

## **ESTIMATION OF GALLIC ACID, EPICATECHIN AND QUERCETIN IN CAESALPINIACEAE SPECIES BY HPLC**

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### **Summary**

Flavonoid and catechin are a group of polyphenolic compounds which are widely distributed throughout the plant kingdom. Gallic acid, epicatechin and quercetin possess many biochemical effects like inhibition of enzymes and pharmacological activities like antimicrobial, antioxidant and anticancer. An HPLC method was developed for the estimation of gallic acid, epicatechin and quercetin from methanol extract of *Caesalpinia pulcherrima* and *Caesalpinia decapetala* (Caesalpiniaceae).

**Key words:** *Caesalpinia pulcherrima*, *Caesalpinia decapetala*, gallic acid, epicatechin, quercetin, HPLC.

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## Introduction

*Caesalpinia pulcherrima* and *Caesalpinia decapetala* (Caesalpiniaceae) were traditionally used for treatment of rheumatism, bronchitis and kidney stone<sup>1</sup>. Further these plants act as emmenagogue and abortifacient<sup>2</sup>.

Gallic acid, epicatechin and quercetin are phenolic compounds structurally they have phenolic groups which serve as a source of readily available hydrogen atoms such that the subsequent radicals produced can be delocalized over the phenolic structure<sup>3</sup>. It is known that phenolics have various physiological benefits such as antioxidants, antibacterial and anticancer<sup>4</sup>. While flavonoids are a group of polyphenolic compounds which are widely distributed throughout the plant kingdom. To date 300 varieties of flavonoids are known<sup>5</sup>. Many have low toxicity in mammals and some of them are widely used in medicine for maintenance of capillary integrity. The interest in these compounds is due to their pharmacological activity as radicals scavengers. They have been proved to have potential preventive and therapeutic effects in many diseases, where the oxidative stress has been implicated, including cardiovascular diseases, cancer, neuro degenerative disorders and in aging. The phenolics are also of interest in food, cosmetic and pharmaceutical industry, as substituent for synthetic antioxidants<sup>6</sup>.

The method of determination of phytoconstituents includes UV-Vis spectrophotometry, HPLC and LC-MS<sup>7,8,9</sup>. Determination of contents of Gallic acid, epicatechin and quercetin in *Caesalpinia pulcherrima* and *Caesalpinia decapetala* was not reported. High performance layer chromatography (HPLC) method is the suitable method for estimation of chemical constituents present in plant material. Hence in this study, Gallic acid, epicatechin and quercetin from methanol extract of *Caesalpinia pulcherrima* and *Caesalpinia decapetala* wood and pericarp were estimated by HPLC method.

## Materials and Method

### Extraction of Plant material

The wood and pericarp of *Caesalpinia decapetala* and *Caesalpinia pulcherrima* were collected from Nashik, Maharashtra, India. The plant was authenticated by Mr. P. G. Diwakar, Botanical Survey of India, Pune (Voucher no. CRP-1 and CRP-2) and preserved in the herbarium of the department. The dried wood and pericarp (100 g) was extracted with 95% methanol for 48 h in soxhlet apparatus. The extracts were filtered and concentrated to vacuum under reduced pressure in rotary evaporator and dried in desiccators. Methanol extract was subjected for estimation of Gallic acid, epicatechin and quercetin

### Solvent and Chemicals

Gallic acid, epicatechin and quercetin obtained from Sigma (MO, USA). Chromatographic grade methanol, formic acid and acetonitrile (AR), were obtained from Merck (Mumbai, India).

### Instrumentation

The Shimadzu class LC-10AT HPLC, Hichrom C18 and a Rheodyne 7725i injector fitted with a 20 µl loop, column oven, and a photodiode array detector. The output signal was monitored and processed using chromquest version3.0 software on Pentium computer (Hewlett Packard).

