

HPTLC Finger Printing of *Solanum nigrum* L. Variants Black and Orange Fruits

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Abstract

A simple and rapid HPTLC analysis was developed for the quantitative determination of diosgenin and solasodine from hydroethanolic extract of *Solanum nigrum* L. The two variants of *Solanum nigrum* - black and orange fruits - are used in various parts of the world as food and medicine drastically. These two variants showed distinctive features in morphology and anatomy. To probe the phytoconstituent variation among these two variants, HPTLC analysis was done and the results of present HPTLC analysis showed important differences in peak values. So this study may provide scientific validation on spectral characteristics of two variants of *Solanum nigrum* L. with black and orange fruits.

Keywords: Diosgenin, HPTLC, Hydroethanolic Extract, *Solanum nigrum*, Solanaceae, Solasodine

1. Introduction

Solanum nigrum L. is herbaceous annual plant which belongs to the family Solanaceae. The plants are usually 10 to 15 cm long with a tender green smooth stem. It is widely found in river bund, wet wood, waste land, quagmire, old field, road side and in wet cultivated land. Our field observations and in earlier work of (Dhasmana *et al.*, 2007)¹ revealed the presence of variations among *Solanum nigrum* populations where three variants viz. Black, Orange and Yellow fruits were observed. In Tamil Nadu and Pondicherry, black and orange fruit variants were observed dominantly. Both variants have been used as green for treating diseases like ulcer (mouth and stomach) under a vernacular name Manathakkali or Milakuthakkali. This can be used as a basic food and medicine without prejudice.

It is used in fever, hepatitis, stomach complaints and dysentery. The plant juice is administered for ulcer

and other skin diseases. The fruits are used as stimulant, appetite laxative, for treating asthma and excessive thirst. Leaves of this plant are effectively taken to treat mouth ulcer during winter periods. It is used in cooking like spinach. Leaf and berry decoctions are used to cure liver related problems including jaundice. It is widely used in oriental medicine because it is utilized to be antioxidant, antitumorogenic, anti-inflammatory, diuretic, hepatoprotective and antipyretic activity. Nyeem *et al.*² also confirmed use of this plant for cervical carcinoma through Chinese experiments.

This genus *Solanum* is medicinally important for the presence of steroidal alkaloids mainly solasodine and other related glycosides³. Eltayeb *et al.*⁴, quantitatively estimated the content of solasonin and solamargin through an optimized isolation method (HPTLC) in various part of *Solanum incanum* plants at different stages of development. The steroidal glycoalkaloids (solasonine) and aglycone (solasodine) from *Solanum nigrum*

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through HPLC and GC-MS analysis were analyzed and 5.85 mg/g of solasodine and the aglycone solasodine with significantly higher amount (75.94%) was reported by Gheewalaa *et al.*⁵.

With the above information, the present work has been initiated with a view to establish pharmacopoeial standards for *Solanum nigrum* L variants whole plants. The main object of this study is to evaluate quality, safety and efficacy of the whole plants of the two variants.

2. Materials and Methods

2.1 Plant Material

In the present investigation the whole plants of *Solanum nigrum* L variants black and orange fruit (Plate – 1 and 2) were selected and collected during same season and time (March – April 2021) from Thirumalairayan Pattinam, 609 606, Karaikal Region, Pondicherry, Union Territory of South India.

2.2 Plant Identification

Floras like Gamble⁶; Matthew⁷; Nair and Henry⁸; Anonymous⁹; Chatterjee¹⁰; Kirtikar and Basu¹¹; the collected plant specimens were identified and confirmed with the help of type specimens available in the Herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu. In addition, as per the method of Jain and Rao 1976¹²; the herbarium were prepared and deposited in Tamil University Herbarium TUH-300(A) – *Solanum nigrum* L. (black fruit). TUH-300(B) – *Solanum nigrum* L. (orange fruit).

2.2 Preparation of Extract

The hydroethanolic extract was prepared using 70% ethanol. The whole plants were dehydrated in an oven at 40°C, ground and macerated in ethanol for 72 hrs. This was filtered and condensed. The collected extracts were used for further spectral studies.

