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# HPLC METHOD FOR THE QUANTIFICATION OF SOME ACTIVE FLAVONOIDS IN ETHYL ACETATE EXTRACT OF LEAVES OF BUTEA *MONOSPERMA* LINN

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## Abstract.

**Aim:** The aim of the present investigation is to study HPLC process to evaluate Some Active Flavonoids in Ethyl Acetate Extract of Leaves of *Butea monosperma* Linn.

**Material and methods:** Using a soxhlation device, the leaves of *Butea monosperma* Linn. were extracted in stages. Each powdered batch (500g) was extracted in stages with polarity-graded solvents such as petroleum ether (Pet. Et) (60-80º), chloroform (CHCl3), ethyl acetate (EtOAc) using a soxhlet extractor. Alkaloids, flavonoids, glycosides, tannins, phenols, and steroids, among other chemical families of components, were identified through qualitative phytochemical screenings of each extract. To make a 10 g/ml stock, standard phenolic markers like quercetin, rutin, catechin, gallic acid, and chlorogenic acid were dissolved in methanol. Phytoconstituents were separated and identified from extracts using various solvents and combinations of solvents, which were chosen after consulting the literature.

**Results and Discussion:** Preliminary phytochemical screening showed the revealed that the leaves contain steroid, triterpenoids, fatty acid and alkaloids. While the ethyl acetate extract found to contain therapeutically important phytoconstitutes such as steroids, triterpenoids, saponins, flavonoids, and tannins. Bioactive extracts of *Butea monosperma* were found to include flavonoids and phenolic substances. In ethyl acetate extract, various flavonoids and phenolic compounds were discovered.

**Conclusion:** This is a preliminary report on the identification of phytochemical and HPLC evaluation of ethyl acetate extract of leaves of *Butea monosperma* Linn. and to unravel the mechanisms driving bioactive qualities and the existence of putative synergy among these substances, more research is needed on the isolation and characterization of individual Flavonoids or phenolic compounds.

**Key words.** HPLC method, quantification of some active flavonoids, ethyl acetate extract, *Butea monosperma* Linn., quercetin, rutin, catechin.

## Introduction.

Nature continues to fulfill its role as man's primary source of healing. Functional nutrition has been shown to be important in reducing the risk of some chronic diseases, according to research in preventive medicine. The human body's natural defence mechanism may be insufficient to counteract the harm produced by oxidative stress [1]. Quercetin and other flavonoids are structured to operate as potent antioxidants. This has been demonstrated in vitro numerous times. Quercetin, as a prominent flavonoid ingredient, is able to prevent various chronic degenerative illnesses [2]. Synthetic antioxidants are connected to a variety of negative side effects, such as liver damage and mutagenesis, according to growing scientific data [3]. As a result, there has been a surge in interest in natural goods as antioxidants, which restrict free radical reactions and protect the human body from illnesses like cancer and diabetes. Plants and other herbal extracts, Polyphenolic substances (such as gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin, and sitosterol) might have high antioxidant capabilities, according to recent study [4].

In conclusion, high-performance liquid chromatography (HPLC) is a versatile analytical procedure that takes cheaper equipment and less experience. The investigation aims to develop a rapid, sensitive, and accurate analytical process for assessing flavonoids in plant extract bioactive extracts and their pharmaceutical dosage form, which could be used to examine various plant extract samples and formulations on a regular basis [5]. The goal of this research is to figure out how much flavonoids are in *Butea monosperma* Linn extract.

## Materials and Methods.

### Extraction of plant materials:

The leaves of *Butea monosperma* Linn. are subjected for successive extraction using soxhlation apparatus. Each powdered batch (500g) was extracted in stages with solvents of varying polarity, such as petroleum ether (Pet. Et) (60-80º), chloroform (CHCl3), ethyl acetate (EtOAc) using a soxhlet extractor. The extraction was carried out till to exhaust. The extract was dried under vacuum and processed for the further HPLC analysis [6].

### Preliminary phytochemical evaluation:

The existence of main bioactive phytocompounds or components (alkaloids, flavonoids, glycosides, tannins, phenols, and steroids) was investigated employing qualitative screens of each extract [7].

### Marker based estimation of extracts by HPLC:

Agilent liquid chromatography system series 1200 with quaternary pump, Rheodyne injector with fixed loop of 20 l, and UV detector is employed. At room temperature, the separation was performed using a Waters Symmetry C-18 Column (250 X 4.6mm, particle size 5m) followed by an ODS guard column (10m, 10mm X 5mm ID) [8].

**Preparation of sample solution:** The bioactive extract i.e. EtOAc was dissolved in respective solvents to get 1 mg/ml as a reference solution [9].

