

# Estimation of Biomarker Epicatechin in Ethanolic Bark Extract of *Acacia catechu willd* by HPLC Method

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## Abstract

The objective of our study is to estimate the epicatechin content present in *Acacia catechu* ethanolic bark extract by HPLC method. Estimation of epicatechin content in *Acacia catechu* ethanolic Bark extract was performed using C18 column (Luna, 5 $\mu$ m, 4.9mm x 150mm) in isocratic mode with a flow rate of 1.0 ml/minute. The mobile phase consisted of 5% acetic acid, methanol with a ratio of (85:15 v/v) with Ultraviolet-Visible detection at 280 nm. The method was validated in compliance with International Conference on Harmonization [ICH] guidelines. In conclusion, the epicatechin content present in the ethanolic bark extract of *Acacia catechu* is found to be 4.156% w/w.

## KEYWORDS

*Acacia catechu* Bark, Epicatechin, HPLC, ICH guidelines.

## INTRODUCTION

*Acacia catechu Willd.* (Family: Fabaceae and subfamily: Mimosoideae.) is widely used in ayurveda for treatment of various disease condition. It is highly valuable for its powerful astringent and antioxidant activities. It is useful in dental oral, throat infections and as an astringent for reducing oozing from chronic ulcers and wounds. The extracts of *Acacia catechu* exhibit various pharmacological effects like antipyretic, anti-inflammatory, antidiarrhoeal, hypoglycaemic, hepatoprotective, antioxidant and antimicrobial activities.<sup>2, 3-10</sup>

The phytochemical constituents present in *Acacia catechu* includes catechuic acid, catechu tannic acid, catechu red, quercetin, catechin, epicatechin, phlebotannin etc., It also contains active principles like cyanidol, tannins, flavonoids and polyphenols.<sup>5</sup>

Epicatechin is present in many plants. High quantities can be found in cocoa, tea and grapes. Pure epicatechin is an odorless white powder. Epicatechin is a flavonol belonging to the group of flavonoids and it is a powerful Antioxidant agent. Flavonoids are a group of polyphenolic compounds, which possess many biochemical effects like inhibition of enzymes, regulatory role on different hormones and pharmacological activities like antimicrobial, antioxidant, and anticancer, antihepatotoxic, protection of cardiovascular system.<sup>11</sup>

Various studies have proved the efficacy of Epicatechin in management of heart problems. It

reduces lipid peroxidation and inhibits platelet aggregation. Epicatechin cause blood vessel dilation by regulating nitric oxide, a molecule secreted by the blood vessel endothelium to signal surrounding muscle to relax.<sup>12</sup>

The review of literature revealed that no method is yet reported for the estimation of Epicatechin in *Acacia catechu* Ethanolic Bark extract. This prompted us to develop simple, accurate, precise and sensitive simultaneous estimation of Epicatechin by HPLC method. The method was validated as per International Conference on Harmonization (ICH) guidelines.

## MATERIALS AND METHODS

### Extract and Chemicals

The Ethanolic Bark extract of *Acacia catechu willd* was obtained from Green Chem, Herbal Extracts & Formulations, Bangalore. Epicatechin reference standard was purchased from Extrasynthese (Genay, France) Acetic acid and methanol HPLC grade solvents; all analytical grade solvents obtained from E-Merck Ltd, Mumbai, India.

### HPLC ANALYSIS FOR ESTIMATION OF EPI CATECHIN

#### Instrumentation

The Shimadzu class LC-20AT HPLC, Phenomenex C18 and a Rheodyne 7725i injector fitted with a 20  $\mu$ l loop, column oven, and a SPD-M20A photodiode array detector. The output signal was monitored and processed using LC Solutions

Version 1.21 SP1 software on a Pentium computer (Hewlett Packard).

#### Preparation of Standard Solution

A stock solution of Reference Standard of Epicatechin was prepared by accurately weighing about 5mg Epicatechin standard in 20 ml of Mobile phase in a volumetric flask. It was then sonicated for 15-20 minutes and the final volume of the solution was made up to 25ml with Mobile phase.

#### Preparation of Sample Solution

25 mg Sample (*Acacia catechu* Bark extract) is accurately weighed and dissolved in 20 ml of Mobile phase in a volumetric flask. It was then sonicated for 15-20 minutes then the contents of the flask were filtered through Whatman No. 41 paper (Merck, Mumbai, India). The final volume of the solution was made up to 25 ml with Mobile phase.