### Preparation of Standard

Gallic acid, epicatechin and quercetin 5 mg were accurately weighed into a 10 ml volumetric flask, dissolved in 5 ml methanol to give 1000 µg/ml concentration of extract. The extract was then filtered using Whatman filter paper.

**Preparation Sample Solutions** *C. pulcherrima* and *C. decapetala* wood and pericarp extract was accurately weighed (10 mg) into a 10 ml of volumetric flask and shaken on a mechanical shaker for 10 min filtered through Whatman filter paper No. 42. After filtering through a filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into HPLC by autosampler.

### Chromatography

Injection quantity : 50 µl

Column used : Exsil ODS column (250cm x 4.5mm, 5µ particle size)

Column temperature : 30°C

Flow rate : Gallic acid- 0.7, Epicatechin- 0.3, Quercetin- 0.9

Detection : Gallic acid and epicatechin- 280 nm, Quercetin- 340.

Mobile phase : Gallic acid- 0.1 % H<sub>3</sub>PO<sub>4</sub>: Acetonitrile (85:15)

Epicatechin- Acetonitrile: water (80:20)

Quercetin- 0.5 % formic acid: Acetonitrile (85:15)

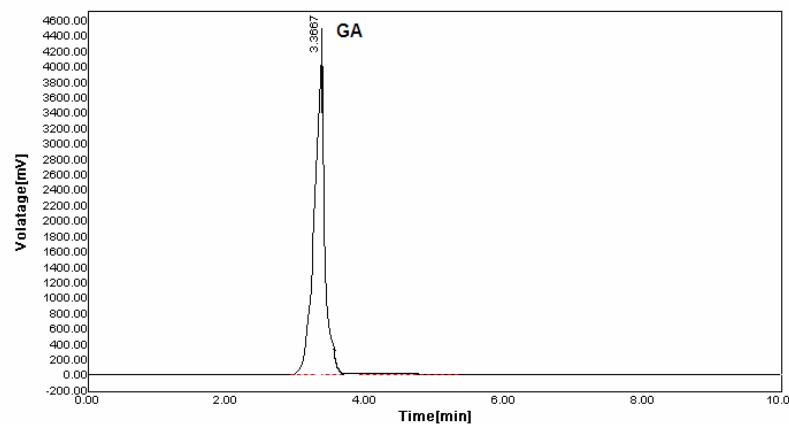
### Results and Discussion

This HPLC procedure provided excellent identification and quantification of these three phenolic compounds presented in *C. pulcherrima* and *C. decapetala* wood and pericarp with a short analysis time (15 min). The experimental results (Table 1) indicated that *C. pulcherrima* wood extract contained an especially high concentration of gallic acid and epicatechin (0.2246 % w/w and 5.7048 % w/w). It was also shown that gallic acid and epicatechin were detected in wood and pericarp of both the plants and wood of *C. decapetala* (Figure 1, 2) contained highest amount of quercetin (0.0239 %w/w) (Figure 3). Since the phenolic compounds have been of interest of health benefits, the present analytical study could be a potential application to identify and quantify the phenolic compounds in other species of Caesalpiniaceae.

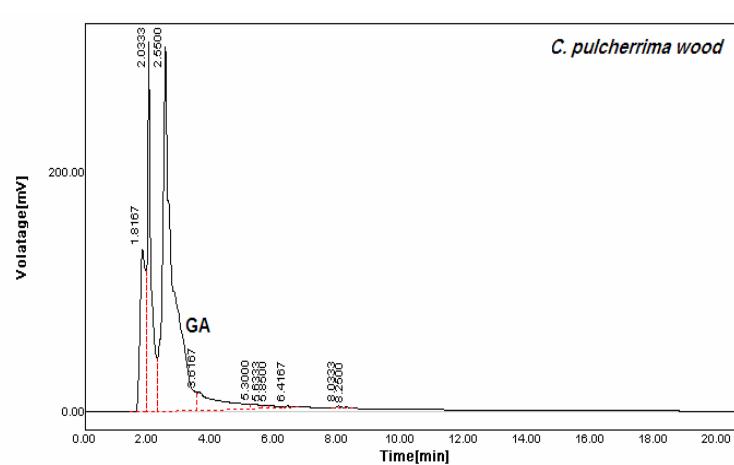
Table 1 Contents of phenolic compounds in the methanol extract of *C. pulcherrima* and *C. decapetala* (n=5)

