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Quantitative determination of Gallic Acid in Hydroalcoholic extract of flowers of Nymphaea Stellata using HPTLC Technique

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Abstract

This method has been developed for quantitative determination of gallic acid in the isolated, dried flowers of Nymphaea Stellata. Hydroalcoholic extract of N. Stellata was used on silica gel 60 F_{254} plates with Toluene: ethyl acetate: formic acid, 8.7:7:0.7 (v/v/v), as mobile phase. Quantitation and detection were performed by densitrometric scanning. The proposed method provide an enough resolution of gallic acid from other constituents within hydroalcoholic extract used in this study.

Key word: Gallic acid, HPTLC, Nymphaea Stellata.

Nymphaea Stellata is commonly known as Indian blue, water lily often referred as, blue lotus of Indian, but it is not a lotus'. In Ayurveda for the highest heat, fever and pitta conditions bitter, fire purging and heat dispelling herbs are used, that is also bitter, as Nymphaea Stellata is bitter this may also increase agni (fire) by their drying action in addition they do not aggregate pitta. It is bitter in taste, due to the presence of chemical constituents like alkaloids, Monoterpenes, Sesquiterpene, Lactones, diterpenes, Triterpenes and rarely floceanones, acyl phloroglucides, and steroids[1-3]. The plant also has antihepatotoxic antidiabetic[4,5] and antihyperlipidaemic activities.

The basic theme of this proposed work is to establish phytochemical evaluation of this herbal drugs isolated from Nymphaea Stellata using densitrometric HPTLC method.

Materials

The flowers of Nymphaea Stellata has been collected from Khandwa Road (Simroal) Indore and were authenticated by the Botanist Dr. O.P. Joshi, P.M.B. Gujarati Science College, Indore.

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Methods

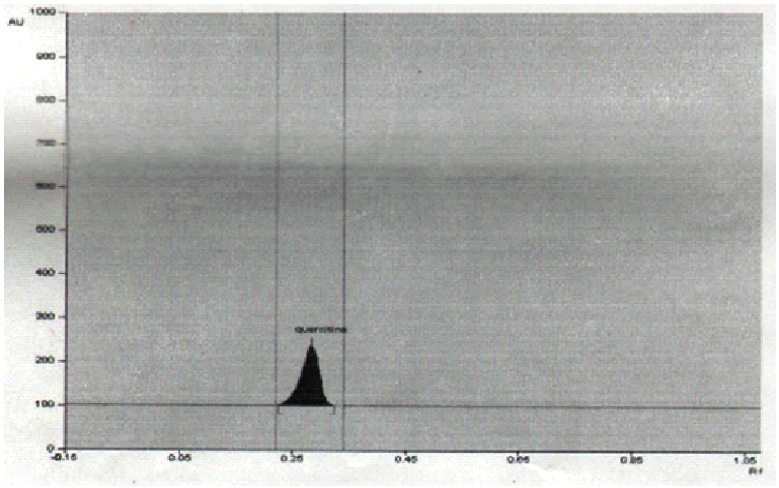
The flowers were dried under the shade[7] finely powdered and passed through 60 mesh sieve and stored in airtight container at room temperature (32 ± 2°C). About 250 gm of the powder was extracted with hydroalcohol in a extractor. The powdered material of dried flowers of N. Stellata was treated with hydroalcohol (70% ethylalcohol and 30% water.) The extract was collected and freeze at - 37°C for 2 days and again Lyophilized at temperature - 45°C and pressure to dryness.

The extract obtained was stored properly in the dessicator for further analysis.

The method is used as per ICH guidelines HPTLC was performed on 20cm × 10cm Aluminum backed plates coated with silica gel 60 f_{254} standard solution of gallic acid and sample solution were applied to the plates as 7.0 mm wide, 35.0 mm apart and 11.0 mm from the bottom edge of the same chromatographic plate. Ascending development to a distance of 80 mm was performed at room temperature (32 ± 2°C) with toluene : ethyl acetate : formic acid, 8.7 : 7:0.7 (v/v/v) as mobile phase vapour for 20 min. after complete development, the plates were air dried and then scanned using deuterium lamp[1-8].

Isolation of gallic acid from the extract of N. Stellata.

Level	0	1	2	3
Sample (mg)	250	250	250	250
Standard added (mg)	0.00	60	100	140
Overall recovery %	78.33	-	-	-



Fg. 1. Chromatogram of extract of dried flower of N. Stellata.

The mobile phase toluene: ethyl acetate: formic acid (8.7 : 7 : 0.7, v/v/v) gave good resolution with R_f = 0.26 for gallic acid but typical peak shape was missing. At last, the mobile phase consisting of (8.7:7:0.7, v/v/v) gave sharp and well defined peak at R_f = 0.24 (Fig. 1) a well apparent spot were obtained after saturation with mobile phase for 20 min. at room temperature.

There was no interference with the gallic acid peak from other constituents present in the plant.

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