INTRODUCTION

Flavonoids are a diverse group of polyhydroxy compounds found in almost all fruits (apples and berries), onion, tea, vegetables, and some beverages. Quercetin and kaempferol are the best-known flavonoids. Nature's 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, hematomatological 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 pathologic 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 x 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 wavelength.

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)

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

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

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 (5:5:0.7 v/v) was used as a solvent system using silica gel 60 F254 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 $y=603.12x$ (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 inflorescence (Table 2).

### 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        | 99.40                |
|                 | 35                           | 29                | 64                     | 65                | 101.56       |                      |
|                 | 35                           | 35                | 70                     | 68.6              | 98           |                      |

HPTLC: High-performance thin-layer chromatography.
DISCUSSION

The developed method has been found to be sensitive, accurate, precise, 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 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, holusindol, holusindal, angkhyperoside, hyperside, 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*.

ACKNOWLEDGMENT

The authors would like to express their profound gratitude and sincere appreciation to UGC-BSR Single Girl Child Fellowship (Award letter no. and dated. F7-152/2007 BSR; 16-12-2013) and DBT-IPLS project (Project no. BT/PR-4548/INF/22/146/2012) sanctioned to Punjabi University, Patiala, for using the facilities and financial support for this study. The authors are also thankful to Head, Department of Botany, Punjabi University, Patiala.

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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 (R²)   | 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
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