Compounds	Standard	<i>C. pulcherrima</i>				<i>C. decapetala</i>			
		Wood		Pericarp		Wood		Pericarp	
	Retention time (min)	Retention time (min)	Contents (% w/w)	Retention time (min)	Contents (% w/w)	Retention time (min)	Contents (% w/w)	Retention time (min)	Contents (% w/w)
Gallic acid	3.3667	3.6167	0.2246	3.6167	0.02842	3.2333	0.06867	3.6167	0.0059
Epicatechin	2.5500	2.5500	5.7048	2.5667	0.3539	2.5500	1.3699	2.5667	0.1171
Quercetin	16.9333	14.9800	0.0127	ND <sup>a</sup>	ND <sup>a</sup>	17.200	0.0239	ND <sup>a</sup>	ND <sup>a</sup>

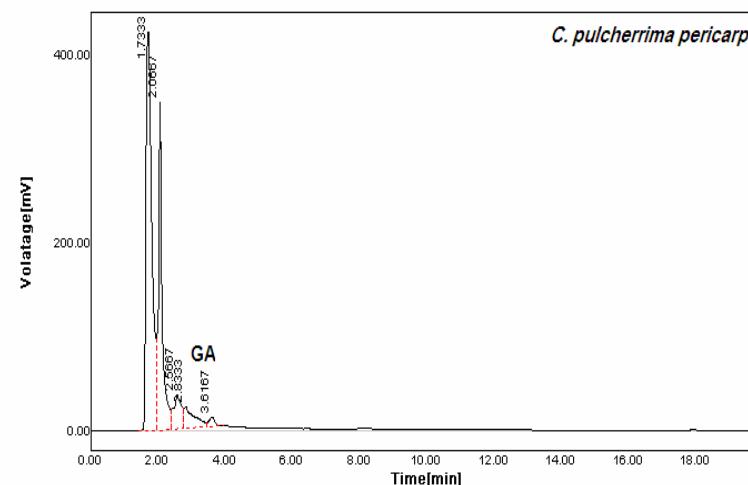
<sup>a</sup>ND = not detected



(a)



(b)



(c)

