

Estimation of Catechin in Leaf Extract of *Guazuma tomentosa* by HPLC

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Received: 7th Dec, 17; Revised: 9th Jan, 18, Accepted: 24th Jan, 18; Available Online: 25th Feb, 2018

ABSTRACT

Catechin is one of the major polyphenolic compounds found in numerous plant species and processed food products. It acts as a primary antioxidant and can be helpful for improving or preventing a number of chronic diseases. A new and simple analytical method using HPLC is developed for estimation of catechin. The method reported here can be applied for the quantitative analysis of catechin in leaf extract of *Guazuma tomentosa*, other medicinal plants, herbal formulation and food products.

Key Words: Catechin, HPLC, *Guazuma*, *Guazuma tomentosa*

INTRODUCTION

From the history of civilization herbal medicines were used to cure human ailments in every possible condition. In modern era we have the option to use them over the synthetic molecules because herbal drugs have lesser side effects¹. From the literature it is very clear that herbal drugs show their pharmacological action either due to specific constituent or due to blend of constituents. But the amount of constituents varies batch to batch due to ecological factors, time of collection of plant. Pharmacological effect of the drug can be obtained only when its concentration ranges within the therapeutic range. Any fluctuations above or below the therapeutic concentration lead to either toxic effects or no response². So it is necessary to determine the amount of secondary metabolite in plant or plant extract. *Guazuma tomentosa* is a plant native to tropical America, Ecuador and Colombia. Traditionally whole plant is used for its multipurpose benefits, e.g. As astringent, in cold, in cough, in diarrhoea, as diuretic, in dysentery, in venereal diseases, etc. Its non-medicinal uses involve, as a fuel wood, in making of charcoal, ropes (bark and stem; because of their tough and fibrous nature). Despite of its ethno-pharmacological uses, presently it is proven to have many therapeutic valuable uses because of the presence of many phyto-constituents E.g. colistin, colatannins, catechins, caffeine, kaempferol, procyanidin B-2, procyanidin B-5, procyanidin C-1, tartaric acid, theobromine, xanthan gum, etc^{3,4}. As most of the therapeutic potential of drug is due to tannins, so an

attempt has been taken to determine catechin content using HPLC. Various methods are available for the estimation of Catechin. The usual methods are spectroscopy method, HPTLC, and HPLC etc. Among these methods HPLC method is much more sensitive and provided superior resolution⁵.

MATERIALS AND METHODS

Plant material

The leaves of *Guazuma tomentosa* were collected in the month of September, 2011 from the Veermata jijabai bhosale udyan Byculla, Mumbai and get authenticated at Chinmaya College of Sciences, BHIL, Haridwar. The specimen of herbarium of plant is kept in domain of Pharmacognosy and phytochemistry, School of

Table 1: Chromatographic condition

Instrument	SHIMADZU (LC-10AT VP)
Column	C18 (5 μ , 250 \times 4.6mm)
Mobile Phase	Water (containing 0.1% acetic acid) and methanol (95:5)
Drug	Catechin
Injection volume	20 μ l
Flow rate	1ml/min
Detector	UV
Wavelength	280nm
Retention time	4.892

Table 2 Phytochemical screening of different extract of *Guazuma Tomentosa*

Phytochemical	Dichloromethane extract	Ethanol extract
1.Alkaloids		
a. Wagner's test	+	+
b. Hager's test	+	+
c. Mayer's test	+	+
d.Dragendroff's test	+	+
2.Carbohydrates		
a. Molisch's test	-	+
b.Fehling's test	-	+
3.Glycosides		
a. Borntrager's test	-	-
b.Modified Borntrager's test	-	-
4.Saponin		
Froth test	-	-
5.Phytosterols		
a.Salkowski's test	+	+
b.Libermann Burchard's test	+	+
6.Fixed oil & Fats		
Stain test	-	-
7.Resins	+	+
8.Phenols		
Ferric chloride test	+	+
9.Flavonoids		
a.Alkaline reagent test	+	+
b.Lead acetate test	+	+
10.Diterpenes		
Copper acetate test	-	+
11.Tannins		
Gelatin test	+	+
Matchstick test	+	+
12.Protein & amino acid		
Ninhydrin test	-	+

Table 3: Concentration vs. AUC value of Catechin

Concentration (µg/ml)	Rt	AUC
0.2	4.892	1250391
0.4	4.917	2600701
0.6	4.983	3861404
0.8	4.917	5111124
1	4.783	6294005

Pharmaceutical science, Lovely Professional University. The leaves were washed with tap water followed by drying under shade (temperature 30-40°C). Dried leaves were grinded to coarse powder and packed in suitable container for further study.