**Preparation of standard solutions:** Standard phenolic and flavonoids markers (quercetin, rutin, catechin, gallic acid, and chlorogenic acid) are dissolved in methanol to make a 10 g/ml stock [10].

**Chromatographic conditions:**

Phytoconstituents were separated and identified from extracts using various solvents and combinations of solvents, which were chosen after consulting the literature. The best possible combination of mobile phase is employe for each marker [11].

**HPLC specifications for analysis of extract.**

Specifications for HPLC analysis of ethyl acetate extract (Table 1).

***Table 1.*** *Specifications for HPLC analysis of ethyl acetate extract.*

| Sr. no | Active Phytocompounds | Mobile phase (v/v) | Flow rate (ml) | Standard Rt (Min) | Detection (nm) |
|---|---|---|---|---|---|
| 1 | Gallic acid | Methanol+ (polar solvent) Water (55:45) pH-2.0 with OPA | 0.6 | 2.47 | 280 |
| 2 | Chlorogenic acid | | 0.6 | 4.2 | 280 |
| 3 | Catechin | | 0.6 | 5.0 | 280 |
| 4 | Rutin | | 0.6 | 5.8 | 280 |
| 5 | Quercetin | | 0.6 | 11.02 | 280 |

## Results and Discussion.

Preliminary phytochemical screening showed the revealed that the leaves contain steroid, triterpenoids, fatty acid and alkaloids. While the ethyl acetate extract found to contain therapeutically important phytoconstitutes such as steroids, triterpenoids, saponins, flavonoids, and tannins (Table 2). Polyphenols have a hydroxyl group in their structure, as well as the potential to transfer electrons, making them a powerful antioxidant. The qualitative chemical test clearly revealed that the potent antioxidant phytoconstituents i.e. flavonoids, tannins were highly accumulated in ethyl acetate extract [12,13].

***Table 2.*** *Phytochemical analysis of different extracts of leaves.*

| Tests | Leaves Extracts of *Butea monosperma* Linn. | | |
|---|---|---|---|
| | Petroleum ether | Chloroform | Ethyl acetate |
| **Alkaloid** | - | + | + |
| **Glycoside** | - | - | - |
| **Saponins** | - | - | + |
| **Steroid** | + | + | + |
| **Triterpenoids** | + | + | + |
| **Tannins and Phenolic** | - | - | ++ |
| **Flavonoid** | - | + | +++ |
| **Test for carbohydrates** | - | - | - |
| **Proteins** | - | + | - |
| **Fats and Lipids** | + | + | - |

*(-): Not present, (+): present in less quantity, (++): Moderate presence, (+++): Strong Presence.*

**Marked Based Estimation of *Butea monosperma* by HPLC:**

Bioactive extracts of *Butea monosperma* were found to include flavonoids and phenolic substances. In ethyl acetate extract, presence of different phenolic (gallic acid, chlorogenic acid) and flavonoids i.e. quercetin, rutin, catechin were discovered. These are well documented for their different pharmacological activities i.e. antioxidant, anti-inflammatory, immunomodulatory, antimicrobial and anticancer activity (Table 3) [14-16].

***Table 3.*** *HPLC analysis of Butea monosperma leaves extracts.*

| Sr. No | Phytoconstituents | *Rt* (min) | *Butea monosperma* Linn. Ethyl acetate Extract |
|---|---|---|---|
| 1 | Quercetin | 11.02 | 11.2 |
| 2 | Rutin | 5.8 | 5.87 |
| 3 | Catechin | 5.0 | - |
| 4 | Gallic acid | 2.47 | - |
| 5 | Chlorogenic acid | 4.2 | 4.09 |