### METHODOLOGY

#### High-performance liquid chromatography method development

##### HPLC instrument :

Shimadzu LC –20AT Prominence Gradient system, comprising a quaternary pump, an automatic sampler and a photodiode array (PDA) detector was used with data acquisition by SPI software. The output signal was monitored and processed using LC Solutions Version 1.21 on a Pentium computer (Hewlett Packard).

#### Chromatography system:

- HPLC Column: Phenomenex Stainless steel column, Luna, 150mm x 4.9 mm packed with octadecylsilane bonded [C18] to porous silica ( 5  $\mu$ m ).
- Mobile phase: a mixture of 85 volumes of 5.0% *acetic acid* solution and 15 volumes of methanol
- Flow rate: 1 ml per minute
- Spectrophotometer set at 280 nm
- Injection volume: 20  $\mu$ l

**Reference Solution:** A 0.005 percent w/v solution of *Epicatechin RS* in *methanol*

**Test Solution:** Weigh accurately about 25 mg of the sample and dissolve in 100 ml of *methanol*. Sonicate in a sonicator for 5-10 min. Make up the volume to 100 ml, with *methanol* and filter.

Inject the reference solution. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 percent.

Inject the test solution and the reference solution. Calculate the content of the Epicatechin in the test sample.

##### Calculation of Assay:

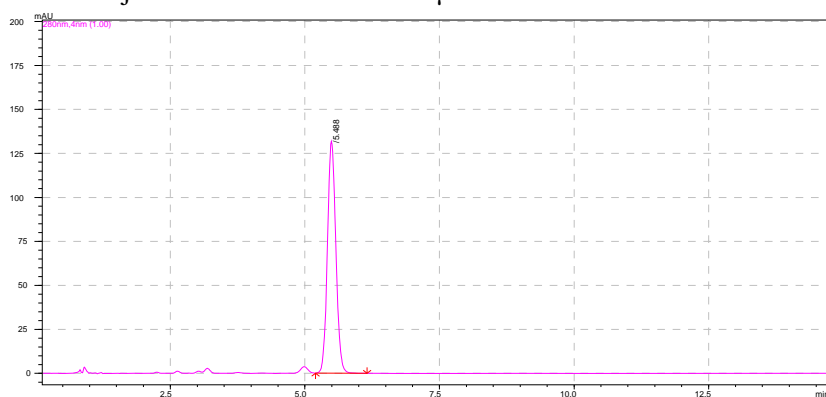
$(\text{Std Wt})/(\text{Sample wt}) \times (\text{Sample area}/\text{Std area}) \times \text{Std Purity} = \% \text{ Epicatechin in sample}$

The developed method was validated according to International conference on Harmonization guidelines (ICH)<sup>26,27</sup>

### CHROMATOGRAM ANALYSIS(HPLC)

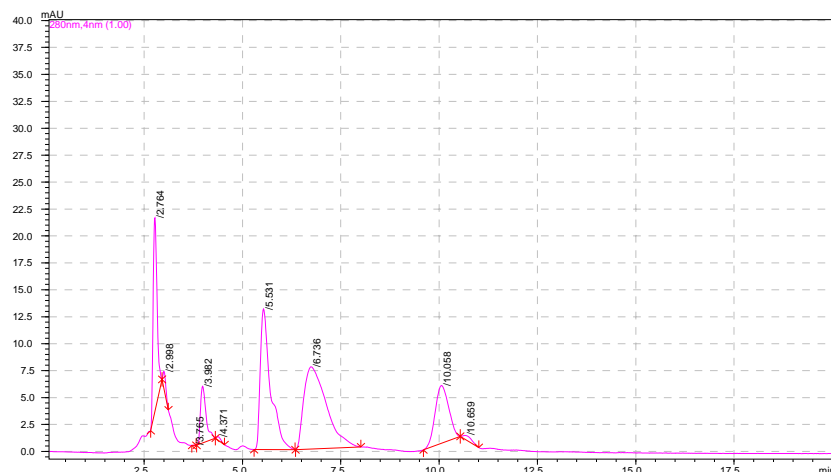
#### GRAPH 1 ESTIMATION OF STANDARD (EPICATECHIN)

