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Estimation of flavonoid-luteolin in different plant parts of *Bacopa monnieri* (L.) Wettst. by using HPTLC method

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ABSTRACT

Bacopa monnieri (L.) Wettst., commonly known as *Brahmi* or *Jalanimba* in India is distributed throughout the warmer regions of the world. A simple, sensitive and accurate HPTLC method has been developed for quantitation of a flavonoid Luteolin from micropropagated, native and cultivated plant parts (roots, leaves and stem) of *Bacopa monnieri* collected from two different locations (Bhayander and Chembur) of Maharashtra. Samples were extracted with methanol and spotted on HPTLC silica gel 60 F_{254} pre-coated plates using automatic sample spotter (Linomat IV) with toluene: ethyl acetate: formic acid (3:3:0.8; v/v/v). Spectra of Luteolin were comparable only with the leaf collected from Bhayander and stem collected from Chembur. Densitometric evaluation of the plates was performed using deuterium lamp with Camag Scanner II equipped with WinCats 3 software at 254 nm. Luteolin response was linear over the range 20 µg/mL to 150 µg/mL. Method was validated as per ICH guidelines.

KEYWORDS

Bacopa monnieri (L.) Wettst;
Micropropagation;
Morphological parts;
Regional variation;
HPTLC;
Luteolin.

INTRODUCTION

Bacopa monnieri (L.) Wettst. (Scrophulariaceae) commonly called as *Brahmi* is widely distributed throughout India and the warmer regions of the world[1]. In India and the tropics it grows naturally in wet soil, shallow water, and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet and easily cultivated if adequate water is available. *Bacopa monnieri* is reported to have many therapeutic uses like memory enhancer, broncho-vasodilator, hepatoprotective, antidepressant, calcium antagonistic, anti-inflammatory, anti-epilepsy, anti-allergic, anti-oxidant, anti-microbial and anticancer[2].

Bacopa monnieri is used in many herbal formulations (e.g. Bacomind tablets) by several herbal industries as memory enhancer. It is reported to possess alkaloids (Brahmine and herpestine), saponins (d-mannitol, hersaponin, monnierin, bacopasaponins, bacosides A, bacosides B, bacopaside II, bacopaside I, bacopaside X, bacopasaponin C, bacopaside N2), flavonoids (apigenin) and betulic acid, stigmastarol, betasitostero, tannins, terpenoids in significant amount[3].

Flavonoids (polyphenols) are large heterogeneous group of secondary plant metabolites widely distributed in the plant kingdom. A number of factors influence their concentration in the herbal plants such as; the time and period of collection, geographical origin

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and climatic conditions. Sometimes, the influence of these factors may leads to even absence of active constituents in the same plant collected from different regions[4].

Aerial parts of the plant contain a flavonoid Luteolin (Figure 1); a bioactive marker with antioxidant, anti-inflammatory, antimicrobial, anticancer[5], cardiovascular and antidiabetic activities[6].

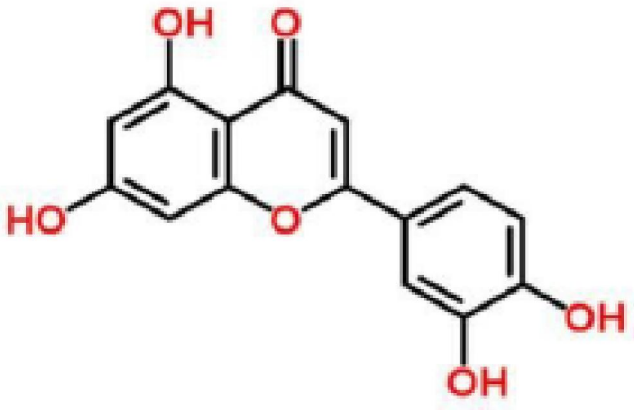


Figure 1: Structure of Luteolin

Quantitation of Luteolin has been done from other plants like *Achyrocline satureioides*[7], *Bauhinia variegate, Bacopa monnieri* whole plant; *Vitex negundo* (leaves) *Centella asiatica* etc[8, 9]. The literature reveals that there is no HPTLC method reported for estimation of Luteolin from micropropagated, native and cultivated plant parts (roots, leaves and stem) of *Bacopa monnieri*. In the present work, a sensitive, simple and accurate HPTLC method has been developed for the estimation of Luteolin from micropropagated and native, cultivated (from two different locations of Mumbai) different plant parts of *Bacopa monnieri*.