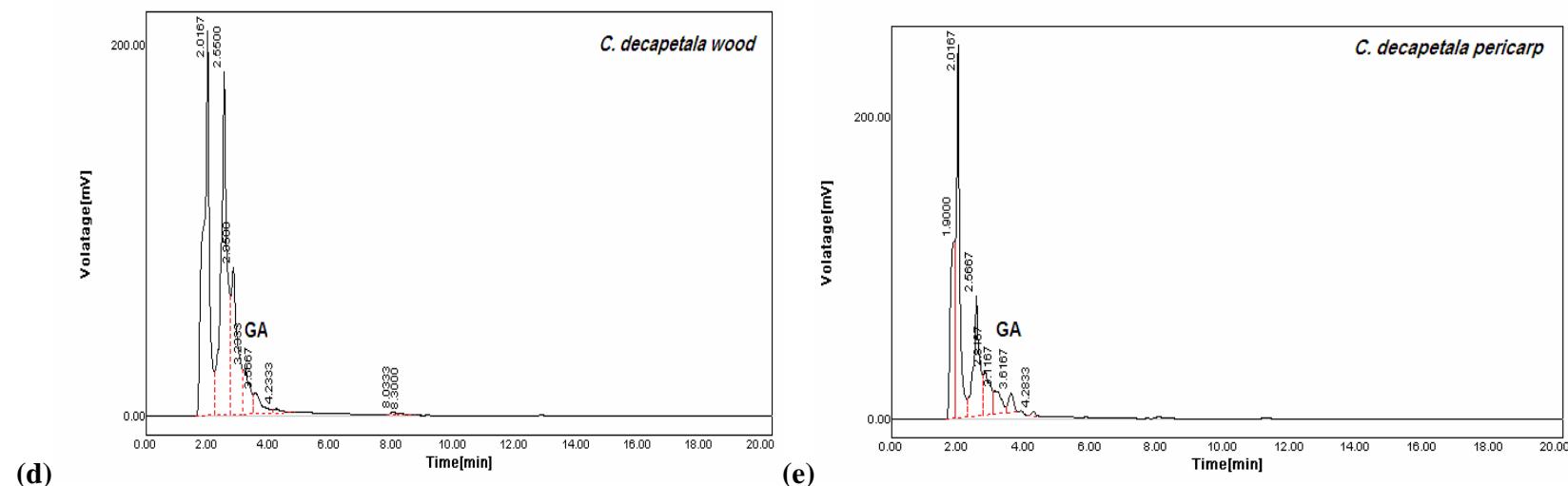
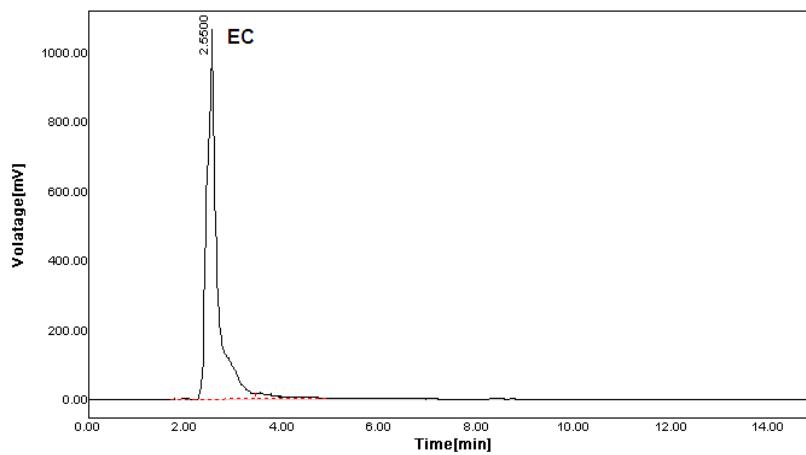
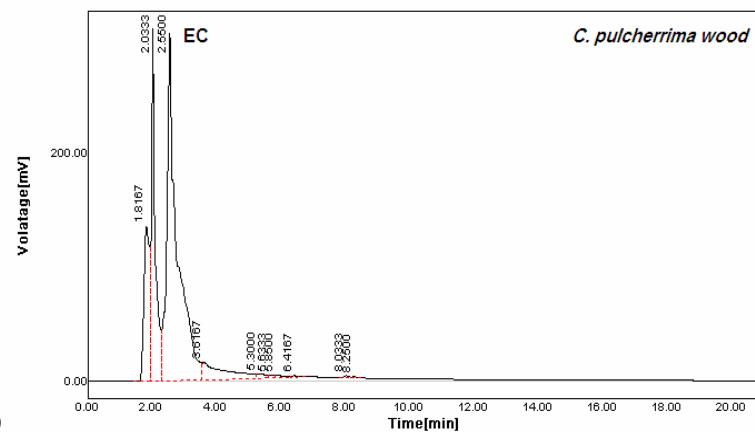


