INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

HPTLC Method for the Simultaneous Estimation of Catechol, Caffeic Acid and Anthraquinone in the Leaf Extract of Xanthium Strumarium and Its Formulation

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Abstract:
A simple, precise, accurate and rapid High-Performance Thin Layer Chromatographic method has been developed and validated for the simultaneous estimation of of catechol, caffeic acid and anthraquinone in the leaf extract of Xanthium strumarium and its Formulation. The stationary phase used was precoated silica gel 60F254.The mobile phase used was a mixture of n-hexane: chloroform: ethyl acetate: methanol (6:2:2:1 v/v/v/v). The detection of spots were carried out at 254 nm. This HPTLC method was validated statistically and recovery study was performed to confirm the accuracy of the method. It can be used for routine quality control of herbal raw materials as well as formulations containing any or all of these compounds.

Keywords: *Simultaneous estimation, HPTLC, Catechol, Caffeic acid and Anthraquinone*

1. Introduction

"Traditional systems of medicine have been a integral part of healthcare for centuries all over the world. One of which is use of herbs as medicine, which has been used in all cultures throughout history. By definition, 'traditional' use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines' Traditional medicine is "the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness"[ii]

Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value[iii]. Nowadays herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs. There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice[iv].

Xanthium strumarium L. (Family: Compositae) is a cocklebur or burweed commonly found as a weed in roadsides, rice fields, hedges throughout the tropical parts of India.[v],[vi]The literature surve showed that the plant parts posses anti inflammatory activity.[vii][viii] Scientists have identified different components of *X. strumarium* to be responsible for its activity. The aerial parts of the plant contain a mixture of unidentified alkaloids, which are said to be toxic. Besides alkaloids, the aerial parts of the plant contain sesquiterpene lactones, viz. xanthinin; its stereoisomer, xanthumin, xanthatin (deacetylxanthinin); a toxic principle, a sulphated glycoside: xanthostrumarin, atractyloside, carboxyatractyloside; phytosterols, xanthanol, isoxanthanol, xanthinosin, hydroquinone; xanthanolides; [ix],[x],[xi],[xii] Leaves was found to contain anthraquinone, cardenolide, leucoanthocyanin, simple phenolics (Catechol) and triterpenoids. Eleven free amino acids were found to be present in leaves. These are glutamic acid, tyrosine, alanyl glycine, glycine, glucosaminel, threonine, Dl alanine, argenine mono hydrochloride, proline, valine and isoleucine[xiii][xiv]. The anti-inflammatory effect found was due to caffeic acid and phenolic compounds like catechol present in *X. strumarium.* [xv].

2. Materials and Methods

2.1. Materials

A CAMAG TLC system comprising of a Linomat-5 applicator and CAMAG TLC III scanner. Stationary phase used was silica gel G60F254, 20x10 cm TLC plate.

The Reference standard catechol, caffeic acid and anthraquinone was obtained from Sigma-Aldrich Corporation, Bangalore India. The plates were developed in a CAMAG twin trough glass chamber (20 x 10 cm) by ascending method. Distance of solvent front 80mm, band length 6mm, slit dimension 5.00 x 0.45 mm and detection wavelength 254 nm were used for the present study.

2.2. Plant Material

Leaves of *X. strumarium* were collected from Buldana, Maharashtra. Herbarium samples of *X. strumarium* were prepared in duplicate and authenticated by Botanical Survey of India (BSI), Pune, India. A voucher specimen numbered SP-1 has been retained in the herbarium section of BSI, Pune for future reference. The leaves were washed with water to remove any dust particles, dried in shade, powdered and then sieved through BSS mesh size 85 and stored at 25^{o}C in an airtight container.

2.3. Method

2.3.1. Preparation of Catechol, Caffeic acid and Anthraquinone Standard Solution

Weighed 100 mg of Catechol, 50 mg each of Caffeic acid and Anthraquinone standard and transferred into 100 ml volumetric flask. Added 70 ml of diluent, sonicated to dissolve, it is then equilibrated to room temperature and diluted upto mark with diluent.

2.3.2. Chromatographic Conditions

Chromatography was performed on (100 mm x 200 mm) prewashed aluminum HPTLC plates, coated with silica gel 60F_{254} (E.Merck, Germany). 10µL of each of the standard solutions were spotted with the help of by use of a CAMAG (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100-ul Hamilton (USA) syringe. Ascending double development to a distance of 90 mm was performed at room temperature (28±2^{o}C), with n-Hexane: ethyl acetate: chloroform: Methanol 6:2:2:1 (v/v) as mobile phase, in a CAMAG glass twin-trough chamber previously saturated with mobile phase vapor for 20 min.

After development, the plates were dried in air first and then by keeping on the CAMAG TLC plate heater at 90^{o}C for 5 min. The plates were then scanned at 254 nm with a CAMAG TLC Scanner with winCATS3 software, using the deuterium lamp.

