

# Determination of Rutin Content and Antioxidant Activity of Extracts of *Butea monosperma* Flowers Extracted Using Various Extraction Methods

Edwin Jarald E\*, Nalwaya Narendra, Meena Manish, Jain Anurekha, Edwin Sheeja<sup>1</sup>

\*TIFAC-CORE In Green Pharmacy, B.R. Nahata College of Pharmacy, Mhow-Neemuch Road, Mandsaur – 458001, Madhya Pradesh, India.

<sup>1</sup>Unijules Life Sciences Ltd. & Associated Companies, Nagpur.

## ABSTRACT

In our present study, we quantified rutin and also determined the *in vitro* antioxidant potential of aqueous extracts of *Butea monosperma* flowers prepared using soxhlet, decoction, ultrasonic and maceration methods. Presence of rutin was identified using chemical test and TLC method. Quantification of rutin in extracts was done by HPLC method. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitro blue tetrazolium (NBT) assay method was performed at different concentrations (100–400 µg/ml). All the extracts showed the presence of rutin. The percentage yield of crude extract and the quantity of rutin was found to be more in the extract prepared by soxhlet method than the other extracts. The antioxidant activity of extracts increased in a dose dependent manner. Concentration of soxhlet, decoction, ultrasonic and maceration extract needed for 50% inhibition of DPPH were found to be 52.80, 58.02, 60.86 and 52.62 µg/ml respectively. Similarly concentration needed for superoxide scavenging activity was found to be 543.92, 906.61, 752.44 and 680.04 µg/ml respectively. The results of our study showed that the *B. monosperma* possess a significant antioxidant activity and the maceration extract showed better antioxidant activity than the other extracts in DPPH model and in NBT model, the extract prepared using soxhlet showed better activity compared to other extracts.

**Keywords:** Antioxidant activity; *Butea monosperma*; Decoction; Maceration; Soxhlet; Ultrasonic.

**Editor** Mueen Ahmed KK, Phcog.Net

**Copyright:** © 2009 Phcog.net

**\*Author for correspondence:** E-mail address: ejeru@rediffmail.com

## INTRODUCTION

*Butea monosperma* Linn. (family-Leguminoceae) is a species of *Butea* commonly found all over India and Brahmadesh. The flowers are large 15 cm long, beautiful, bright orange red colored in rigid racemes, grow in spring (1). Rutin is the most widespread of all quercetin glycosides, which is also found in flowers of *B. monosperma*. Chemically it is quercetin-3-rutinoside and is sparingly soluble in water but freely soluble in methanol. After hydrolysis it affords an aglycone quercetin and two sugars i.e. rhamnose and glucose. Rutin as well as its aglycone quercetin both are used as vitamin P, in coronary thrombosis and purification of blood. Rutin is also used as antioxidant (2). Most of the diseases are mainly linked to oxidative stress due to free radicals. Antioxidant compounds play an important role as a health-protecting factor (3). Extraction is one of the most crucial points in the analytical chain in the effort of achieving a complete recovery of targeted compounds (4).

The present study deals with the comparative estimation rutin content and *in-vitro* antioxidant potential of aqueous extract of *Butea monosperma* flowers extracted using various extraction methods.

## MATERIALS AND METHODS

### Plant Material

Flowers of *Butea monosperma* were collected from local area of Mandsaur region. The collected material was dried and powdered.

### Apparatus and Chemicals

HPLC System (Merck), UV Spectrophotometer (Thermospectronic Model of Elico India SL-159), Soxhlet assembly, Ultrasonicator (Scinentech), Water bath, TLC plates & chamber, Rutin Standard, HPLC grade methanol

and other chemicals & reagents of analytical grade were used.

### **Preparation of Test and Standard Solutions**

The extracts were separately dissolved in ethanol and used for the *in-vitro* antioxidant study. The extracts and the pure rutin were dissolved in HPLC grade methanol separately and used for quantification of rutin. The stock solutions were serially diluted with HPLC grade methanol to obtain the lower dilutions.

### **Extraction Methods**

#### *Soxhlet extraction*

Plant material (flowers) (25 g) was accurately weighed, packed in a soxhlet apparatus and extracted using water as solvent for 24 hrs at 50°C. After extraction the content of the flasks were filtered through filter paper and the filtrate was evaporated.

#### *Maceration extraction*

Plant material (flowers) (25 g) was taken in a 250 ml conical flask and 100 ml water was added. Then the flask was placed on a mechanical shaker for 24 hrs. After extraction the content of the flask was filtered through filter paper and the filtrate was evaporated.

#### *Ultrasonic extraction*

Ultrasound assisted extraction was performed in an ultrasonic bath. 25 g of plant material (flowers) was kept with water (100 ml) in a 250 ml conical flask and kept for sonication for 2 hrs at room temperature. After extraction, the contents were filtered and evaporated to dryness.

#### *Decoction extraction*

Plant material (25 g) was taken in a beaker and 100 ml of water was added. The plant material was allowed to boil for 15 mins. After extraction, the contents were filtered and evaporated to dryness.

