oberoi and Ledwani, 2010).

Similar work was observed in flavonoids extracted from marigold flowers which were investigated for their dyeing potential. Patulitrin and patuletin were isolated and their structures established using HPLC-MS (Guinot *et al.*, 2008). Standard extraction procedure for examining chromophoric substances of turmeric was investigated. Acetone and methanol were used as extracting solvents with different extraction procedures and pH levels. GC-MS analysis identified curcumin (6.7 min), feruloylmethane (8.3min), coumaran (6.09min), vanillin (6.2min), and zingiberene (10.5min) as the major products. Curcumin which has been known

as the major chromophoric substance of turmeric was not detected in any samples (Cheunsoon and Obendorf, 2006).

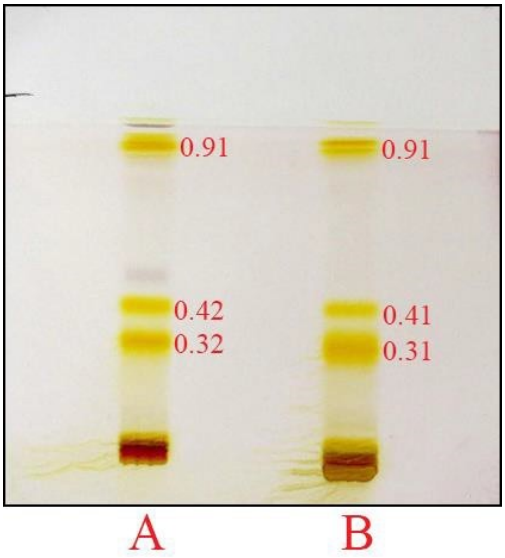

Plate 1. HPTLC fingerprint of methanolic and aqueous dye extracted from *Butea monosperma* (Lamk.) Taub. flowers at white light

Keywords: A – Methanolic dye, B – Aqueous dye

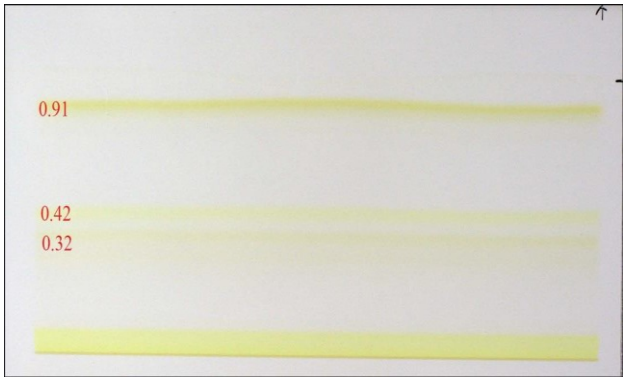

Plate 2. Preparative HPTLC fingerprint of methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. flowers

