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Phyto-Analytical Evaluation of Rutin in Viola odorata L., (Banafshan)-An Expectorant

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Abstract:

Viola odorata L., commonly known as banafshan, is widely distributed in Pakistan. Medicinally it used as expectorant. In present study a rapid and inexpensive qualification method for the quality control of Viola odorata on thin layer chromatography (TLC) was developed and validated on silica gel using solvent ethyl acetate–glacial acetic acid–formic acid-water (10 : 1.1 : 1.1 : 2.6 v/v)/v/v) (Rf of silybin 0.48 ± 0.05) in the absorbance mode at 254 nm. The method showed a good linear relationship (r2 = 0.999) in the concentration range 25–1500 ng per spot. It was found to be linear, accurate, precise, specifi c, robust and stability-indicating and can be applied for quality control and standardization. Also occurrence of rutin was figured out in different parts of plant. It was found that corolla of Viola odorata flower contain rutin in much higher concentration than calyx, while calyx have more rutin content than dried leaves, which in turn contain higher content of analyte than fresh leaves (figure-1&2).

Key words: Identification, *Viola odorata*, Thin layer Chromatography

INTRODUCTION

Viola odorata or sweet violet is native to many regions of Africa, Asia-temperate, Asia-tropical and Europe. It is also found in Pakistan and known as Banafshan. Frequently cultivated in gardens as medicinal as well as ornamental plant[1,3]. It has expectorant and anti-tussive activity. With respect to Habitat it is Acaulescent, annual to perennial prostrate plant. Its rhizome is long, pointed, branched, slender, giving rise to a stoloniferous stem. Leaves are 2.0-3.5 x 1.5-3.0 cm, broadly ovate, obtuse, pubescent, cordate, serrate, 5-7-veined, sinus deep, petiole covered with deflexed hairs, often twice or thrice the length of lamina, upto 15 cm long; stipules free, broad lanceolate to ovate, acuminte, fringed or fimbriate. Flowers are odorous, violet, upto 2.0 cm long on upto 20 cm long peduncle. Sepals are 4.0-7.0 x 1.0-3.0 mm, ovate, obtuse, entire, cuneate. Petals are obovate-oblong, obtuse, entire, up to 12 mm long, c. 5.0 mm broad; lateral petal 11-16 x 7.0 mm, large, oblong, obtuse, entire, spur straight or subcurvate, obtuse, c. 4 mm long and 5-8 mm dry fruit splits open when ripe [1,2].

The literature survey shows the presence of rutin in viola species. Many fruits, vegetables and medicinal plants contain rutin like Citrus fruits [6], Rue [5,

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11], Buckwheat leaf flour [7, 11] and seeds [8], Fennel [9], Sage [10], Thyme [10, 11], Flowers of rose [11] etc. Rutin with molecular formula C27H30O16 .3H20 [4] is a chemical marker of Viola odorata and is also known as Vitamin P [4], Rutoside[4], and Quercetin-3-O-rutinoside[5].

E. Sofic et al. 2010 performed quantitative analysis of rutin in leaves and flowers of 50 medicinal plants by using HPLC-ED system and results showed highest concentration in leaves of rue (86.0 mg/gm) followed by flowers of buckwheat (53.5mg/gm) and one of viola species i.e., Viola tricolor L. (33.6mg/gm) [11].

For the evaluation of rutin content in whole herb and its different parts a simple and fast method was developed in this study.

MATERIAL AND METHODS

Reference standard of Rutin was purchased from Roth, Germany. All reagent were of analytical grade. Plant material were provided by PARC Agrotech Company (Pvt) Ltd.

Experimental Work

The samples were spotted as bands of 6 mm width with a Camag microliter syringe on a precoated silica gel aluminium sheet 60F-254 (20 × 10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat V applicator (Switzerland). The application rate of 150 nL/s was constant throughout the study and a space of 14 mm was kept between the two bands. The slit dimension was kept at 5 × 0.45 mm, and the scanning speed was 20 mm/s. Ethyl acetate–glacial acetic acid–formic acid-water (10 : 1.1 : 1.1 : 2.6 v/v) was used as the solvent system. Linear ascending development was carried out in twin trough glass chamber (20 × 10 cm) saturated with the mobile phase for 30 min at room temperature. The solvent front was run up to 80 mm. Subsequent to the development, TLC plates were dried with the help of an air-dryer. Densitometric scanning was done by using TLC scanner III (CAMAG, Switzerland) in the absorbance mode at 254 nm. Deuterium lamp was the source of radiation utilized. The content of rutin in different parts of herb was determined by comparing the area of the chromatogram of samples with standard. For peak responses see figure 3, 4, 5, 6, 7, & 8).