*(-) absent*

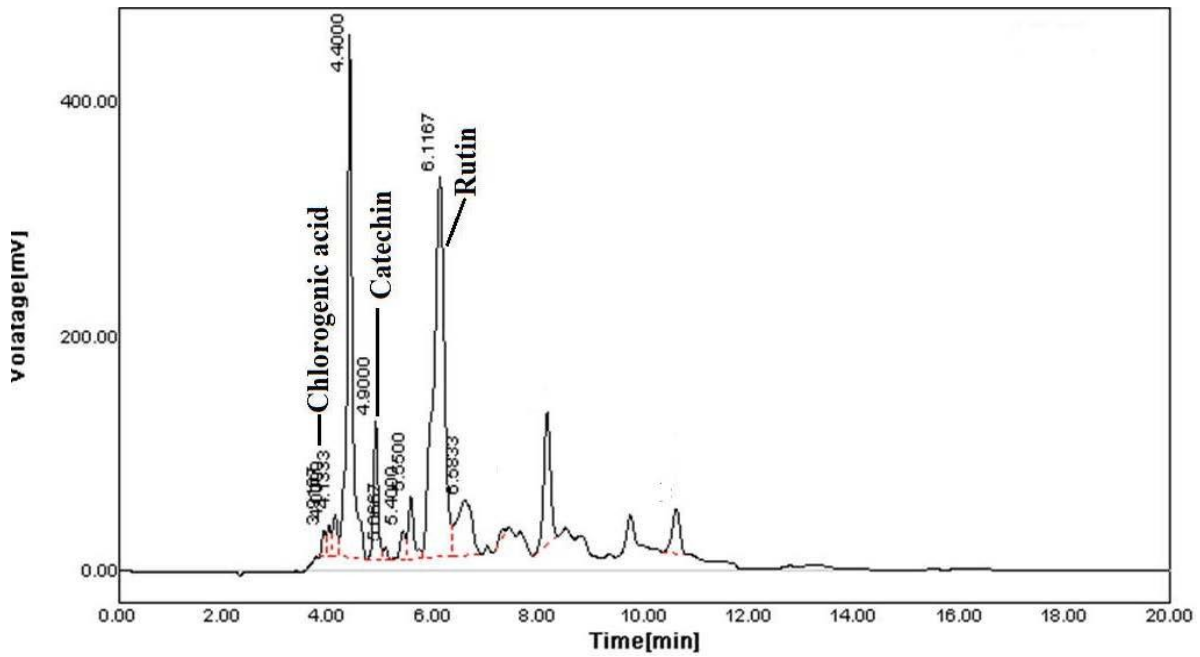


***Figure 1.*** *Chromatogram of leaves ethyl acetate extract for presence of different flavonoids.*

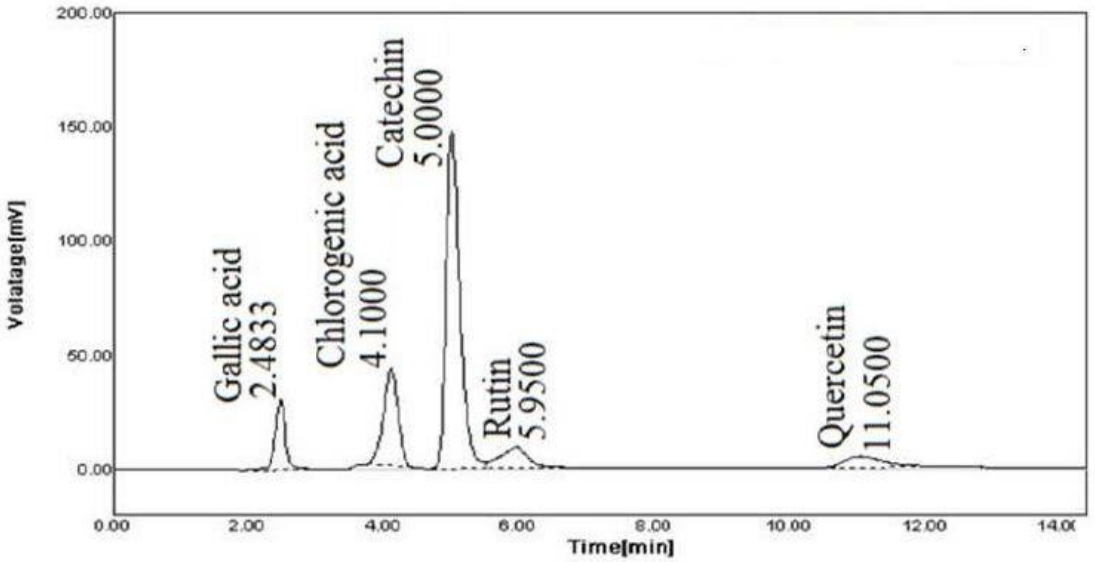


***Figure 2.*** *Chromatogram of standard different flavonoids.*

With the emergence of new terminologies like neutraceuticals, food supplements, botanicals, dietary supplements, multifunctional foods, etc., the market of natural substances has intensely changed. But to maintain their sustain use; there is also a need of adequate analytical tool which will ensure the quality and purity of these herbal products [17,18]. These analytical tools should be such that they can withstand with the complexity of natural product mixture. As a result, it is now possible to develop acceptable procedures for quality analysis and standardisation of herbal medicines in order to preserve as much uniformity as possible in the plant extract [19,20]. High-performance liquid chromatography can be used to standardise and control the quality of raw materials, extracts, and final herbal remedies, among other things, alone or in combination (Figures 1 and 2).

