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Research Article

TO QUANTIFY THE LUTEOLIN CONTENT FROM THE AERIAL PARTS OF *HETEROPOGON CONTORTUS* (L.) BEAUV. (SPEAR GRASS) THROUGH HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Objective: The objective of this study was to quantify the luteolin content present in the extracts from different aerial plants (leaves, stem, and inflorescence) of *Heteropogon contortus* through high-performance thin-layer chromatography.

Methods: The chromatographic fingerprint analysis of the different plant extracts has been developed using optimized mobile phase toluene: ethyl acetate: formic acid (5:5:0.7 v/v) and the developed plate is derivatized with freshly prepared anisaldehyde-sulfuric acid. Then, the plate is heated at 110–120°C. The plate is scanned for densitometry measurements and to record the overlay spectra at 366 nm absorbance/reflectance wavelength. Quantification of luteolin marker compound in different extracts of *H. contortus* is estimated using 2–12 ng/spot.

Results: The yellow colored bands appearing on the chromatogram confirm the presence of luteolin marker compound in the different plant samples of *H. contortus.* Further, the presence of the luteolin marker is confirmed by comparing the R_f values (0.21) of the standard and the samples and from densitometry measurements by scanning the plate at 366 nm absorbance/reflectance. Line-to-line overlay spectra are obtained.

Conclusion: From this, it is concluded that leaf sample of *H. contortus* contains maximum amount of luteolin, i.e., 37.13 ± 0.11 mg/g of dry wt. than inflorescence (1.60 ± 0.013 mg/g of dry wt.) and stem (0.53 ± 0.014 mg/g of dry wt.). The leaves are good source of luteolin and can be used as an alternate natural source to synthesize herbal drugs to cure cancer, hypertension, and inflammatory diseases.

Keywords: *Heteropogon contortus*, Flavonoid, Luteolin, Different plant samples.

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INTRODUCTION

Flavonoids are a diverse group of polynutrients found in almost all fruits (apples and berries), onion, tea, vegetables, and some beverages. Quercetin and kaempferol are the best-known flavonoids. Natures' biological compounds are flavonoids that have the characteristics to modify the reactions taking place in the human body due to allergies, viruses, and carcinogens [1]. Quercetin and kaempferol are used as antioxidants and are investigated for inhibition of carcinogenicity [2]. Luteolin is an important flavonoid with a yellow crystalline appearance. It is found in vegetables and fruits such as celery, broccoli, onion leaves, carrots, peppers, cabbages, and apple skin and *Chrysanthemum* flowers [3-5]. Luteolin rich plants have been used in medicines to prevent hypertension, inflammatory diseases, and cancer [6]. The molecular structure of luteolin is given below (Fig. 1).

Heteropogon contortus (L.) Beauv. (Syn. *Andropogon contortus* L.) is a member of the family Poaceae (Gramineae), distributed in Southern Asia, Southern Africa, and Northern Australia. The spear grass is reported to have myo-inositol, galactinol, raffinose, and polysaccharides [7,8]. The grass exhibits medicinal importance as used in toothache, fever, atrophy, emaciation, muscular pain, hematological disorders, dysentery, and scorpion sting [9,10]. Roots of the plant have diuretic and stimulant properties. The whole plant is used to cure asthma [11].

The earlier studies observed that the methanolic extract of *H. contortus* is used for the treatment of pathological infections caused by mast cell destabilization, membrane destabilization, and free radical generation. It mainly includes acute and chronic inflammatory response such as asthma, arthritis, cardiovascular, and neural diseases. *H. contortus* extract inhibits bronchoconstriction induced by histamine or acetylcholine [12]. It also hinders inflammation induced by carrageenan and egg albumin. High-performance thin-layer chromatography (HPTLC) method was developed for the simultaneous quantification of luteolin and apigenin from *Cardiospermum halicacabum* and *Hydnocarpus pentandra* [13]. The main aim of the present research is to develop chromatographic fingerprint analysis of luteolin compound present in methanolic extracts of leaves, stem, and inflorescence of *H. contortus.*

METHODS

Standard marker

Luteolin is used as a reference standard for HPTLC. For chemical compounds, collection of plant material and sample preparation, preparation of stock solution, and thin-layer chromatography are seen [14].