Extraction

DCM and ethanolic extract of *Guazuma tomentosa* was prepared by soxhlet extraction. Both extracts were subjected to phytochemical screening for qualitative analysis for presence and absence of secondary metabolite⁶.

Preliminary phytochemical screening

All the extracts were subjected to phytochemical screening for qualitative analysis for presence and absence of secondary metabolite^{7,8}.

Table 4: Concentration vs. AUC value of Catechin

Extract (1mg/ml)	Rt	AUC	Conc. of Catechin µg/ml	Amount of Catechin in µg/mg of extract	Amount of Catechin in mg of extract
DCM	4.800	1450278	0.231	0.231	0.231
Ethanol	4.892	1289372	0.206	0.206	0.206

Quantitative estimation of Catechin in extracts of leave of *Guazuma tomentosa* by HPLC

Chromatographic condition

Chromatographic condition is shown in Table 1

Calibration curve of Catechin

Different conc. of Catechin (0.20µg/ml, 0.4µg/ml, 0.6µg/ml, 0.8µg/ml, and 1µg/ml) was prepared in HPLC grade methanol and was subjected to HPLC analysis. The graph was plotted between conc. versus area under curve (AUC).

Preparation of test solution of ethanolic extract

Accurately weighed 10mg of ethanolic extract was dissolve in 10ml of methanol to get 1mg /ml of solution.

Preparation of test solution of Dichloromethane extract

Accurately weighed 10mg of Dichloromethane extract was dissolve in 10ml of Dichloromethane to get 1mg /ml of solution.

RESULTS AND DISCUSSION

Preliminary phytochemical screening showed the presence of blends of chemical constituent as mention in Table 2.

High Performance Liquid chromatography (HPLC)

The chromatograms obtained for standard catechin is shown in fig 1. The retention time (Rt) as well as area under curve (AUC) is shown in table 3.

Quantification of Catechin in ethanolic and Dichloromethane extract

The prepared test solution was subjected to HPLC analysis and AUC were noted. Finally amount of Catechin was estimated with help of calibration curve (Fig: 2).

The chromatogram of DCM and ethanolic extract is shown in fig 3 and 4 respectively. The Value of Rt and AUC of both extract is reported in table 4. The catechin content in DCM and etanolic extract was found to be 0.231 and 0.206 mg/g.

CONCLUSION

The increasing significance of catechin as a therapeutic necessitated its estimation in medicinal plants. A simple HPLC method has been developed and the results

