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A HPTLC Method for Qualitative and Quantitative Estimation of Gallic Acid from *Emblica officinalis* Gaertn in Polyherbal Formulation

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ABSTRACT

HPLC is an analytical technique used for the qualitative and quantitative evaluation of polyherbal formulations. Amla an important medicinal plant with wide medicinal value is frequently used in a large number of traditional herbal preparations. Gallic acid a major bioactive was selected as a chemical marker of Amla. A selective HPTLC analytical method has been developed for the finger printing of Amla and analysis of Gallic acid in Amla fruit extract and its polyherbal formulation. For HPTLC stationary phase is silica gel F_{254} plate. Toluene-ethyl acetate-acetic acid-formic acid (20:45:20:05) v/v as the mobile phase.

Keywords: *Emblica officinalis*, Polyherbal formulation, HPTLC, Gallic acid.

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INTRODUCTION

Emblica officinalis Gaertn. or *Phyllanthus emblica* Linn, commonly known as Indian gooseberry or Amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda [1]. Various parts of the plant are used to treat a range of diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments such as common cold and fever; as a diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, alterative, antipyretic, anti-inflammatory [2], hair tonic; to prevent peptic ulcer and dyspepsia, and as a digestive. Preclinical studies have shown that amla possesses antipyretic, analgesic [3], antitussive, antiatherogenic, adaptogenic [4],cardioprotective,gastroprotective,antianemia,antihypercholesterolemia, cytoprotective [5], wound healing, antidiarrheal, antiatherosclerotic, hepatoprotective [6], Antimutagenic, anticarcinogenic[7], nephroprotective, memoryenhancing activity and neuroprotective properties. In addition, experimental studies have shown that amla and some of its phytochemicals such as gallic acid, ellagic acid, pyrogallol, some norsesquiterpenoids, corilagin, geraniin, elaeocarpusin, and prodelphinidins B_1 and B_2 [8] also possess antineoplastic effects. Amla is also reported to possess radiomodulatory [9], chemomodulatory, chemopreventive effects, free radical scavenging [10], antioxidant [11], anti-inflammatory, antimutagenic and immunomodulatory activities, properties that are efficacious in the treatment and prevention of cancer. Our formulation, polyherbal Tablet for management of Parkinson disease contains extract of three herbal plants. One of the major plant extract was *Emblica officinalis*. It was necessary to develop HPTLC method for estimation of Gallic acid in polyherbal tablet.

MATERIALS AND METHODS

Procurement of plant materials

Dried fruits of Amla were procured from yucca enterprises Mumbai.

Preparation of extract

Procured plant materials Amla pericarp was dried and then coarsely powdered in a blender. The coarse powder 1 kg was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by using solvents 60% ethanol. The solvents was decanted and filtered with filter paper and recovered by distillation with help of rotary vaccum evaporator at 75^0C to 80^0C. The extracts were dried under desiccators and stored in airtight container at room temperature [12].

HPTLC procedure

A Camag linomat HPTLC system equipped with an automatic TLC sampler, TLC scanner, and integrated software was used for the analysis. HPTLC was performed on a pre-coated silica gel HPTLC 60 F_{254} (10 cm × 10 cm) plate of 0.20 mm layer thickness. Chromatography was carried in twin through chamber which was pre-saturated with 10 ml mobile phase Toluene-

ethyl acetate-acetic acid-formic acid (20:45:20:05) v/v ratio for 30 min at room temperature (25 ± 2 °C). The samples and standards were applied on the plate as 6 mm wide bands with an automatic TLC sampler under flow of inert N_2 gas, 20 mm from the bottom, application speed 100 nl/s. The length of solvent front position 83mm from the base. After that, TLC plates were dried in a current of air, followed by heating on Camag HPTLC plate heater at 60°C for 5 min. The separated bands on the HPTLC plates were scanned over the wavelength of 200 - 400 nm. The source of radiation utilized was the tungsten lamp (or deuterium lamp) [13].