Detection and estimation of luteolin

The linear fingerprint development was carried out in presaturated twin trough chamber (20 cm×10 cm) with toluene:ethyl acetate: formic acid (5:5:0.7 v/v) used as mobile phase. The length of the chromatogram was carried out up to a distance of 75 mm. The plate was dried with hair drier and was then dipped in the freshly prepared anisaldehyde-sulfuric acid for derivatization. Then, the plate was heated at 110–120°C in hot air oven. Yellow colored bands appear on the plate. For the quantitative estimation of luteolin, the plate was scanned in absorption-reflection mode at 366 nm with 100 µm/step data resolution and at 20 mm/s scanning speed.

Method validation

The method is validated as per ICH guidelines. Specificity, linearity, limit of detection (LOD) and quantification, precision, accuracy, robustness,

and stability were checked for confirming method validation (Table 1). All the parameters were performed in triplicates [15].

LOD and limit of quantification (LOQ)

$$LOD = 3.3\frac{SD}{S}$$

$$LOQ = 10\frac{SD}{S}$$

Where, SD stands for standard deviation, S for slope

Recovery
Recovery was determined by adding known concentrations of standard to a preanalyzed sample. The analysis was done by the proposed HPTLC method and the analysis was carried out in triplicate.

Calibration curve of luteolin
The standard stock solutions (1 mg/1 ml) of luteolin (2–10 µg/spot) were applied in triplicate on an HPTLC plate. These plates were developed with the mobile phase toluene: ethyl acetate: formic acid (5:5:0.7 v/v). After development, the plates were air dried and scanned at 366 nm absorbance using deuterium lamp. The resolved peak area was recorded for the standard. The calibration curve of luteolin was plotted by taking peak area versus concentrations of standard.

Method specification
For luteolin, toluene:ethyl acetate:formic acid (5:5:0.7v/v) was used as a solvent system using silica gel 60 F_{254} precoated plates (20 cm×10 cm). Automatic Linomat V application was used for spotting. The plates were developed in ascending mode up to 75 mm and scanned at 366 nm under UV-Vis mode. The content of luteolin in leaves, stem, and inflorescence of *H. contortus* was determined by comparing the peak area of standard luteolin with a calibration curve of *H. contortus,* considering the isolated compound to be 100% pure.

RESULTS

Optimization
At present, HPTLC fingerprint profile of luteolin and plant samples (leaves, stem, and inflorescence) has been developed under optimized chromatographic conditions using toluene: ethyl acetate: formic acid (5:5:0.7 v/v) as a mobile phase. Freshly prepared anisaldehyde-sulfuric acid is used as derivatizing reagent, and yellow colored bands have been observed during HPTLC profiling (Fig. 2). Then, the plates are scanned under UV-Vis absorbance/reflectance at 366 nm wavelength to obtain densitometry measurements (Fig. 3).

Calibration curve and linearity
The calibration curve was performed by plotting peak area versus concentration (µg/spot). The linear regression equation and correlation coefficient for luteolin are y=6031.2x and R^2=0.99. Thus, the graph obtained is linear (Fig. 4).

Accuracy and recovery
The presently obtained results showed that the average percentage recovery at three different levels of the luteolin compound is found 99.40% (Table 1).

The highest amount of luteolin is present in leaves of the selected plant, i/e., 37.13 ± 0.11 mg/g of dry wt. It is very low in stem sample, 0.53 ± 0.014 mg/g of dry wt. and 1.60 ± 0.013 mg/g of dry wt. in inflorescence. The calculated amount of luteolin presents in the different plant parts of *H. contortus* falls in the decreasing order of leaves>inflorescence>stem, i.e., 37.13 >1.60>0.53 mg/g of dry wt. (Table 2).