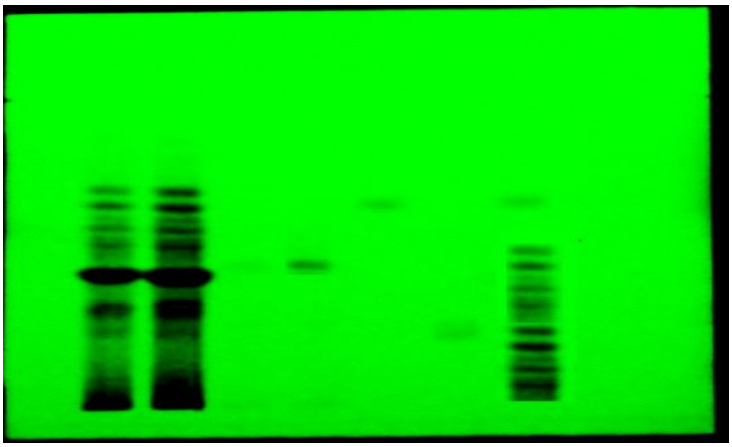
The densitograms were recorded and the peak areas of Catechol, Caffeic acid and Anthraquinone for each applied concentration of Catechol, Caffeic acid and Anthraquinone were noted.

2.3.4. Sample Preparation

About 5 gm of dried leaf powder of *X.strumarium* was weighed into a round bottom flask. 30 mL of methanol was added to the flask and the mixture was refluxed on a boiling water bath for about 30 min. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India). The same procedure was performed twice and filtrate obtained was combined together and made up to 100 mL with methanol.

2.3.5. Formulation Sample

For analysis of the formulation sample 1 gm was accurately weighed into a round bottom flask. 30 mL of methanol was added to the flask and the mixture was refluxed on a boiling water bath for about 30 min. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India). The same procedure was performed twice and filtrate obtained was combined together and made up to 100 mL with methanol.



a b c d e f g

a,b : Methanolic extract of leaves of *X. strumarium*
c,d : Standard Catechol
e : Standard Anthraquinone
f: Caffeic acid
g : Methanolic extract of formulation containing *X. strumarium*

3. Method Validation

ICH harmonized tripartite guidelines were followed for the validation of the developed analytical method.[xvi]

3.1. Specificity
The specificity of method was ascertained by standards and samples (extracted from leaves and extracted from formulation). The spots of blank-methanol, standards (catechol,caffeic acid and Anthraquinone), extracted samples (extracted from leaves and extracted from formulation) were spotted on HTLC plate.The interference due to blank was checked.

3.2. Precision

3.2.1. Repeatability
Repeatability of sample applications and measurement of peak area was carried out using the six replicates of same spot 10 µl//spot. Repeatability is also termed Intra-assay precision.

3.2.2. Intermediate Precision
The intra-day and inter-day variations for determination of glycyrrhizin were carried out at three different concentration levels 2, 5, 10 µl/spot.

3.2.3. Recovery Studies
Recovery Study was performed by spiking LOQ level, 50%, 100% and 1500 % of standard components externally to the pre analyzed samples. The experiment was conducted in triplicate and applied onto the plate in triplicate. This was conducted to check the recovery of drugs at different levels.

3.2.4. Robustness
The robustness of method was performed by small but deliberate change in two parameters, i.e injection volume(±2%) and mobile phase composition of one of the solvent (±2%) and its impact on area and Rf values were recorded.

3.3. Summary
The method was validated for linearity, precision, specificity, recovery, robustness and stability. The method was found to be linear from 50-500 µg/mL for Catechol and Anthraquinone and from 20-200 µg/mL for Caffeic acid. The correlation coefficient was found to be ≥0.99 for all the three components. The precision (%RSD) of the method was found to be ≤ 2%, indicating that the proposed method is precise. The recovery values for all the three components were within acceptable limits (90.0 to 110.0%). Solution stability were evaluated by monitoring the peak area response. Standard solutions were analysed right after its preparation and after 72 hrs. There was no significant change (% RSD ≤ 2%) in the Rf and area values of standard peak.

Parameter		**Catechol**	**Caffeic acid**	**Anthraquinone**
Specificity		Specific	Specific	Specific
Linearity(µg/ml)		40-500	20-300	20-300
Correlation coeff		0.9997	1.0	1.0
LOD (µg/mL)		13.38	6.85	6.79
LOQ (µg/mL)		40.13	20.56	20.36
Precision (RSD)		≤ 2 %	≤ 2 %	≤ 2 %
Assay	Plant	0.34%	0.24%	0.27%
	Formulation	0.21%	0.15%	0.16%
Stock soln. stability (2-8°C)		Stable till 72hrs	Stable till 72hrs	Stable till 72hrs
Robustness		Robust	Robust	Robust

Table 1: Summary of method validation parameters

4. Result and Discussion

A normal phase high performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of catechol, caffeic acid and anthraquinone from leaf powder of X.strumarium (Linn.) was developed in the present research work.

5. Conclusion

The proposed method is simple, rapid, precise and accurate. The method was found to be suitable for qualitative and simultaneous quantitative analysis of catechol, caffeic acid and anthraquinone in the methanolic extract of *X.strumarium*. The method established in this work can therefore be used as quality-control method for other market formulations or dietary supplements containing leaf powder of *X.strumarium*

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