Figure-3: Peak responses of Rutin STD

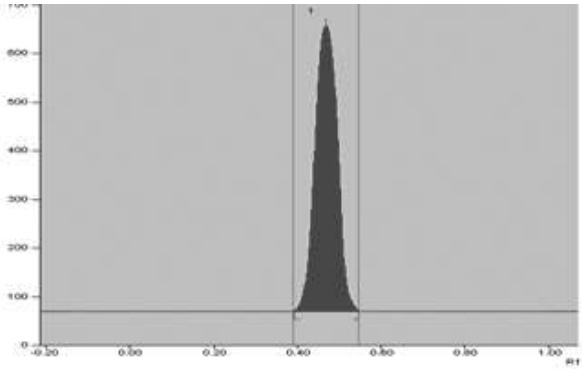


Figure-4: Peak responses of rutin in Calyx of Viola odorata

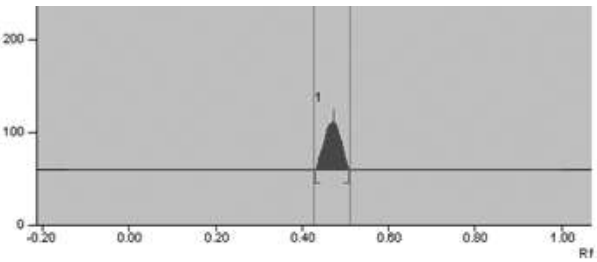


Figure-5: Rutin in Corolla of Viola odorata

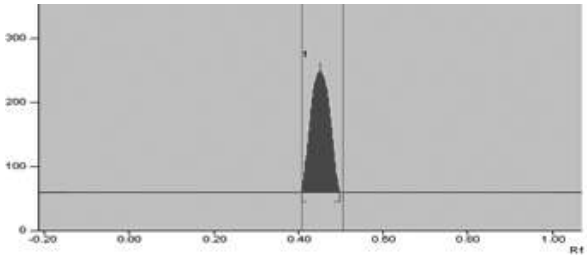


Figure-6: Peak responses of rutin in dry leaves of Viola odorata

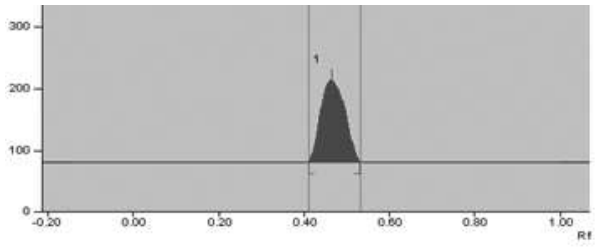


Figure-7: Peak responses of rutin in Fresh leaves of Viola odorata

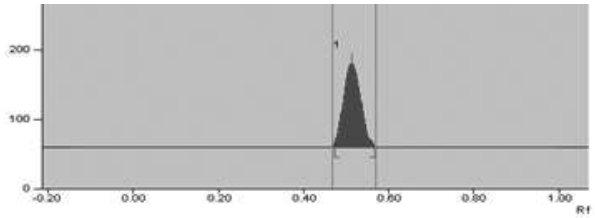
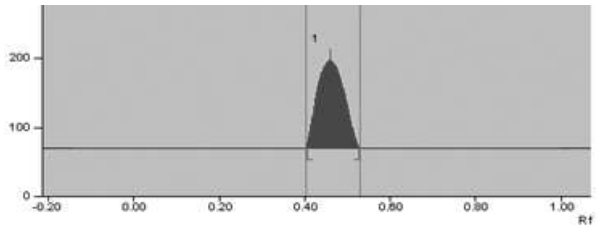


Figure-7: Peak responses of rutin in whole herb of Viola odorata



RESULTS AND DISCUSSION

Development of the Optimum mobile phase

TLC procedure was optimized to develop an accurate method. Initially, Ethyl acetate–glacial acetic acid–water in varying ratio and combinations were tried and the mobile phase with Ethyl acetate–glacial acetic acid-water (10.0 :1.1 : 2.6 v/v) gave good resolution for the separation of rutin. Addition of formic acid reduced the tailing of the peak and, finally, the solvent system consisting of Ethyl acetate–glacial acetic acid–formic acid-water (10.0 : 1.1 : 1.1 : 2.6 v/v) was found to give compact spots for rutin in diff erent concentration levels at an Rf value of 0.48 ± 0.05. Densitometric analysis of rutin was carried out in the absorbance mode at 254 nm ?max (Fig. 1 & 2).

Figure-1: TLC image of Rutin analysis of Corolla

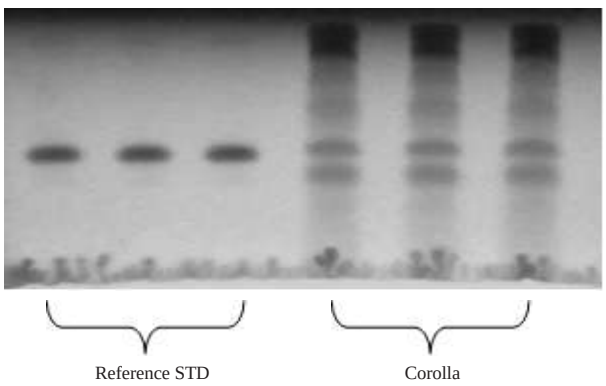
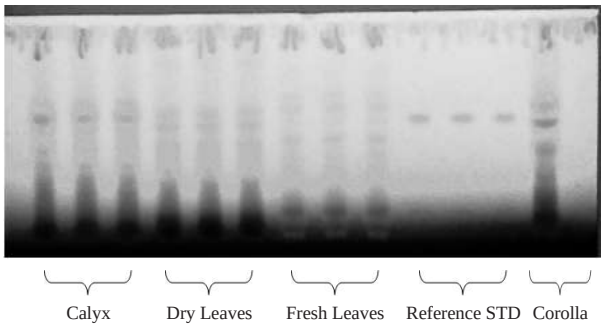