The presently developed method is validated as per the ICH guidelines in terms of precision, repeatability, and accuracy (Table 3). The linearity range for luteolin was found to be 2–12 µg/spot with 0.99 as a correlation coefficient and the obtained linear regression equation is y=6031.2x (set intercept zero). Linear calibration curves were obtained for the standard compound as described above. LOD value for standard compound is 0.015 ng/spot, whereas, LOQ value is 0.04 ng/spot.

Specificity
An overlain spectrum is recorded to check the identity and specificity of luteolin present in methanolic extracts of leaves, stem, and

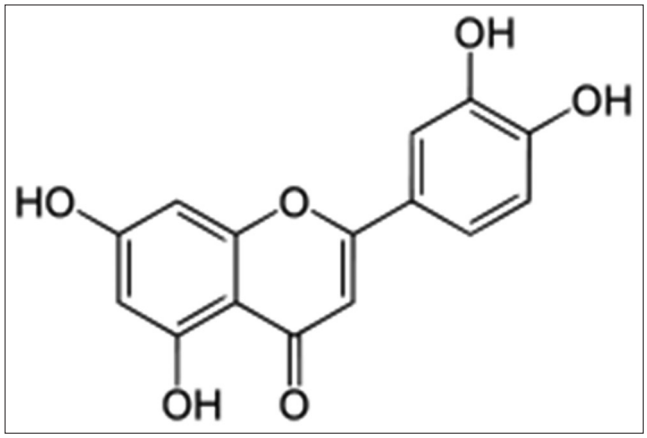

Fig. 1: Molecular structure of luteolin

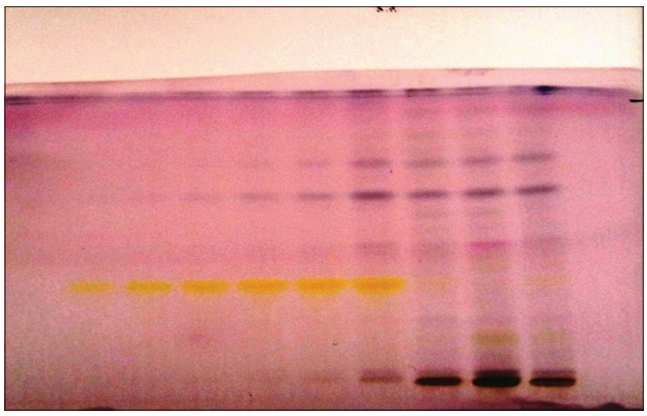

Fig. 2: High-performance thin-layer chromatography profile of luteolin (track 1–6) and methanolic plant samples of *Heteropogon contortus* (racks 7–9); 7 (HCLM), 8 (HCSM), and 9 (HCIM)

Table 1: Recovery study of luteolin by the proposed HPTLC method

Marker compound	Amount present in sample (µg)	Amount added (µg)	Theoretical amount (µg)	Amount found (µg)	Recovery (%)	Average recovery (%)
Luteolin	35	25	60	59.2	98.66	
	35	29	64	65	101.56	99.40
	35	35	70	68.6	98	