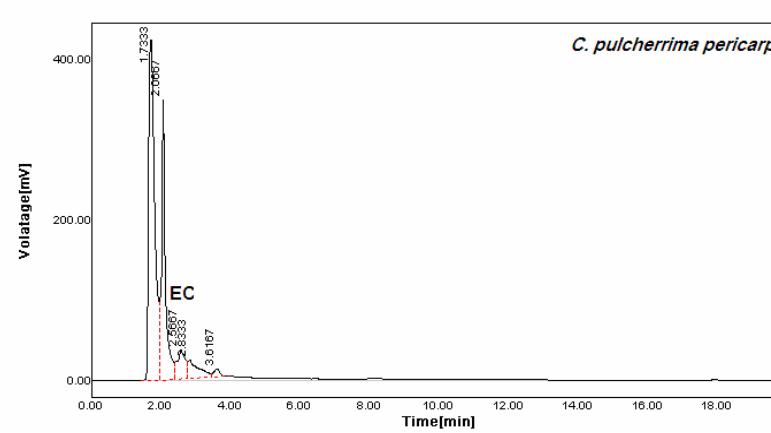
Figure 1. HPLC chromatograms of (a) Standard gallic acid (GA) (b) *C. pulcherrima* wood (c) *C. pulcherrima* pericarp (d) *C. decapetala* wood (e) *C. decapetala* pericarp methanol extracts.



(a)



(b)



(c)

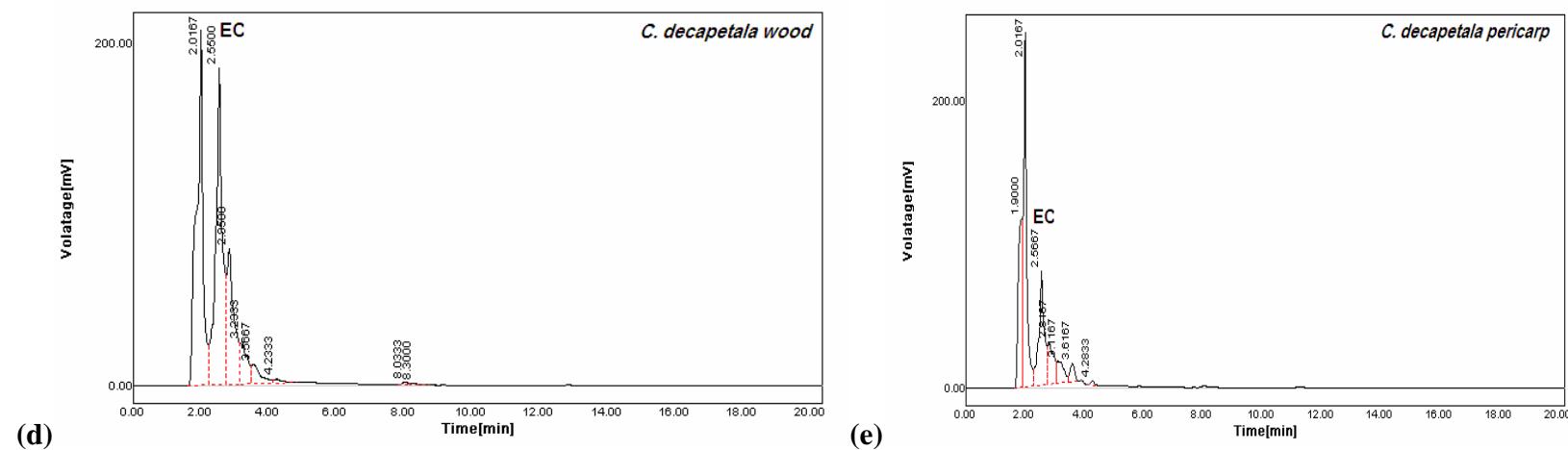


Figure 2. HPLC chromatograms of (a) Standard epicatechin (EC) (b) *C. pulcherrima* wood (c) *C. pulcherrima* pericarp (d) *C. decapetala* wood (e) *C. decapetala* pericarp methanol extracts.

