Simple Method for Standardization and Quantification of Linoleic Acid in Solanum nigrum Berries by HPTLC

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Abstract
Linoleic acid is a polyunsaturated omega-6 fatty acid found as phytoconstituent in many plant species. It is reported to have activity such as 5-alpha-reductase-inhibitor, antianaphylactic, antiarthritic, antiatherosclerotic, anticancer, anticoagulant, antieczemic, antibifibrinolytic and many others. In this research work we have developed a method by HPTLC to identify and standardize linoleic acid in methanolic extract of Solanum nigrum berries. Calibration curve of Linoleic acid was plotted and was found to have r2 value of 0.99451. The percentage content of Linoleic acid in Solanum nigrum berries methanolic extract was found to be 9.32% w/w.

Keywords: Linoleic acid; Solanum nigrum; Finger print; HPTLC

Abbreviations: HPTLC: High Performance Thin Layer Chromatography; µg: Microgram; mL: Millilitre; Rf: Retention Factor; nm: Nanometer; µL: Microlitre; RSD: Relative Standard Deviation; TLC: Thin Layer Chromatography; ºC: Degree Centigrade.

Introduction
Solanum nigrum (black nightshade) is a medicinal plant member belonging to the family Solanaceae. S. nigrum has been extensively used traditionally to treat various ailments. The juice of the berries used as an antiarrhetic, ophthalmopathy and hydrophobia. It is also used in anasarca and heart disease. Berries are reported to possess tonic, diuretic and cathartic properties. Seeds are useful in dippisa and giddiness [1].

Linoleic acid is a polyunsaturated omega-6 fatty acid. It is reported to have a number of useful physiological activities including 5-alpha-reductase-inhibitor, antianaphylactic, antiarthritic, antiatherosclerotic, anticoagulant, antieczemic, antibifibrinolytic, antigranular, anti-inflammatory, antimicrobial, antiprostatitic, hepatoprotective, hypcholesteremic, immunomodulatory, insecticidal, metastatic, nematicide [2]. Solanum nigrum oil is reported to have linoleic acid as the most abundant unsaturated fatty acid found in it [3,4].

For the establishment of a consistent biological activity of any herbal item, standardization is an important step. It provides a consistent chemical profile, or simply a quality assurance program for production and manufacturing of an herbal drug. Standardization thus, is a tool in the quality control process. HPTLC is a modern adaptation of Thin Layer Chromatography with better and advanced separation efficiency and detection limits. HPTLC provides a number of advantages including easy separation process for with colored compounds. HPTLC can be used for different modes of evaluation, allowing identification of compounds having different light-absorption [5]. HPTLC is a method to standardize and identify the chemical ingredients which is expected to be present in a medicinal plant. This is done from regulatory perspective to ensure the efficacy, quality as well safety of the herbal drugs present in a plant. Thus it provides a very reliable way of determining the purity and percentage content of the active biomarker in the plant extracts.

Standardization of herbal products is a current issue of interest. For quality control of these herbal materials or their extracts one needs to proceed by selecting one of the different phytoconstituents of the product, preferably the one showing maximum desired bioactivity and subsequent method of quantification of that specific constituent is required to be developed. The method so accepted should be simple and cost effective. Previously mentioned standardization methods of Solanum nigrum berries states use of gas chromatography, high performance liquid chromatography which by quantifying linoleic acid or other phytoconstituents [6,7]. But these methods are expensive. Standardization and quantification of linoleic acid in Solanum nigrum berries by HPTLC for quality control of this herbal material is an effective and simple method and may be used with necessary modifications for estimation of the afore said phytoconstituent in other plant materials containing the same.

Materials and Methods

Instrumentation and reagents
The CAMAG HPTLC system used comprised of WINCATS software, LINOMAT V automatic sample applicator, and automatic development chamber, scanning densitometer CAMAG scanner 3 and photo documentation apparatus CAMAG reprostar-3. Stationary phase was used as aluminum based silica gel plate 60 F254 (Merck, Mumbai) with 10 cm × 10 cm in a particle size of 5-10 µm. All the solvents were used of analytical grade. 100 µL syringe (Hamilton, Switzerland) was used for sample application on HPTLC plates. Linoleic acid was purchased from Sisco Research Laboratories (SRL). Methanol, n-hexane and ethyl acetate (analytical grade) were procured from Merck (Mumbai, India).

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All the samples were filtered through Whatman's syringe filter (NYL 0.45 μm). The plant material was collected from local area of Kolkata, West Bengal, India and the identity was confirmed by taxonomist. The shade dried powdered berries of Solanum nigrum were extracted with methanol by cold maceration. The extract solution was dried under reduced pressure with the help of rotary evaporator.

