



QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS OF *CAESALPINIA PULCHERRIMA*

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Abstract

Caesalpinia pulcherrima belonging to family Caesalpiniaceae is distributed throughout India. Commonly it is known as Peacock-flower. Plant shows diterpenoids, isovouacaperol, sitosterol and flavonoids. The plant is considered as emmenagogue, purgative and stimulant, bark is powerful emmenagogue, abortifacient and infusion of flower is pectoral and febrifuge, also used in bronchitis, asthma and malarial fever, leaves used as antipyretic, antimicrobial. Flower also shows antioxidant and antiviral activity.

Wood of *Caesalpinia pulcherrima* was collected and extracted with petroleum ether and methanol. Then the fractionation of extracts was done and active phytoconstituents were isolated from the fractions with the help of column chromatography. US-Q and US-P were isolated from petroleum ether extract and EA-F and EA-I was isolated from methanol extract. Quantitative estimation of isolated phytoconstituent was carried out using Camag HPTLC system equipment. Percentage content of these constituents was found as US-Q: 0.1749, US-P: 4.2847, EA-F: 6.0039 EA-I: 5.3093.

Key Words: *Caesalpinia pulcherrima*, HPTLC, Quantitative estimation.

INTRODUCTION AND MATERIAL AND METHODS:

Introduction

Caesalpinia pulcherrima (Caesalpinaceae) is a shrub or small tree up to 5m in height. It is a commonly known as Guleture, distributed through India^[1]. Literature survey reveals the presence of diterpenoids, isovouacapenol C, pulcherrimin A in root^[2]. Stems contain peltogynoids, bhonducellin, and 6-methoxypulcherrimin, homoisoflavonoids^[3]. Flowers showed the presence of lupeol, B-sitosterol, flavonoids, and myricetin^[4]. The leaves contain hydrocyanic acid, tannins, and benzoic acid^[5]. The bark contains terpenoid that gives antimicrobial and cytotoxic activities^[6]. Plant is used as emmenagogue, purgative, stimulant, and abortifacient, also used in bronchitis, asthma, malarial fever^[7]. A literature survey revealed no analytical method had been reported for quality control of *Caesalpinia pulcherrima* wood powder. We have developed a sensitive, simple, rapid, cost effective and reproducible HPTLC method with good recovery for determination of isolated phytoconstituents from *Caesalpinia pulcherrima* wood.

HPTLC offers a number of advantages for the analysis of herbals. An HPTLC technique is usually suitable for comparison of sample based on finger prints. It provides the means, not only the flexible screening, but also the quantitative analysis.

Material and methods:

Plant Collection and Identification:

For this study the wood of *Caesalpinia pulcherrima* were collected from Nashik, Maharashtra, India. The plant was identified at the Botanical Survey of India, Koregaon, Pune and a voucher specimen no. (CRP-2).

Preparation o Wood Extract:

Petroleum ether and Methanol Extracts:

The sun dried wood were powdered and extracted with Petroleum ether (60-80^oc) and methanol individually by hot soxhlet extraction method. The crude extracts were evaporated to dryness and the residues (Petroleum ether 2.02% w/w, Methanol 13.13% w/w) were maintained.

Isolation of phytoconstituents:

The separation of phytoconstituents was done by column chromatography. The unsaponifiable matter of Petroleum ether extract was subjected to column chromatography. The column was packed with silica gel for column chromatography (#60-80) and the column was eluted by benzene. Two compounds were isolated from column coded as US-Q and US-P.

The methanol extract was treated with ethyl acetate. Then the ethyl acetate insoluble fraction was subjected to column chromatography. Mixture of benzene and methanol was used as mobile phase for column. Two compounds were isolate from column coded as EA-F and EA-I.

Experimentation

The amount of isolated compounds was determined by using high performance thin layer chromatography (HPTLC).

Samples US-P and US-Q were spotted on precoated silica gel F254 aluminium plate I (E-Merck grade) and EA-F and EA-I on plate II as narrow bands 4mm wide at a constant rate of 10 μ ls-1 using a Camag Linomat IV model applicator under nitrogen atmosphere. Benzene was used as a mobile phase for plate I and a mixture of benzene and methanol (95.5 v/v) for plate II. The plate I was sprayed with vanillin-H₂SO₄ reagent (0.5 % vanillin in sulphuric acid-ethanol (4:1)) and heated at 105^oc for 5 min. which gave well resolved spots at Rf 0.10 (US-Q) and Rf 0.35 (US-P). Purple color developed was quantified using Camag TLC Scanner with CATS 4 software at 490 nm. Plate II was sprayed with Alcoholic FeCl₃ reagent (5 % w/v ferric chloride in 90 % alcohol). Which gave well resolved spot at Rf 0.66 (Ea-I). The bluish black color developed was quantified using Camag TLC Scanner at 490 nm and EA-F at 360 nm. Chromagraphical conditions were mention in the table.

Table: Chromatographical condition

Extract	Pet ether	Methanol
Samples	US-P, US-Q	EA-f, EA-I
Plate	Plate I	Plate II
Mobile phase	Benzene : Methanol	Benzene
Spraying reagent	Vanillin- H ₂ SO ₄	Alcoholic FeCl ₃
Color of spots	Purple	Blue
Rf value	US-P: 0.35 US-Q: 0.10	EA-F; 0.65 EA-I: 0.66
Scanning	490 nm	360 nm, 490nm.

Result

Quantitative estimation of isolated phytoconstituent from *Caesalpinia pulcherrima* wood was carried out using Camag HPTLC system equipment. A photograph of the chromatographic plates is shown in Figure 1 & 2. Percentage content of these constituents was found as US-Q-0.1749, US-P-4.2847, EA-F: 6.0039, EA-I-5.3093. The proposed HPTLC method for quantitative monitoring of phytoconstituents is sensitive, simple, rapid, cost effective and reproducible with good recovery.

Acknowledgement

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FIGURES :



Figure.1 : Petroleum ether extract

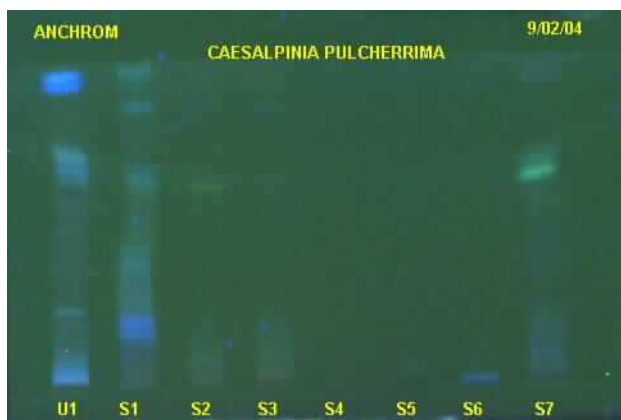


Figure.2 : Methanol extract