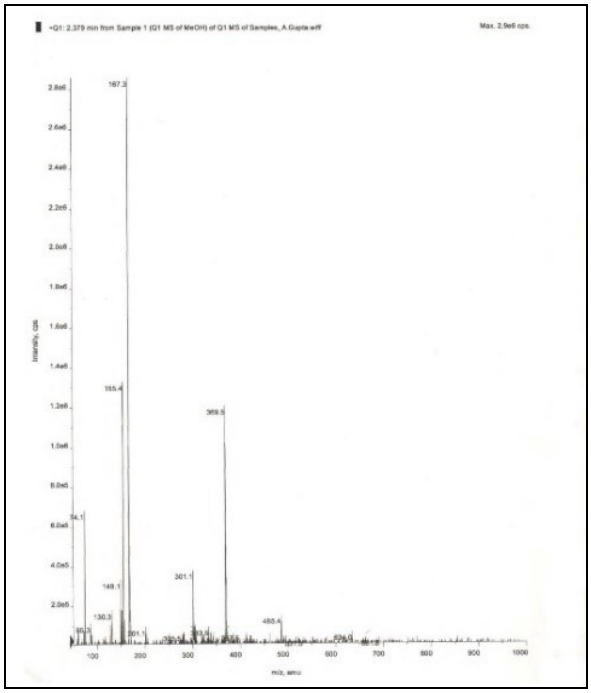

Figure 2. Q1-MS observation for methanol

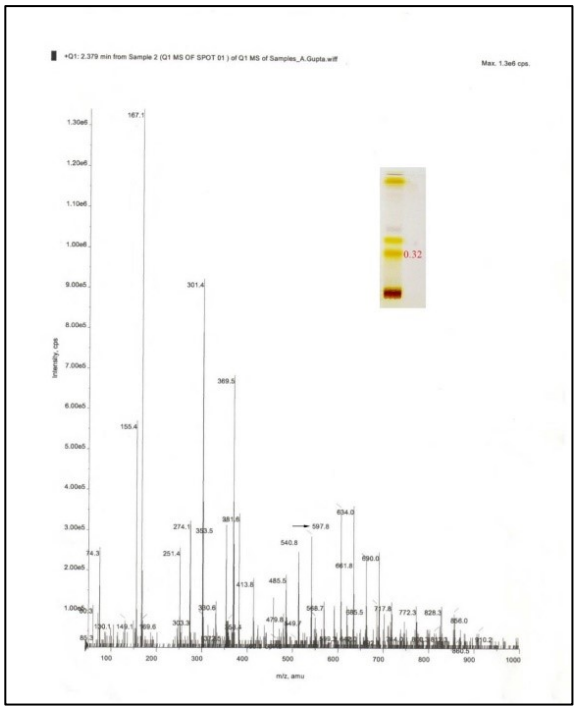

Figure 3. Q1-MS observation for methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. flowers for spot 1

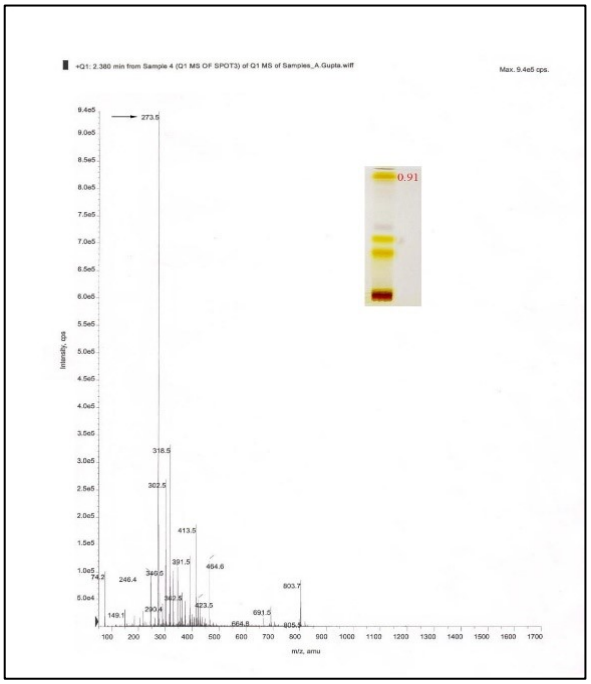

Figure 4. Q1-MS observation for methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. flowers for spot 3

IV. CONCLUSION

It was observed from the present study that the methanolic and aqueous dye extracted from *Butea monosperma* (Lamk.) Taub. flowers showed intense yellow colour. The HPTLC and LC-MS analysis confirmed the presence of flavonoids responsible for the yellow colour in *Butea monosperma* (Lamk.) Taub. flowers. The flowers are good source of flavonoids which can be used as natural colour. Flavonoids also have many medicinal properties and can be used in therapeutic treatment.

Table 2. Q1-MS observation for methanolic dye extracted from *Butea monosperma* (Lamk.) Taub. Flowers

Q1 MS of MeOH	Q1 MS of Spot 1	Q1 MS of Spot 2	Q1 MS of Spot 3
74.10	74.30	74.10	74.20
85.30	85.30	-	-
130.30	130.10	130.30	-
149.10	149.10	-	-
155.40	155.40	155.40	-
167.30	167.10	-	-
201.10	-	-	-
-	251.40	-	-
-	274.10	-	**273.50**
301.10	301.40	301.50	-
-	-	-	**302.50**
305.50	-	-	-
-	-	-	318.50
-	353.50	353.50	-
367.50	-	-	-
369.50	369.50	369.50	-
-	381.60	381.50	-
383.50	-	-	-
-	413.80	-	413.50
-	-	437.40	-
-	-	-	464.60
485.40	485.50	-	-
531.50	-	-	-
-	540.80	-	-
-	568.70	-	-
-	**597.80**	**597.50**	-
-	-	619.80	-
634.00	634.00	-	-
661.80	661.80	-	-
-	685.50	-	-
-	690.00	-	-
-	717.80	-	-
-	-	739.80	-
-	-	-	803.70

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