**Methods**

**Preparation of standard solution:** About 1 mg of linoleic acid standard was taken in 1 mL eppendorf tube 1.0 mL of methanol was added to it and mixed in vortex mixture till the material got completely dissolved. It was then filtered through 0.45 μ syringe filter and kept for further study.

**Preparation of calibration curve of linoleic acid:** HPTLC analysis was performed using isocratic technique. The mobile phase was optimized with n-hexane and ethyl acetate in a ratio of 5:4 v/v. The temperature was kept at 25°C and mobile phase was developed in a twin trough glass chamber. The standard solution was applied 2, 4, 6, 8, 10 μL in a band wise fashion. After development the plate was dried. Then the dry plate was treated by spraying sulphuric acid-anisaldehyde reagent. The plate was kept at 110°C for 5 minute in hot air oven and evaluation was carried out at 366 and 540 nm. Calibration curve of linoleic acid was obtained by plotting peak areas versus concentrations of linoleic acid applied.

**Identification and quantification of linoleic acid in Solanum nigrum berries**

**Preparation of sample solution:** About 5 mg of Solanum nigrum berries methanolic extract was dissolved in 1 mL methanol in an eppendorf tubes. It was then mixed in vortex mixture and subjected to ultrasonication bath till the material completely dissolved. Then it was filtered through 0.45 μ syringe filter. Sample solution was applied consequently in the range of 4, 8 and 12 μL and was subjected to a fore mentioned chromatography.

**Evaluation of method by some parameters**

- **Instrumental precision:** Instrumental precision was checked by repeated scanning (n=5) of the same spot of linoleic acid (2 μg/spot) and expressed as relative standard deviation (%RSD).

- **Repeatability:** The repeatability of the method was affirmed by analyzing 2 μg/spot of linoleic acid TLC plate (n=5) and expressed as %RSD.

- **Recovery:** The accuracy of the method was assessed by performing recovery study at three different levels (50%, 100% and 125% addition of linoleic acid). The percent recoveries and the average percent recoveries were calculated.

**Results**

**Calibration curve of linoleic acid**

The calibration curve was found to be linear with the equation of Y=3141.508 × X+1366.840 (correlation coefficient=0.9954), where X represents amount of linoleic acid and Y represents area under the curve. R, value of standard linoleic acid was found to be 0.65.

**Quantification of linoleic acid in Solanum nigrum berries**

The percentage content of linoleic acid in Solanum nigrum berries methanolic extract was found to be 9.32% w/w. R, value of standard linoleic acid was found to be 0.65. Specificity was confirmed by comparing the R, of standard and sample. Figures 1 and 2 represents HPTLC Chromatogram of standard Linoleic acid and that of extract of Solanum nigrum berries. Photodocumentation of Solanum nigrum berries methanolic extract at 366 nm and 540 nm are shown in Figures 3 and 4. Figure 5 represents all tracks at 540 nm having 5 standard and three samples respectively from left hand side.

**Discussion**

Chromatographic fingerprint analysis has proven to be a rational and feasible approach for the assessment of quality and authentication of species of traditional medicine [8-10]. It effectively uses chromatographic techniques to form specific patterns of recognition for phytochemicals. The developed fingerprint pattern of components can thereafter be utilized to check the presence of markers of interest as well as the ratio of all detectable analytes. Though there are some shortcomings of high performance thin layer chromatography, such as...
the limited developing distance and lower plate efficiency as compared to high performance liquid chromatography and gas chromatography, still it is an effective tool for evaluation of herbal drugs due to its simplicity. Moreover, the formerly mentioned limitations can be curbed by separately developing fractions of different polarity on two or several thin layer plates. The unique feature of the picture like image of HPTLC coupled with the digital scanning profile is an attractive and useful tool for construction of herbal chromatographic fingerprint. The linoleic acid in sample of extract was identified and its presence was confirmed by comparing the Rf value of standard linoleic acid to that of the extract. This method is reproducible and has shown satisfactory results on precision, accuracy and recovery study data. There is no report of detection and quantification of linoleic acid in Solanum nigrum berries by HPTLC. Hence, we developed a simple and precise method for quantification of this marker.

Conclusion
The developed method provides a simple, precise and accurate analytical method for the identification and quantification of linoleic acid in berries of Solanum nigrum. Quick and easier process for sample preparation, high method sensitivity and reproducible results by the mobile phase are the important features of the developed method. It can be expected that this HPTLC technique may be applied successfully for evaluation of linoleic acid as marker in different herbal extracts.

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