Figure-2: TLC image of Rutin analysis of Calyx, Dry Leaves, Fresh Leaves, Corolla



Calibration Curve

The linear regression analysis data for the calibration plots (n = 3) in the concentration range of 25–1500 ng per spot, showed a good linear relationship (r2 = 0.999 ± 0.002) with respect to peak area. The mean values of slope and intercept were 6.5768 ± 0.1452 and 314.7 ± 4.256, respectively. There was no significant difference in the slopes (n = 3) of calibration curves ANOVA, $p > 0.05$), indicating linearity of the slope. The regression equation thus obtained from calibration plots, $y = 6.5768x + 314.7$ (where y = area obtained, x = concentration in ng/spot), was used for quantitative estimation of rutin in different samples.

Validation of the Method

The method proposed was validated using precision, accuracy as recovery, robustness, LOD, LOQ and specificity parameters as per the ICH guidelines [12].

Precision

The intra- and inter-day precision of rutin was obtained at three diff erent concentrations of 200, 500 and 1000 ng/spot. The %RSD was found in the range of 0.30–1.51 and 0.58–2.04% for repeatability and reproducibility, respectively, indicating good precision of the method proposed.

Recovery studies

When this method was used for extraction and consequent estimation of rutin from formulation after spiking with 50, 100 and 150% of additional drug, the mean recovery was 100.05%. The % RSD

values after spiking with 50, 100 and 150% of additional drug were found in the range of 0.85–1.26.

CONCLUSION

Statistical analysis has proved that the HPTLC method developed in this study is accurate, precise, robust, reproducible, specific and stability-indicating for the determination of rutin. The proposed, developed and validated HPTLC method was applied for quantitative estimation of rutin in different part of the plant and it was found that corolla of sweet violet flower have higher rutin content than all other parts . This method could also be used to estimate rutin content in different formulation of viola odorata as well. The method proposed was found to be more easy, economic and less time-consuming as compared with the reported HPLC and LCMS methods with applicable in general laboratory conditions. The method is also well validated, sensitive, accurate, specific, and precise as compared with the reported TLC–photodensitometric method as well.

REFERENCES

1. Flora of Pakistan page number 4. URL: http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=200014395

2. English violet. CT, MA, ME, RI. Fields, roadsides, waste areas, lawns, areas of cultivation. Reports of this species in VT are based on misidentified collections URL: https://gobotany.newenglandwild.org/species/viola/odorata/

3. USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - (GRIN) [Online Database]. National Germplasm Resources Laboratory, Beltsville, Maryland. URL: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?41733 (16 April 2014)

4. British Pharmacopoeia 2004 Addendum (electronic)

5. Sofic E, Copra-Janicijevic A, Salihovic M, Tahirovic I, Kroyer G, Screening of medicinal plant extracts for quercetin-3-rutinoside (rutin) in Bosnia and Herzegovina., Medicinal Plants - International Journal of Phytomedicines and Related Industries, 2(2), 97- 102 (2010).

6. Bilbao, Andre´s-Lacueva C, Ja´uregui O, Maria R, Lamuela-Ravento´ s, Maria de Lourdes M, Determination of flavonoids in a Citrus fruit extract by LC–DAD and LC–MS, Food Chemistry, 101, 1742–1747 (2007).

7. Kreft I, Fabjan N, Yasumoto K, Rutin content in buckwheat (Fagopyrum esculentum Moench) food materials and products, Food Research International, 98(3), 508–512 (2006).

8. Jiang P, Burczynski F, Campbell C, Pierce G, Austria J. A, Briggs C. J, Rutin and flavonoid contents in three buckwheat species Fagopyrum esculentum, F. tataricum, and F. homotropicum and their protective effects against lipid peroxidation. Food Research International, 40(3), 356–364 (2007).

9. Abdul-Jalil T Z. Screening Of Rutin From Seeds And Leaves Extracts Of Dill, Coriander And Fennel Cultivated In Iraq. Pharmacie Globale (IJCP), 03 (02) (2013).

10. Huseinovic S, Salihovic M, Topcagic A, Kalcher K, Cavar S, Sofic E, Screening of sage and thyme plant extracts for some phenolic acids and rutin.. Planta Med, 76, 294 (2010).

11. Sofic E, Salihovic M, Huseinovic S, Cavar S, Copra-Janicijevic A, Kalher K, Screening of 50 medicinal plant extracts for quercetin-3-rutinoside. Planta Med, 76, 291 (2010).

12. ICH guidelines Q2A, Text on Validation of Analytical Procedure, international conference of harmonization, Geneva, 1-5 (1994).