## Conclusion.

*Butea monosperma* Linn. grows abundantly in tropical and subtropical places under a variety of climatic circumstances, and they can be mass-produced as value-added products on a huge scale. As a result, the findings may provide a scientific foundation for the usage of plant species in commercial products. This is a preliminary study on the phytochemical identification and HPLC analysis of an ethyl acetate extract of *Butea monosperma* Linn leaves. Further research is needed to determine the processes driving the bioactive qualities of specific Flavonoids or phenolic compounds, as well as the existence of probable synergies, if any, among these molecules.

## REFERENCES

1. Fageria D, Rao DV. A Review on *Butea monosperma* (Lam) kuntze: A Great Therapeutic valuable Leguminous Plants. International Journal of Science and Research Publications. 2015;5:1-9.

2. Madhavi A. An overview of *Butea monosperma* (Flame of forest). World Journal of Pharmacy and Pharmaceutical Science. 2013;3:307-319.

3. Mishra A, Verma S, Mishra AP. A plant review: *Butea monosperma* (Lam.) Kuntze. Research Journal of Pharmaceutical, Biological and Chemical Science. 2012;3:700-714.

4. Mohammad T. Evaluation of antibacterial and antioxidant activity of ethanolic extract of *Butea monosperma*. World Journal of Pharmaceutical Research. 2018;7:730-740.

5. Tiwari P, Jena S, Sahu PK. *Butea monosperma*: Phytochemistry and Pharmacology. Acta Scietific Pharmaceutical Science. 2019;3:19-26.

6. Bhargava SK. Estrogenic and post coitalanticonceptive activity in rats of butin isolated from *Butea monosperma*. Journal of Ethanopharmalogy. 1986;18:95-101.

7. Liao W, Liu J, Zhang D, et al. Butein inhibited in Vitro Hexokinase -2 mediated tumor glycolysis in hepatocrcinoma by blocking epidermal growth factor receptor. Medical Science. 2018;24:3283-3292.

8. Moon DO, Kim MO, Chori YH, et al. Butein induces G2/M phase arrest and apoptosis in human hepatoma cancer cell through ROS generation. Cancer Letter. 2010;288:204-213.

9. Huo SX, Wang Q, Liu XM, et al. The effect of butin in vitilligo mouse model induced by Hydroquinone. Phytochemistry Research. 2017;31:740-746.

10. Shahidi F. Nutraceuticals, functional foods and dietary supplements in health and disease. J. food drug. Anal. 2012;20:226-230.

11. Tandon R, Shivanna K, Mohan Ram H. Reproductive biology of *Butea monosperma* (Fabaceae) Ann. Botany. 2003;92:715-723.

12. Alaa G. Al-Hashimia, Sinan J. Abdul-Abbas, Najla Hussen Saper Al Garory, et al. Isolation and diagnosis of phytochemicals extracted from pomegranate husks and study of the biological effect of extract (O-Veratramide) as an antimicrobial activity. International Journal of Green Pharmacy. 2018;12:310-321.

13. HNK. AL-Salman, Ekhlas Qanber Jasim, Hussein H. Hussein, et al. Theophylline Determination in Pharmaceuticals Using a Novel High-performance Liquid Chromatographic Process. Neuro Quantology. 2021;19:196-208.

14. Wagner H, Geyer B, Fiebig M, et al. Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers. Planta Med. 1986;4:77-79.
15. Yadava R.N, Tiwari L. A potential antiviral flavone glycoside from the seeds of *Butea monosperma* O. Kuntze. J. Asian Nat. Prod. Res. 2005;7:185-188.
16. Owoyele B.V, Oguntoye S.O, Dare K, et al. Analgesic, anti-inflammatory and antipyretic activities from flavonoid fractions of Chromolaena odorata. J. Med. Plants Res. 2008;2:219-225.
17. Gunakkunru A, Padmanaban K, Thirumal P, et al. Anti-diarrhoeal activity of *Butea monosperma* in experimental animals. J. Ethnopharmacol. 2005;98:241-244.
18. AL-Salman HN, Ali ET, Almukhtar OA, et al. 2-benzhydrylsulfinyl-N-hydroxyacetamide Extracted from Fig: A good Therapeutic Agent against Staphylococcus Aureus. AIP Conference Proceedings. 2020;2213:020223-5.
19. Al-Salman HNK, Ali ET, Jabir M, et al. 2-Benzhydrylsulfinyl-N-hydroxyacetamide-Na extracted from fig as a novel cytotoxic and apoptosis inducer in SKOV-3 and AMJ-13 cell lines via P53 and caspase-8 pathway. European Food Research and Technology. 2020;246:1-18.
20. Alassadi EAS, Jasim EQ, AL-Salman HNK, et al. Comparative Study of an In Vitro Release Patterns of Ceftaroline Fosamil fom Chemically – Prepared Coated Hydroxyapatite Nanoparticles. Systematic Reviews in Pharmacy. 2020;11:797-805.