



SIMULTANEOUS QUANTIFICATION OF ELLAGIC ACID, GALLIC ACID, CHLOROGENIC ACID AND QUERCETIN IN THE EXTRACT OF TERMINALIA TOMENTOSA PLANT AND ITS FORMULATION USING HPTLC

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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 ellagic acid, chlorogenic acid, gallic acid and quercetin in the leaf extract of Terminalia tomentosa and its Formulation. The stationary phase used was precoated silica gel 60F254. The mobile phase used was a mixture of Butyl acetate: Formic Acid: Distilled Water 14:5:5 (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, ellagic acid, chlorogenic acid, gallic acid and quercetin.

1. Introduction:-

Validation of analytical methods is mandatory in implementing a quality control system in any analytical laboratory. It provides an assurance of reliability during normal use and can be referred as a process of providing documented evidence of quality for several herbal and traditional drugs. Separation techniques such as chromatography and electrophoresis have been extensively used for quality control of herbal medicine because of their high efficiency and speed.

Terminalia tomentosa Roxb (ex DC) Wight & Arn (*T. tomentosa*). (Synonyms: *Terminalia alata* Heyne ex. Roth, *Terminalia crenulata* Roth, *Terminalia elliptica* Willd. It is commonly known as Crocodile Bark Tree, Indian Laurel in English, Asan in Marathi, Saj in Hindi, Banappu in Kannada, Sahaju in Odiya, Karu-Maruthu in Tamil, Nalla Maddi in Telugu. It is a tall tree growing as high as 30 meters; belonging to the flowering plant family *Combretaceae*. The bark is bitter & styptic, useful in vitiated conditions of pitta, ulcers, vata, fractures, haemorrhages, bronchitis cardiopathy, strangury, wounds, haemoptysis, dysentery, cough, verminosis, leucorrhoea, gonorrhoea & burning sensation (Ayurveda).

Phytoconstituents such as tannins like arjunic acid, arjunolic acid, arjunetin, ellagic acid, gallic acid, chlorogenic acid triterpenoids like oleanolic acid, betulinic acid flavanoid like quercetin and steroid like β -sitosterol have been reported to be present in *T. tomentosa*. The plant is known to possess many pharmacological properties like antifungal, antioxidant, anti-hyperglycaemic, antidiarrhoeal, anti-leucorrhoeal. From the literature survey, it is learnt that no substantial work has been carried out on the leaves of *T. tomentosa* in terms of physicochemical and preliminary phytochemical screening of *T. tomentosa*. However pertaining to our knowledge there is no any hyphenated HPTLC technique available anywhere else for simultaneous quantitation of ellagic acid, gallic acid, chlorogenic acid, quercetin and its formulation in methanolic extract. So the attempt has made to accept this challenge towards development and validation of ellagic acid, gallic acid, chlorogenic acid and quercetin simultaneously by such a hyphenated technology like HPTLC for the betterment of herbal quality standards.

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 ellagic acid, gallic acid, chlorogenic acid, quercetin 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 and Chemicals

T. tomentosa fresh plant was collected from the field area of Kankeshwar, Alibaug, District- Raigad, Maharashtra, India in the month of November 2014; and the specimens (voucher nos:----) were authenticated by Dr. Ganesh Iyer (Taxonomist), Department of Life Science, Ramnarain Ruia College, Matunga, Mumbai. Standards Ellagic Acid, gallic acid, chlorogenic acid and quercetin were purchased from Sigma-Aldrich Chemicals Pvt Ltd, Jigani, Bangalore – 560100. All the solvents used were of chromatography grade and other chemicals used were of analytical reagent (AR) grade.

2.3 Method

2.3.1 Preparation of Standard and quality control (QC) samples

Stock solutions of ellagic acid, gallic acid and chlorogenic acid and quercetin (10mg/ml) were prepared in methanol, and by appropriate dilution standard solutions were prepared in the concentration range of 0.1 to 1.0 mg/ml.