MATERIALS AND METHODS

Materials

Plant samples were collected from Bhayander in the month of January and from Chembur in the month of May of the same year. Plant parts for Micropropagation were collected in the month of January (nodal segments of stem plant part, collected from Bhayander location were used as explants). Micropropagated plant material is represented as sample 1 and their plant parts are- root as 1a, leaf as 1b and stem as 1c and native, cultivated plant material of Chembur location as sample 2 (2a, 2b, 2c) and plant material of Bhayander location as sample 3 (3a, 3b, 3c).

The plant twig was taxonomically identified and authenticated by Blatter Herbarium St Xavier's college, Mumbai. Standard Luteolin (95% purity) was procured from Sigma Aldrich Chemie (Steinheim, Germany). The solvents Toluene, Ethyl acetate and Formic acid were of AR grade, obtained from Qualigens Fine Chemicals, Mumbai, India, were used for the analysis.

Chemicals

Analytical grade solvents Toluene, Ethyl acetate, Formic acid were procured from Qualigens Fine Chemicals, Mumbai. Standard Luteolin (= 98% purity) was procured from Sigma Aldrich, Germany (Figure 1).

Optimized hptlc condition

Chromatographic separation was achieved on HPTLC plates (20 X 20 cm) precoated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness with aluminium sheet support. Samples were spotted using CAMAG Linomat IV Automatic Sample Spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton, 100 µL). Plates were developed in a glass twin trough chamber (CAMAG, 20 X 10 cm) presaturated with mobile phase. Scanning device used was CAMAG HPTLC Scanner 2 equipped with CATS3 software. The experimental condition was maintained at 25 ± 2^0 C.

HPTLC fingerprinting profile

Standard stock and sample solution

The stock solutions were transferred to volumetric flask in order to obtain aliquots of Luteolin (20- 150 µg mL-1) and volume was made upto 10 ml with methanol. Accurately weighed (500 mg) of plant powder was placed in a stoppered tube and 10 mL of methanol was added to it. The sample was vortexed for 1-2 minutes and left to stand overnight at room temperature (28 ± 2ºC). The contents of the tube were filtered through Whatmann filter paper No. 41 (E. Merck, Mumbai, India) and was used for analysis.

Solvent system

Solvent system consisted of Toluene: Ethyl acetate: Formic acid (3: 3: 0.8 v/v/v) has been used in this method

to resolve and to quantitate Luteolin from different plant parts of *Bacopa monnieri*.

Caliberation curve for luteolin

For calibration curve the plate was scanned and absorption spectra were recorded at start, middle and end position of the band to check the purity of the band. The plates were scanned at 254 nm using CAMAG TLC Scanner 2 and CATS software. The peak areas were recorded. Calibration curve of Luteolin was obtained by plotting peak areas vs. concentration of Luteolin applied.

Linearity

The linearity of Luteolin was determined by using working standard solutions of Luteolin, in the concentration range of 20 – 150 µg/mL. The peak areas of Luteolin were recorded for each concentration. The calibration curve of Luteolin was obtained by plotting a graph of peak area vs. applied concentration of Luteolin.

The experiment was performed three times and the mean was used for the calculations. The linearity graph data are given in Figure 2 and TABLE 1 : respectively.

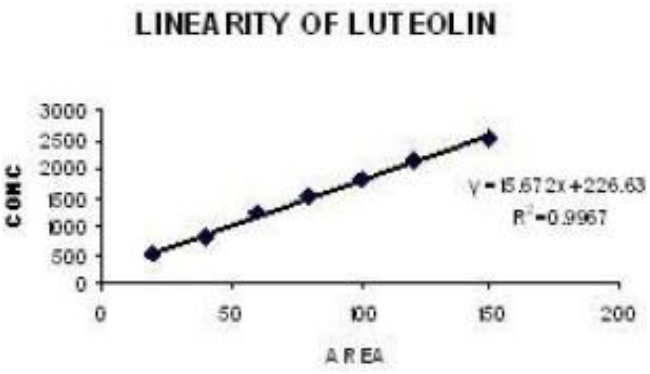


Figure 2 : Linearity of Luteolin

TABLE 1 : Linearity data

Parameters	Result
Linearity range	20 – 150 µg/mL
Slope (m)	15.672
Intercept (c)	226.63
Regression equation*	y = 15.672x + 226.63
Correlation coefficient (r^2)	0.9967
LOD	10 µg/mL
LOQ	20 µg/mL