HPTLC: High-performance thin-layer chromatography

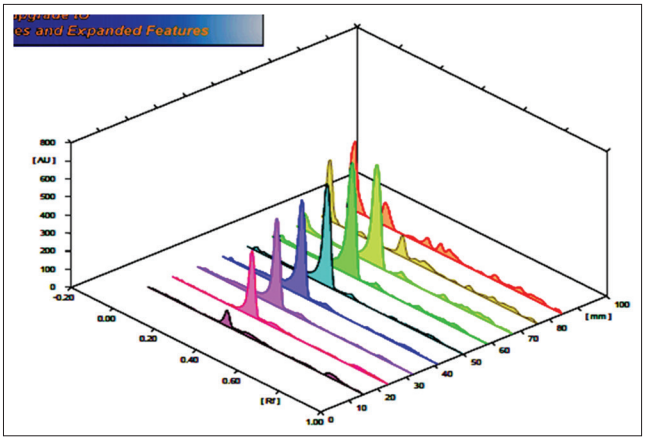

Fig. 3: Densitometric chromatogram of luteolin (track A-F) and plant samples of *Heteropogon contortus* (track G-I) HCLM, HCSM, and HCIM ($\lambda_{max.}$ 366 nm) (3D view)

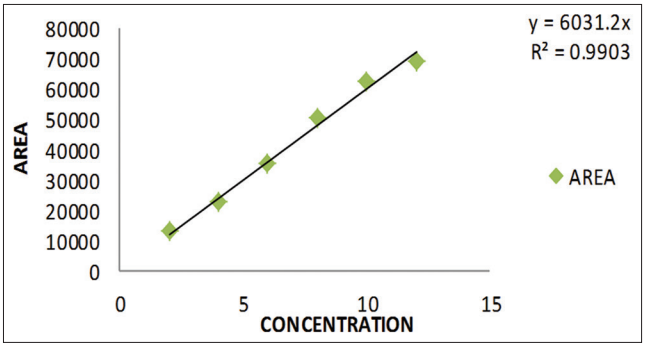

Fig. 4: Calibration curve of luteolin

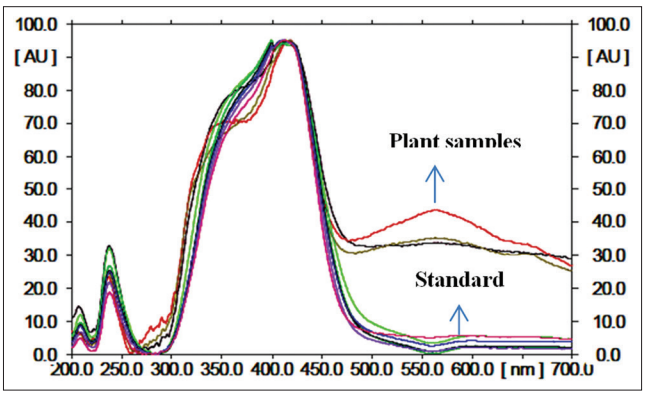

Fig. 5: Absorption spectra of luteolin and plant samples of *Heteropogon contortus* (HCLM, HCSM, and HCIM)

inflorescence of *H. contortus*. The spectrum of luteolin compound with the corresponding plant samples matches exactly. It indicates that there is no interference of the other herbal constituents. A line-to-line overlain spectrum between the standard and the three plant samples is recorded. This shows that the present method is specific (Fig. 5).

DISCUSSION

The developed method has been found to be sensitive, accurate, precise, specific, and robust for the screening and quantification of luteolin. HPTLC is still an effective tool for quality evaluation of medicinal plants, due to its simplicity, low cost, and low requirements. Hence, from the above obtained data, it is clear that *H. contortus* is a good source of flavonoid, i.e., luteolin, and thus the leaves of the species

Table 2: Quantification of luteolin from different plant parts of *H. contortus*

Plant parts	Sample codes	Amount of luteolin in plant sample (% w/w)
Leaves	HCLM	37.13±0.11
Stem	HCSM	0.53±0.014
Inflorescence	HCIM	1.60±0.013

H. contortus: Heteropogon contortus

Table 3: Method validation parameters for the simultaneous quantification of luteolin

Parameters	Luteolin
Wavelength (nm)	366
Rf	0.21
Selectivity	Selective
Specificity	Specific
Linearity range (µg/spot)	2-12
Correlation coefficient (R^2)	0.99
Linear regression equation (y)	6031.2x
LOD (ng/spot)	0.015
LOQ (ng/spot)	0.04
Accuracy (average % recovery)	99.40