### **Phytochemical screening**

In order to determine the presence of rutin, a preliminary phytochemical study (colour reaction) with various extracts was performed.

### **Thin layer chromatography**

To confirm the presence of rutin, TLC was performed using Ethyl acetate: Formic acid: Glacial acetic acid: Water

(100:11:11:27) as the solvent system. Presence of rutin could be detected by the presence of yellow coloured spot (5).

### **Determination of Rutin Content by HPLC**

The extracts and pure rutin were dissolved in HPLC grade methanol separately and used for evaluation of rutin. The stock solutions were serially diluted with HPLC grade methanol to obtain a dilution of 10 µg/ml. Chromatographic separation was performed on a Lachrom liquid chromatographic system equipped with a RP-C18 column, reciprocating pump, UV detector and a rheodyne injector with 20 µl loop volume. Mobile phase of HPLC grade methanol was used and was delivered at a flow rate of 0.5 ml/min with detection at 257 nm. Then the peak area ratios of standard and sample solutions were calculated and the concentration of rutin was determined by using following formula.

$$\text{Concentration of Sample} = \frac{\text{AUC of Sample}}{\text{AUC of Standard}} \times \text{Concentration of Standard} \times \text{Dilution Factor}$$

### **In-vitro Antioxidant Activity**

#### **1, 1 –diphenyl-2-picryl hydrazyl (DPPH) Method**

Different concentrations of each herbal extracts were added at an equal volume, to methanolic solution of DPPH. After 15min at room temperature, the absorbance was recorded at 519 nm (6). It was repeated for 3 times. IC<sub>50</sub> values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

The percentage inhibition was calculated by following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

#### **Nitro Blue Tetrazolium (NBT) Method**

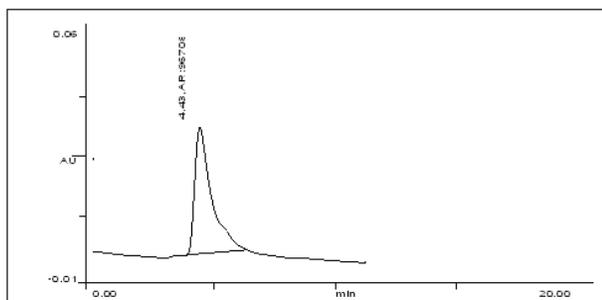
The method was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system. The reaction mixture contains EDTA, riboflavin, nitro blue tetrazolium (NBT), various concentrations of extracts and phosphate buffer (pH 7.6) in a final volume of 3 ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min and absorbance was measured at 560 nm (7).

The percentage inhibition was calculated by following formula:

$$\% \text{ Inhibition} = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

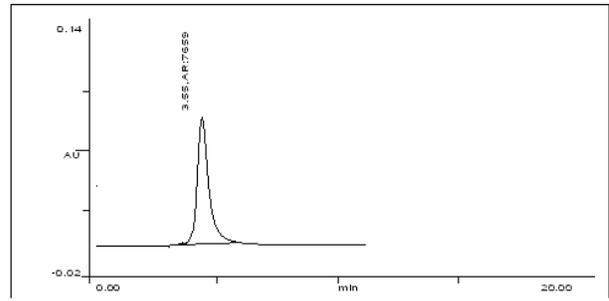
## RESULTS AND DISCUSSION

In our present study, we quantified rutin and also determined the *in vitro* antioxidant potential of aqueous extract of *Butea monosperma* flower extracted using soxhlet, decoction, ultrasonic and maceration methods. Presence of rutin was identified using chemical test and TLC method (Table 1). Quantification of rutin in extracts was done by HPLC method (Fig. 1–5). All the extracts showed the presence of rutin. The percentage yield of crude extract and the quantity of rutin was found to be more in extract prepared by soxhlet method when compared to other extracts (Table 2). The results of our study showed that the *B. monosperma* possessed a significant antioxidant activity and the maceration extract showed better antioxidant activity than the other extracts in DPPH model and in NBT model, the extract prepared using soxhlet showed better activity than the other extracts. The antioxidant activity of aqueous extracts increased in a dose dependent manner. The IC<sub>50</sub> values are presented in Table 3. Flavonoids are used for the prevention and cure of various diseases which is mainly associated with free radicals. Flavonoids acts as an antioxidant in biological systems and scavenge the free radicals thereby increasing the antioxidant defence in the body. The effect of Flavonoids and other putative antioxidants on biomarkers of oxidation have been studied in many pathological states that are thought to result from, or result in oxidative stress (3). In our study, better antioxidant activity was found in extracts prepared using soxhlet and maceration. The content of rutin is high in extract prepared using soxhlet and the content of rutin is less comparatively in the extract prepared using maceration. Even though the concentration of rutin is less in maceration extract the activity was equivalent to soxhlet extract. This may be due to the presence of other