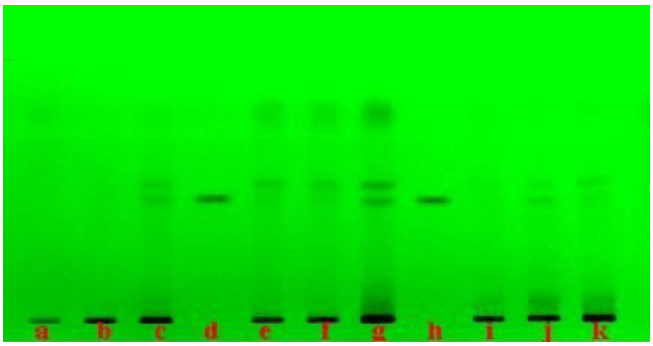
(*y = mx + c, where, y = peak area, m = slope, x= concentration, c = intercept.)

Specificity

Specificity was ascertained by analyzing standard compounds and samples. The band for Luteolin from sample solution was confirmed by comparing the R_f and spectra of the band to that of the standard. The peak purity of the compound was analysed by comparing the spectra at three different levels, i.e. start, middle, and end positions of the bands.

Assay procedure

Of the various solvent systems tried, mixture containing Toluene: Ethyl acetate: Formic acid (3: 3: 0.8 v/v/v) gave the best resolution of Luteolin (R_f = 0.49) from the methanolic extract of different plant parts of micropropagated and native, cultivated plants of *Bacopa monnieri* (L.) Wettst. The identity of bands of Luteolin in plant matrix was confirmed by overlay in UV absorption spectra with that of the standard Luteolin using Camag TLC scanner 2. The purity of band of Luteolin in the plant extract was confirmed by overlaying the absorption spectra at the start, middle and end position of the bands. The UV absorption spectrum of standard Luteolin was matched only with UV absorption spectra of *Bacopa* leaf (3b) of sample 3 and *Bacopa* stem (2c) of sample 2. The chromatogram and peak area data of different plant parts of different samples are shown in Figure 3 and TABLE 2 respectively.



Track a: *Bacopa monnieri* root of sample 1 (1a)
Track b: *Bacopa monnieri* root of sample 2 (2a)
Track c: *Bacopa monnieri* root of sample 3 (3a)
Track d: Luteolin 100 ppm
Track e: *Bacopa monnieri* leaves of sample 1 (1b)
Track f: *Bacopa monnieri* leaves of sample 2 (2b)
Track g: *Bacopa monnieri* leaves of sample 3 (3b)
Track h: Luteolin 100 ppm
Track i: *Bacopa monnieri* stem of sample 1 (1c)
Track j: *Bacopa monnieri* stem of sample 2 (2c)
Track k: *Bacopa monnieri* stem of sample 3 (3c)

Figure 3 : Chromatogram of different plant parts of *Bacopa monnieri* (L.) Wettst. of different locations and standard Luteolin

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TABLE 2 : Peak Area of *Bacopa monnieri* (L.) Wettst. roots, leaves and stems samples of two different locations and micropropagated plant samples

Sample	Rf value	Peak Area(n)
1 a	-	-
2 a	0.49	53.6
3 a	0.49	445.8
1b	0.49	78.4
2b	0.49	111.3
3b	0.49	491.8
1c	0.49	116.7
2c	0.49	374.0
3c	0.49	272.8

n = 3

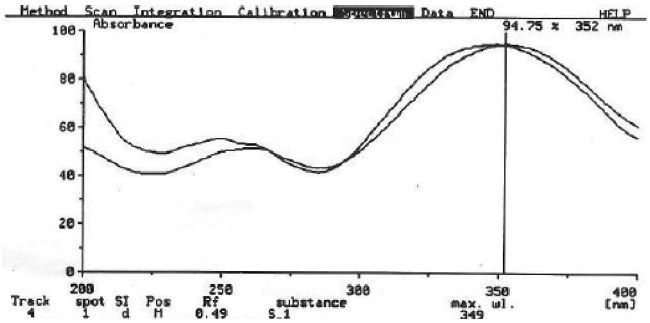

Figure 4 : An overlay of densitometric scan of *Bacopa monnieri* (L.) Wettst. leaves of sample 3 and Luteolin standard