2.3.2 Chromatographic Conditions

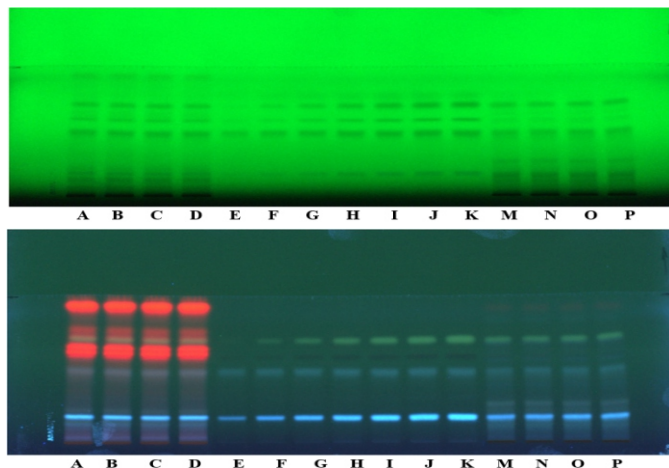
Chromatography was performed on (100 mm x 200 mm) prewashed aluminum HPTLC plates, coated with silica gel 60F254 (E. Merck, Germany). 10 μ L of each of the standard solutions were spotted with the help of by use of a CAMAG (Muttentz, Switzerland) Linomat V sample applicator equipped with a 100- μ L Hamilton (USA) syringe. Ascending double development to a distance of 90 mm was performed at room temperature (28 $\pm$ 20C), with Butyl acetate: Formic Acid: Distilled Water 14:5:5 (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°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 ellagic acid, gallic acid and chlorogenic acid and quercetin for each applied concentration of ellagic acid, gallic acid and chlorogenic acid and quercetin were noted.

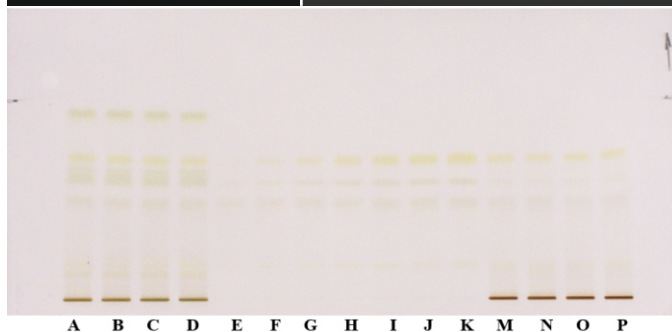
2.3.3 Sample Preparation

5 gm of dried powder of *T. tomentosa* was weighed in a round bottom flask. 100 ml of Methanol was added to the flask and the mixture was extracted by Soxhlate extraction after 12 hrs. The extract was then filtered through Whatman filter paper no. 41 (E. Merck, Mumbai, India) and filtered through syringe filters of mesh size 0.45 μ . The volume was made up to 100ml and used.

2.3.4 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:- Methanolic extract of leaves of *T. tomentosa*

E,F,G,H,I,K:- Mixture of standards chlorogenic acid, ellagic acid, gallic acid and quercetin with different concentrations.

M,N,O,P:- Methanolic extract of Formulation.

3. Method Validation

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

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 (ellagic acid, gallic acid, chlorogenic acid and quercetin), 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 between the range 1 to 2ul/spot. Repeatability is also termed Intra-assay precision.

3.2.2. Intermediate Precision

The intra-day and inter-day variations for determination of Niddwin were carried out at three different concentration levels 4μl/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 100-700 μg/mL for gallic acid, chlorogenic acid and quercetin from 100-220 μg/mL for ellagic acid. The correlation coefficient was found to be ≥0.99 for all the four 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.

Table 1: Summary of method validation parameters

Parameter	Gallic acid	Chlorogenic acid	Ellagic acid	Quercetin
Specificity	Specific	Specific	Specific	Specific
Precision	<2%	<2%	<2%	<2%
LOD μg/mL	30 μg/mL	30 μg/mL	30 μg/mL	30 μg/mL
LOQ μg/mL	100 μg/mL	100 μg/mL	100 μg/mL	100 μg/mL
Linearity μg/mL	100-700 μg/mL	100-700 μg/mL	100-220 μg/mL	100-700 μg/mL
Assay (Plant)	0.22%	0.15%	0.07%	0.18%
Assay (formulation)	0.06%	0.34%	0.06%	0.20%
Stocksolution stability	Stable till 72 hrs	Stable till 72 hrs	Stable till 72 hrs	Stable till 72 hrs

4. Result and Discussion

A normal phase high performance thin layer chromatographic (HPTLC) method for the simultaneous quantification of ellagic acid, gallic acid, chlorogenic acid and quercetin from leaf powder of *T. tomentosa* (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 ellagic acid, gallic acid, chlorogenic acid and quercetin in the methanolic extract of *T. tomentosa*. 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 *T. tomentosa*.

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