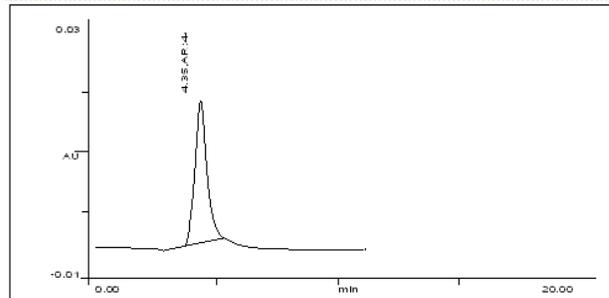
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/Ht
1	4.43	8228	9670871	100.0000	100.0000	BB	0.802
		8e+03	9670871				

**Figure 1.** HPLC of Rutin



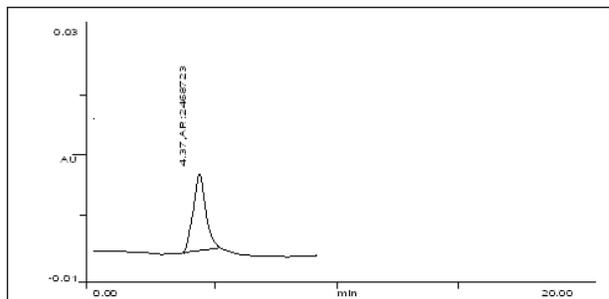
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/Ht
1	3.55	175	76593	0.9188	0.4989	S	0.299
2	4.39	18872	15276451	99.0812	99.5011	BB	0.553
		2e+04	15353044				

**Figure 2.** HPLC of Aqueous Extract of *B. monosperma* Extracted by Soxhlet Method



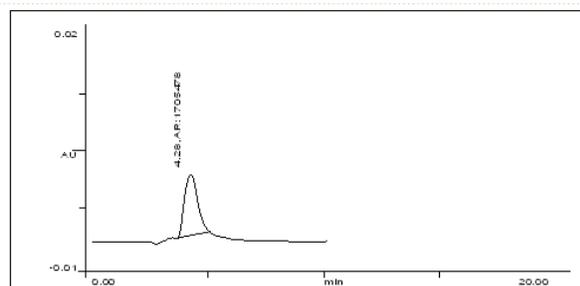
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/Ht
1	4.35	5300	4436234	100.0000	100.0000	BB	0.571
		5e+03	4436234				

**Figure 3.** HPLC of Aqueous Extract of *B. monosperma* Extracted by Decoction Method



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/Ht
1	4.37	2831	2468723	100.0000	100.0000	BB	0.595
		3e+03	2468723				

**Figure 4.** HPLC of Aqueous Extract of *B. monosperma* Extracted by Ultrasonication Method



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/Ht
1	4.28	1778	1705478	100.0000	100.0000	BB	0.655
		2e+03	1705478				

**Figure 5.** HPLC of Aqueous Extract of *B. monosperma* Extracted by Maceration Method

**Table 1. TLC of Rutin in the extracts of *B. monosperma***

Extracts	R <sub>f</sub> Value and Colour
Standard	0.62 (Yellowish Green)
Soxhlet	0.63 (Yellowish Green)
Decoction	0.62 (Yellowish Green)
Ultrasonication	0.61 (Yellowish Green)
Maceration	0.62 (Yellowish Green)

**Table 2. % Yield and Concentration of Rutin in *B. monosperma***

Extraction Methods	Yield* (% w/w)	Concentration of Rutin* (% w/w)
Soxhlet	47.33	82.32
Decoction	35.07	45.87
Ultrasonication	11.28	25.52
Maceration	10.12	17.63

\* Average of the three determination

**Table 3. IC<sub>50</sub> Values\* (µg/ml)**

Extracts	Methods	
	DPPH	NBT
Soxhlet	52.80	543.92
Decoction	58.02	906.61
Ultrasonication	60.86	752.44
Maceration	52.62	680.04

\* Average of the three determination

antioxidant chemicals in maceration extract. The results indicated that the developed procedure could be used for the estimation of rutin.

## REFERENCES

- Gupta D. P., *The Herbs*, 1 edition, (Smt. Prem Lata Gupta, Indore, 2008) 95.
- RangariDr., *Pharmacognosy and Phytochemistry*, Part-II, 1 edition, (Career Publications, Nashik, 2006) 357.
- Velavan S., Nagulendran K., Mahesh R. and Begum Hazeena V. In vitro antioxidant activity of *Asparagus racemosus* root. *Phcog Mag* **3** (9): 26–33 (2007).
- Badami S., Mary Cherian M. and Dongre S. H., Comparative evaluation of conventional and microwave extraction of plant phenolics from five plants. *Indian J Pharm Educ Res* **41** (3): 248–52 (2007).
- Agrawal S. S. and M M., *Herbal Drugs Technology*, 1 edition, (Universities Press (India) Private Limited, Hyderabad, 2007) 560.
- Saravana Kumar A., Mazumder A., Vanitha J., Venkateshwaran K., Kamalakannan K. and Sivakumar T. Evaluation of antioxidant activity, phenol and flavonoid contents of some selected indian medicinal plants. *Phcog Mag* **4** (13): 143–47 (2008).
- Raju I., Moni M. and Subramanian V. Antinflammatory and antioxidant activities of *Cassia fistula* L. bark extracts, *Afr J Traditional, Complementary and Alternative Medicines* **2** (1): 70–85 (2005).