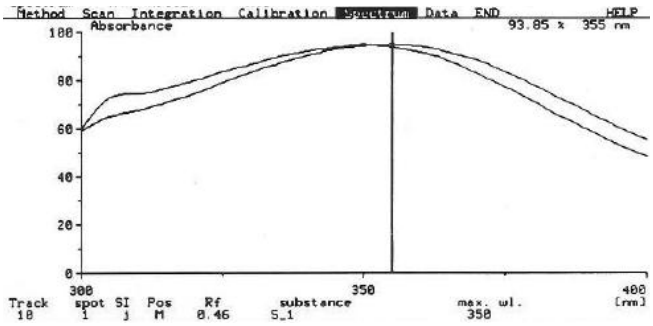

Figure 5 : An overlay of densitometric scan of *Bacopa monnieri* (L.) Wettst. stem of sample 2 and Luteolin standard

RESULTS AND DISCUSSION

In the current study, Luteolin was detected and quantified using HPTLC silica gel 60 F254 pre-coated plates with the mobile phase made of Toluene: Ethyl acetate: Formic acid in the ratio of 3:3:0.8 (*v/v/v*).

The micropropagated and native, cultivated different plant parts of *Bacopa monnieri* (L.) Wettst. exhibited variations in the presence of Luteolin content depending upon the geographical location and different seasons in which they were collected. From the different plant parts used, the spectra of Luteolin was comparable only with the leaf sample collected from Bhayander location and stem sample collected from Chembur location. However, the Rf values of micropropagated plant parts (leaf and stem) and native, cultivated plant parts of Chembur location (root and leaf) and Bhayander location (root and stem) were matched with the Rf value of Luteolin standard, this only Rf matching may be due to the presence of Luteolin glycosides. The Rf value of standard Luteolin was not matched with that of the micropropagated root sample. The identity of the band of Luteolin in the plant extracts (leaf extract of Bhayander sample and stem extract of Chembur sample) were confirmed by overlaying the chromatogram (Figure 4 and Figure 5) of plant part with that of the Luteolin standard. The detection of Luteolin was observed to be linear over a range of 20-150µg/mL with Correlation coefficient (r) 0.997. The concentration of Luteolin was found to be 0.1691 ± 0.0024 mg/500g in leaf of *Bacopa monnieri* (L.) Wettst. collected from Bhayander location, Maharashtra (TABLE 3). The concentration of Luteolin was found to be 0.0940 ± 0.0047 mg/500g in stem of *Bacopa monnieri* (L.) Wettst. collected from Chembur location, Mumbai (TABLE 3).

TABLE 3 : Quantitation of Luteolin from *Bacopa monnieri* (L.) Wettst. leaf of sample 3 and stem of sample 2 using HPTLC technique

Sr. No.	Region	Amount in mg/500mg (n=3)
1.	Bhayander sample 3b (Maharashtra)	0.1691 ± 0.0024
2.	Chembur Sample 2c (Mumbai)	0.0940 ± 0.0047

CONCLUSION

HPTLC Fingerprint analysis is one of the most powerful tools to link the botanical identity of the plant to the chemical constituent profile of the plant. In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can be used to detect adulterations in raw materials. The proposed method is simple, rapid, sensitive, selective and economical and can be used for routine- quality control analysis

and quantitation of Luteolin from different plant parts of *Bacopa monnieri* (L.) Wettst. The present study highlights the significant differences in the presence of Luteolin content in methanolic extracts of *Bacopa monnieri* (L.) Wettst which may be due to the differences in natural climatic conditions or may be due to the collection of plants in different seasons.

Thus, Luteolin can be used as a phytochemical marker to identify the raw material with maximum concentration of Luteolin content for the use in polyherbal formulations. Since the presence of Luteolin was found to be in *Bacopa monnieri* (L.) Wettst. leaf sample collected from Bhayander location and in stem sample collected from Chembur location, it could play a vital role for the use of these plant parts in Ayurvedic companies manufacturing polyherbal formulations containing leaves and stem of *Bacopa monnieri* (L.) Wettst.

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