**Development of the optimum mobile phase**

The TLC procedure was optimized with a view to develop an assay method. The standard and the test solutions were spotted on HPTLC plates and different individual solvents as well as combination of solvents were tried to get a good separation and stable peak. Both the pure drug (gallic acid) and the extract of amla solution were spotted on the TLC plates and run in different solvent systems. The mobile phase Toluene-ethyl acetate-acetic acid-formic acid (20:45:20:05) v/v ratio gave good resolution Well-defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature[14].
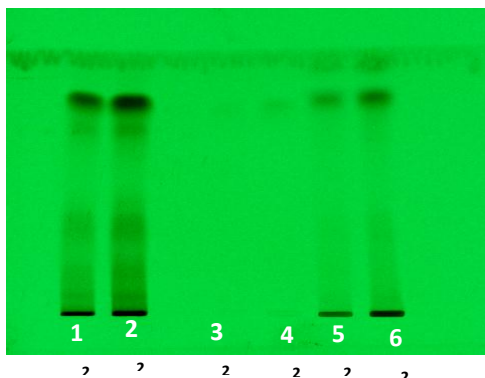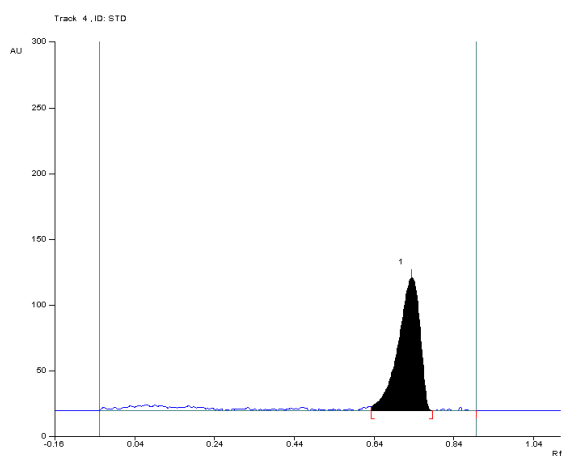

Spot 1 and 2 for Amla Ext., 3 and 4 for Std. gallic acid, 5 and 6 for Tablet at 254 nm
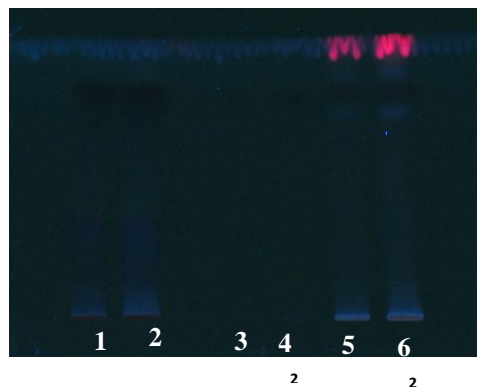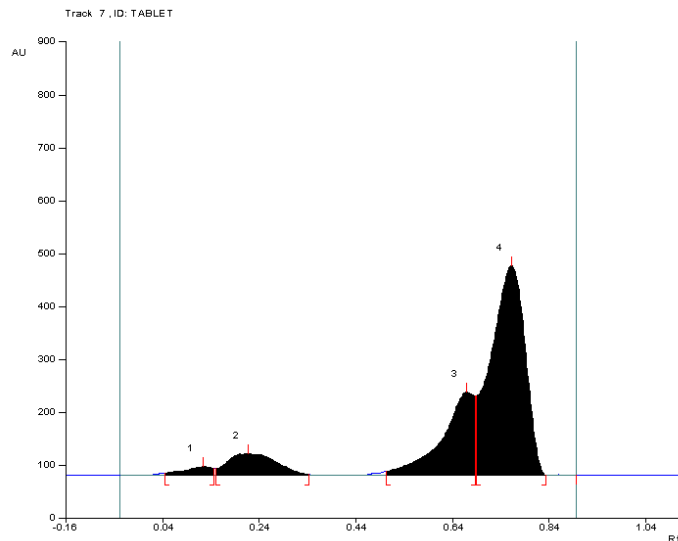