2.3 High Performance Thin Layer Chromatography¹³

On a 10×10 cm pre-activated HPTLC silica gel 60 F₂₅₄ plates were used for Chromatography analysis. Samples were loaded to the plate 6 mm width band with an automatic TLC applicator Linomat 5 with N₂ flow (CAMAG, Switzerland), 8 mm from the bottom. Densitometry scanning was performed on CAMAG

Scanner III. Methanol was used for prewashing of plates and was activated for 5 minutes × 0.45 minutes at 60°C and scanning speed was 20 minutes. Different mobile phases of varying polarities were allowed and 10 ml of mobile phase was chosen. Lean ear ascending development was carried out in twin glass chamber with saturated mobile phase.

The hydroethanolic extracts 1 mg/ml in alcohol was prepared and loaded with the help of CAMAG Linomat 5 applicator. It was optimized by selecting correct mobile phase respectively and plant extracts were also developed in a twin trough chamber, 20 × 10 cm at 25°C and allowed to dry. Mobile phase as Toluene:Ethyl Acetate 6:4 were used. The experiment was carried out in CAMAG-HPTLC instrument under ideal conditions. The spots were detected under ultraviolet chamber at 433 nm and scanned in CAMAG-HPTLC scanner.

3. Results

HPTLC profiles of hydroethanolic extracts were evaluated for Diosgenin and Solasodine. Diosgenin and solasodine were present in hydroethanolic extracts. Hydroethanolic extracts of variants black and orange fruit were chromatographed (Figures 1-6). The chromatograph of black fruit extract for evaluating diosgenin showed spots at Rf 0.58, 0.60 and 0.62 and orange fruit showed spots at Rf 0.57, 0.59 and 0.62 respectively. HPTLC fingerprint of hydroethanolic extract of black fruit showed three peaks. The Rf value 0.62 has maximum (1726.3) peak area in black fruit and that of orange fruit showed maximum peak area (2185.8) at Rf value of 0.62.

The chromatograph of variant black fruit extract evaluating solasodine showed spots at Rf 0.33, 0.36 and 0.40 and the orange fruit extract showed at Rf 0.34, 0.37 and 0.41 respectively (Figures 7 and 8). HPTLC fingerprint of hydroethanolic extract of black fruit showed three peaks. The Rf value 0.40 showed maximum (950.0) peak area (Figure 7) whereas orange fruit plant extracts showed 3 peaks. The peak corresponding to the Rf (0.41) value showed maximum (641.9) peak area (Figure 8).

4. Discussion

Solanum nigrum is one of the most popular and important medicinal plants that have been used traditionally for the treatment of various diseases (Abbas *et al.*, 2014)¹⁴.

HPTLC profile of hydroalcoholic extracts were evaluated for Diosgenin and Solasodine. They were present in hydroethanolic extracts of two variants. (Figures 1-6). Chromatographic analysis is a valid, sensible and practicable method for determination of quantity and authentication of medicinal plants^{15,16,17}. The important feature of the HPTLC was image coupled with the digital scanning profile is an attractive and most useful tool for construction chromatographic fingerprints. HPTLC profile of hydroethanolic extracts revealed the presence of diosgenin and solasodine with reference to standards.

The samples were confirmed and identified by comparing the Rf value of standards with extracts. This is the first comparative report for quantification of diosgenin and solasodine in two variants by HPTLC.

Fingerprint of hydroethanolic extract of orange fruit plant extracts shows 3 peaks. The Rf value 0.40 has maximum 950.0 peak area (Figure 7) compared with the reference studies could speculate that with be one of the following compounds diosgenin and solasodine Eltayeb *et al*⁴. In HPTLC analysis the variants showed much similar profile as the variants belong to the species *nigrum*. To explore the chemotaxonomic comparison the quantitative analysis is essential. Chemical profiles of hydroethanolic extracts of both variants revealed common phytoconstituents like solasonine and solamargin. These chemical profiles could be used for identifying the variants⁴. Comparative studies on alkaloidal profile of delimited species in 5 locally available taxa of *Solanum nigrum* complex highlighted the boundaries among close taxonomic variants. Glycoalkaloids (Solasonine, α -Solamargine, β -Solamargine and α -Solanine) and their aglycones (solasodine and solanidine)¹⁸. Analysis persists to be valuable tool to resolve the international taxonomic controversy on morphological characters. The results of our present finding were with search outcomes of Jeyasree *et al*¹².