LOD: Limit of detection, LOQ: Limit of quantification

can be used in pharmaceutical industries. The flavonoids are known to play modulatory role in almost all neural pathways involved in the pathogenesis of epilepsy. Some of the important flavonoids are hispidulin, rutin, hesperetin, naringenin, eriodictyol, chrysin, gossypin, apigenin, kaempferol, myricetin, quercetin, hoslundin, hoslundal, hoslunddiol, morine, amentoflavone, hyperoside, anghyperoside, epicatechin, rutin, silibin, luteolin, etc [16]. Thus, the present study of *H. contortus* on phytochemical analysis of luteolin exhibits medicinal importance.

CONCLUSION

During the present study, the maximum amount of luteolin is found in leaf extract (36.90 ± 0.11) of *H. contortus* rather than stem and inflorescence. The compound luteolin present in the plant sample is found pure and do not show interference of any other herbal constituents. HPTLC is validated and is the most accurate method for the quantification and identification of luteolin in medicinally important grass *H. contortus*.

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REFERENCES

1. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo state. Plant Sci Res 2009;2:11-13.
2. Joshi C, Savai J, Varghese A, Pandita N. Validated HPTLC fingerprint analysis for simultaneous estimation of Quercetin, Kaempferol and Asiatic acid in leaves of two different chemotypes of *Centella asiatica*. Int Pharma Biol Res 2012;3:202-12.
3. Neuhouser ML. Dietary flavonoids and cancer risk: Evidence from human population studies. Nutr Cancer 2004;50:1-7.
4. Mean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem 2001;49:3106-12.

5. Mencherini T, Picerno P, Scesa C, Aquino R. Triterpene, antioxidant, and antimicrobial compounds from *Melissa officinalis*. J Nat Prod 2007;70:1889-94.
6. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry 2000;55:481-504.
7. Blake JD, Richards GN. Polysaccharides of tropical pasture herbage –I, studies on the distribution of the major polysaccharide components of spear grass (*Heteropogon contortus*) during growth. Aust J Chem 1970;23:2353-60.
8. Beveridce RJ, Dekker RF, Richards GN, Tows M. Identification of myo-isositol, galactikol, and rbffixose in spear grass (*Heteropogon contortus*). Aust J Chem 1972;25:677-8.
9. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi, India: Council of Scientific and Industrial Research; 1956. p. 259-61.
10. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol II. Deharadun, India: International Book Distributors; 1935. p. 1074-6.
11. Ghante MH, Bhusari KP, Duragkar NJ. Evaluation of *Heteropogon contortus* (L.) Beauv. methanolic extract for mast cell, cell membrane and free radical stabilization. Indo Am J Pharma Res 2012;2:1015-26.
12. Ghante MH, Bhusari KP, Duragkar NJ. Bronchorelaxent and anti-inflammatory effect of *Heteropogon contortus* (L.) Beauv. Methanolic extract. Int J Pharm Tech Res 2013;5:99-104.
13. Shambhu N, Dighe V. Development, validation of HPTLC method for simultaneous quantitation of luteolin, apigenin from *Cardiospermum halicacabum* Linn. and *Hydnocarpus pentandra* (Buch-Ham.) Oken. Int J Pharm Pharm Sci 2014;6:408-12.
14. Kaur N, Gupta RC. Determination of sugar biomarkers in different plant parts of *Lasiurus scindicus* henrard (*Poaceae*) through high performance thin layer chromatography. Int J Sci Eng Res 2017;8:1237-49.
15. ICH Q2A. Text on Validation of Analytical Procedures. In: Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva; 1994.
16. Singh P, Singh D, Goel RK. Phytoflavonoids: Antiepileptics for the future. Int J Pharm Pharm Sci 2014;6:51-66.