Spot 1 and 2 for Amla Ext., 3 and 4 for Std. gallic acid, 5 and 6 for Tablet at 366nm
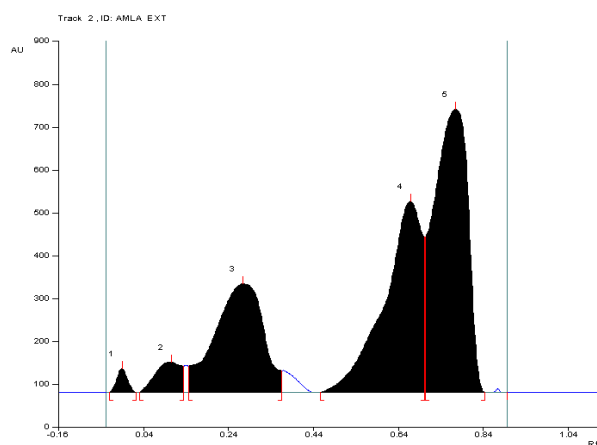
Peak for Amla Extract

Peak for gallic acid standard.

Peak for tablet

Preparation of standard Sample

A stock standard solution of gallic acid (5 mg/ml) was prepared by dissolving 50 mg of accurately weighed gallic acid in 60% ethanol and making up the volume to 10 ml. The stock solution was further diluted with 60% ethanol to give a standard solution of gallic acid (10µl). This concentration was used as the working standard for the HPTLC method.

Preparation of Extract Sample

Accurately weigh 250 mg of 60% ethanolic extract of *Emblica officinalis* Gaertn in a 50 ml volumetric flask. Dissolve in 10 ml of 60% ethanol by sonication and make up the volume with 60% ethanol. The stock solution was further diluted with 60% ethanol to give a standard solution of extract (10µl). This concentration is used for the estimation of gallic acid from the 60% ethanolic extract of *Emblica officinalis* Gaertn.

Preparation of polyherbal tablet Sample

Pharmaceutical polyherbal tablet equivalent to about 500 mg of *Emblica officinalis* Gaertn extract was weighed and transferred to 100ml volumetric flask containing 60% ethanol. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for gallic acid content. 10 µl of the filtered solution was applied on the TLC plate followed by development, visualization and scanning as described in HPTLC procedure.

RESULTS AND DISCUSSION

Qualitative estimation

Table 1: Chromatographic profile of crude extracts of *Emblica officinalis* Gaertn by HPTLC method.

S.NO	crude extract	Solvent system	Detection	Rf
1.	Amla	Toluene: Ethyl acetate: Acetic acid: Formic acid 20 : 45 : 20 : 05	at 254nm&366nm in U.V light	0.02 0.13 0.37 0.70 0.84 0.91

Table 2: Chromatographic profile of Tablet by HPTLC method.

S.NO	Tablet	Solvent system	Detection	Rf
1.	Evaluation for Amla	Toluene: Ethyl acetate: Acetic acid: Formic acid 20 : 45 : 20 :05	at 254nm&366nm in U.V light	0.15 0.34 0.69 0.83 0.91

Table 3: Chromatographic profile of Standard by HPTLC method.

S.NO	Standard	Solvent system	Detection	Rf
1.	Evaluation for Gallic acid	Toluene: Ethyl acetate: Acetic acid: Formic acid 20 : 45 : 20 : 05	at 254nm&366nm in U.V light	0.79

Quantitative estimation

Table 4: Evaluation of Amla extract and poly herbal tablet for gallic acid content per milligram by   HPTLC method.

Standard	Crude extract (Amla extract)	Tablet (for Amla)
Gallic acid content	48.2µg/mg	12.8µg/mg

DISCUSSION

The HPTLC method was developed for amla extract, polyherbal tablet and gallic acid. In qualitative estimation of Amla extract Rf values was found to be 0.02, 0.13,0.37,0.70,0.84,0.91, in poly herbal tablet for amla 0.15, 0.34, 0.69, 0.83, 0.91and for gallic acid standard was 0.79. For quantitative estimation gallic acid content was found to be 48.2µg/mg in Amla extract and 12.8µg/mg in polyherbal tablet.

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