5. Conclusion

The *solanum nigrum* variant finds enormous medicinal usage in various parts of the world and medicine drastically. Variants showed distinctive features in morphological and anatomical characters. To probe phytoconstituent variation among these two variant, HPTLC analyses was done and the results showed important differences in peak values. It would be very important for the field of phytochemistry

researchers to explore and investigate more in these varieties of *Solanum nigrum* L black and orange fruits. It should give attention of the pharmaceutical community to identify the exact drug and other medicinal purposes

6. References

1. Dhasmana M, Simon L, Narayanaswamy P, Rathore RKS, Sreeramu BS. Characterization of *Solanum nigrum* L. genotypes by morphological and RAPD markers. *Med Aro Plant Sci Biotech*. 2007; 1(2):257–62.
2. Nyeem MAB, Rashid AKMMU, Nowrose M, Hossain Md A. *Solanum nigrum* (Maku): A review of pharmacological activities and clinical effects. *Int J Appl Res*. 2007; 3(1):12–7.
3. Giulietti AM. *Solanum elaeagnifolium* Cav. In vitro culture and production of solasodine. *Biotechnology in Agriculture, Forestry, Medicinal and Aromatic Plants* [Bajaj, Y.P.S. (ed.)], Germany, Berlin: Springer Verlag, 1991; 15:432–50. https://doi.org/10.1007/978-3-642-84071-5_26
4. Eltayeb EA, Al-Sinani SS, Khan IA. Determination of the glycoalkaloids solanine and chacorine levels in 18 varieties of potato (*Solanum tuberosum* L.) grown in Oman. *Potato Research*. 2003; 46: 57. <https://doi.org/10.1007/BF02736103>
5. Gheewala NK, Saralaya MG, Sonara,GB, Gheewala TN. Phytochemical evaluation of total glycoalkaloid of dried fruit of *Solanum nigrum* Linn. *Curr Pharm Res*. 2013; 3(4):1010–3. <https://doi.org/10.33786/JCPR.2013.v03i04.005>
6. Gamble JS. *Flora of Presidency of Madras*. Vol.3. Culcutta, India: Botanical Survey of India, 1957. p. 816.
7. Matthew KM. *The Flora of Tamil Nadu Carnatic*, Vol. III. The Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. 1983.
8. Nair NC, Henry AN. *Flora of Tamil Nadu, India, Series I, Vol. I*. Coimbatore, India: Botanical Survey of India, Southern Circle. 1983.
9. Anonymous. *The Wealth of India*. Vol. I. New Delhi: CSIR, 1992; 56–7.
10. Chatterjee A, Pakrashi SK. *The Treatise on Indian Medicinal Plants*, Publication and Information Directorate, New Delhi. 1994; 1:70–5.
11. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. India: Dehra Dun. B.S.M.P. Singh Publishing Co. 1935; 3:1762.
12. Jeyasree J, Jenifer S, Priya S, Sukumaran V, Kezia Laveena D. HPLC spectral analysis of phytochemicals

- in *Solanum nigrum* L. and Target protein identification. *World J Pharm Pharmaceu Sci.* 2014; 3(12):1182–92.
13. Sethi PD. Introduction. HPTLC - High Performance Thin Layer Chromatography, 1st ed. New Delhi: CBS Publishers and Distributors; 1996.
 14. Abbas K, Niaz U, Hussain T, Saeed MA, Javaid Z, Idrees A, Rasool S. Antimicrobial activity of fruits of *Solanum nigrum* and *Solanum xanthocarpum*. *Acta Pol Pharm.* 2014; 71(3):415–21.
 15. Qiao C, Han Q, Song J, Mo S, Kong L. Chemical fingerprint and quantitative analysis of *Fructus psoraleae* by high-performance liquid chromatography. *Journal of Separation Science.* 2007; 30:813–8. <https://doi.org/10.1002/jssc.200600339>. PMID: 17536725.
 16. Lu H, Liang Y, Chen S. Identification and quality assessment of *Houttuyniacor* data injection using GC-MS fingerprint: A standardization approach. *Journal of Ethno Pharmacology.* 2006; 105:436–40. <https://doi.org/10.1016/j.jep.2005.11.018>. PMID: 16384679.
 17. Li K, Wang S. Fingerprint chromatogram analysis of extracts from the leaves of *Tripterygium wilfordii* Hook. F. by High Performance Liquid Chromatography. *Journal of Separation Science.* 2005; 28:653–7. <https://doi.org/10.1002/jssc.200400106>. PMID: 15912735.
 18. Tlkunove YM, Khrustaleva LI, Karlov G. Application of ISSR markers in the genus *Lycopersicon*. *Euphytica*, 2003; 131(1):71–81. <https://doi.org/10.1023/A:1023090318492>