Sample Name : EPICATECHIN/EXTRASYNTHESE /STD/ 100%  
 Vial : 1  
 Injection Volume : 20  $\mu$ l



## GRAPH 2- ESTIMATION OF EPICATECHIN CONTENT IN ACACIA CATECHU ETHANOLIC BARK EXTRACT

Sample Name : ACACIA CATECHU ETHANOLIC BARK EXTRACT  
 Vial : 2  
 Injection Volume : 20 µl



### HPLC ANALYSIS

**Equation 1 Calculation of Assay of Epicatechin**  
 $(\text{Std Wt} / \text{Sample Wt}) \times (\text{Sample Area} / \text{Std Area}) \times \text{Assay of Standard} = \text{Assay of Sample}$

### Calculation of assay of Epicatechin in *Acacia catechu* Bark(Ethanolic)Extract

Sample Weight taken : 25.2mg  
 Area of sample : 237307  
 Standard Weight taken : 5.9 mg  
 Reference number :  
 EPICATECHIN/EXTRASNTHESE /STD/ 100% (France)  
 Area of Standard : 1336910  
 Assay of Epicatechin : 100%

### Assay of Epicatechin in test sample:

$(5.9 / 25.2) \times (237307 / 1336910) \times 100\% = 4.156\%$   
 [w/w]

### RESULT AND DISCUSSION

Recent research indicates the polyphenols, being secondary metabolites, are present in rich amount in various plants. Many of them possess antioxidant, anti-inflammatory and several others therapeutic properties *Acacia catechu* willd

contains many active constituents in it like Catechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, phloroglucin, protocatechuic acid, poriferasterol glucosides, poriferasterol acyglucosides, lupenone, kaempferol, dihydrokaempferol Quercetin, Taxifolin etc.,

Epicatechin may improve blood flow and has potential for cardiac health. Cocoa, the major ingredient of dark chocolate, contains relatively high amounts of epicatechin and has been found to have nearly twice the antioxidant content of red wine and up to three times that of green tea in vitro.<sup>15</sup> In the test outlined above, it appears the potential antioxidant effects in vivo are minimal as the antioxidants are rapidly excreted from the body.

TABLE 1 –HPLC ANALYSIS OF STANDARD EPICATECHIN  
 PDA CH 280nm 4nm

Peak #	Ret. Time	Area	Height	Area %	Height %
I.	5.488	1336910	131700	100.00	100.00
<b>Total</b>		1336910	131700	100.00	100.00

TABLE 2 –HPLC ANALYSIS OF ACACIA CATECHU ETHANOLIC BARK EXTRACT PDA CH 280nm 4nm

Peak #	Ret. Time	Area	Height	Area %	Height %
1.	2.764	127327	18158	14.720	35.163
2.	2.998	7457	1512	0.862	2.927
3.	3.765	99	29	0.011	0.056
4.	3.982	52195	5264	6.034	10.194
5.	4.371	2345	300	0.271	0.580
6.	5.531	237307	13032	27.434	25.237
7.	6.736	303443	7633	35.079	14.781
8.	10.058	128659	5351	14.874	10.363
9.	10.659	6184	361	0.715	0.699
<b>Total</b>		865017	51639	100.00	100.00

TABLE 3 -RF VALUES OF HPLC ANALYSIS

Sample	Retention time (Authentic sample)	Retention time (Plant extract)
Epicatechin	5.488	5.531

Our HPLC studies (table 1 &2) revealed that the plant extract were also rich in Epicatechin (4.156%w/w), the assay of Epicatechin was calculated according to the Equation 1 .The retention times observed for the authentic sample of Quercetin and those of *Acacia catechu* ethanolic bark extract are given in Table 1. Study conducted by Shen D *et al* proved that the leaves and heartwood extract exhibits predominant amount of catechins.<sup>16</sup>

Hence our present study reveals that the antioxidant, antimicrobial, anti inflammatory, anticancer activity of *Acacia catechu willd* is not only due to presence of phytochemical constituent like Catechin, and Quercetin, it is also due to the Epicatechin which possess lots of medicinal value and proved to be a valuable agent to treat various diseases.

### CONCLUSION

In conclusion, our study provides new scientific information about *Acacia catechu ethanolic bark extract*, based on its antibacterial potential and chemical profiling that has never been reported. The antibacterial and antioxidant activity of *Acacia catechu bark extract* may be attributed to the various phytochemical constituents present in the refined extract.

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