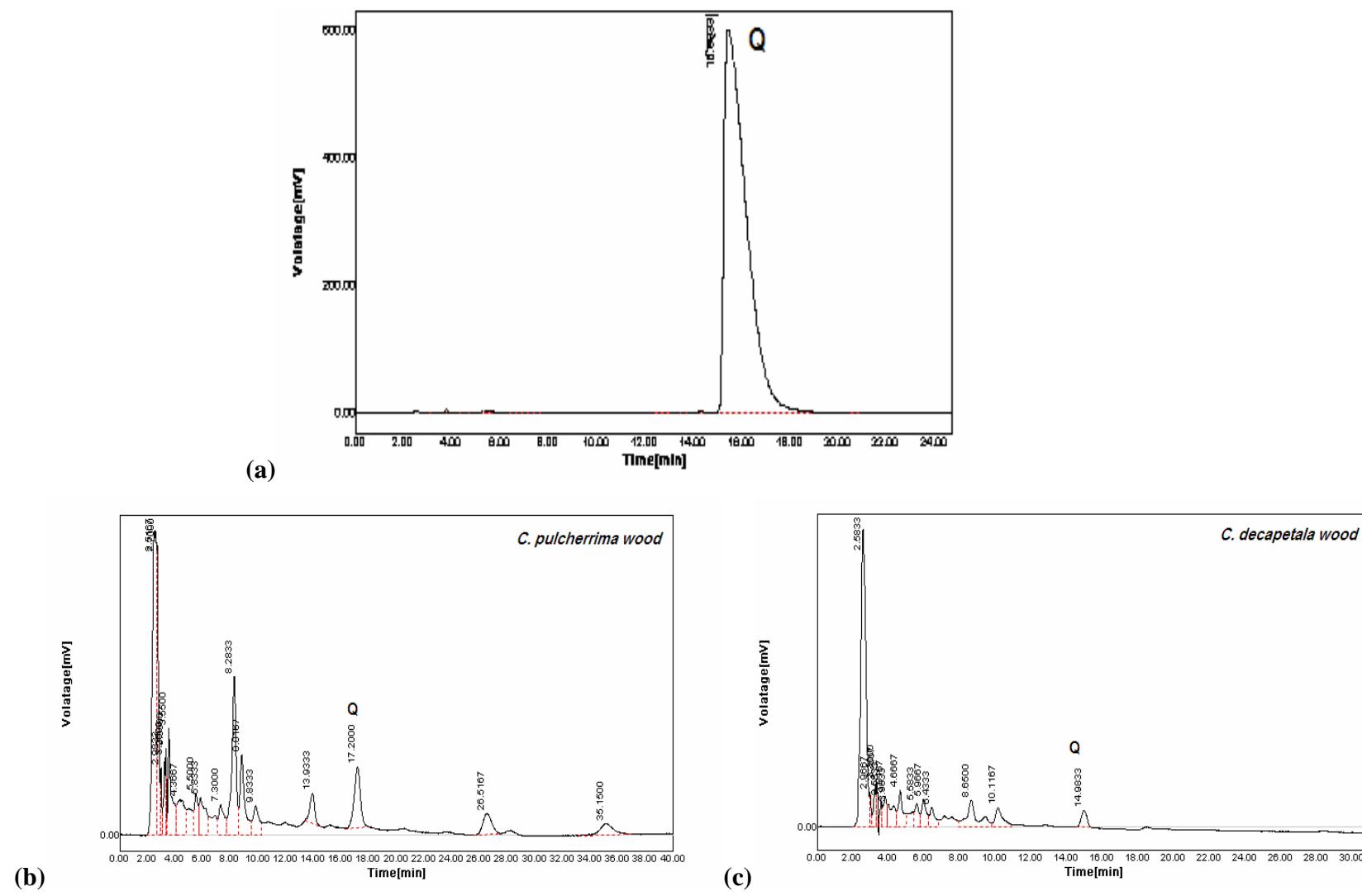


Figure 3: HPLC chromatograms of (a) Standard quercetin (Q) (b) *C. pulcherrima* wood and (c) *C. decapetala* wood methanol extracts.

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