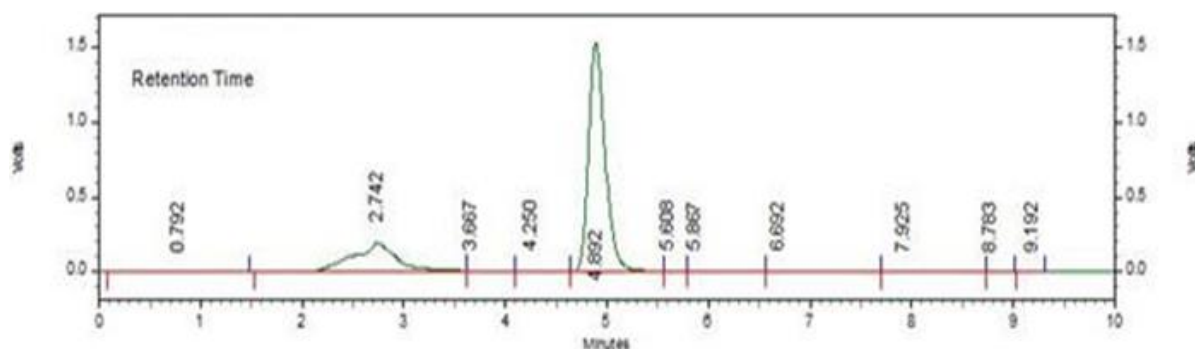


Fig 1: HPLC chromatogram of standard Catechin

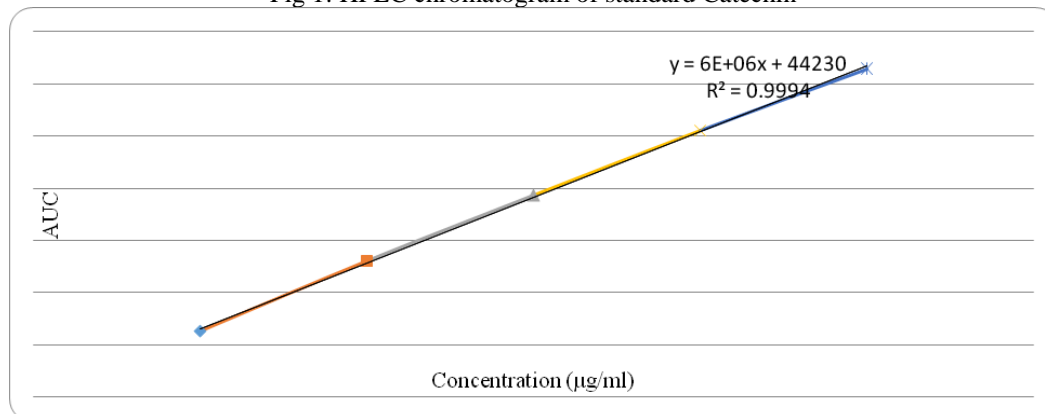


Fig 2: Standard plot of Catechin by HPLC method

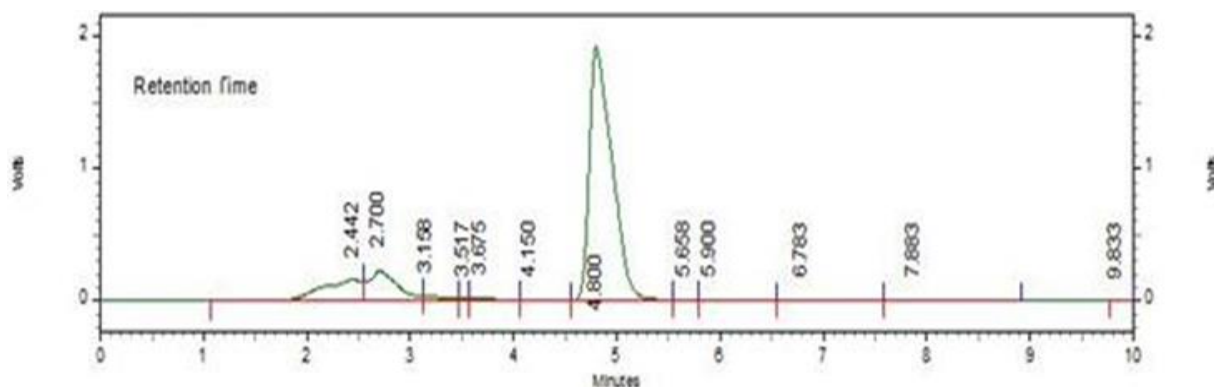


Fig 3: HPLC chromatogram of DCM extract

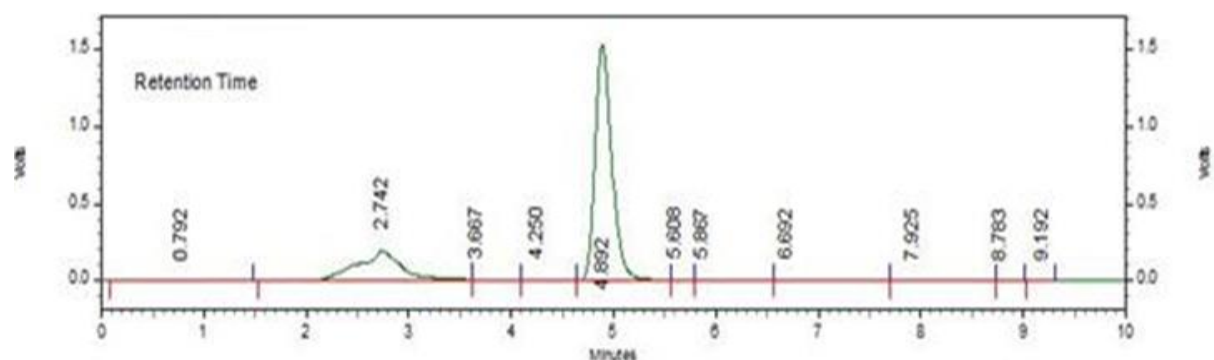


Fig 4: HPLC chromatogram of Ethanolic extract

demonstrate that it be used in the determination of catechin in medicinal plants, herbal formulation and food products.

REFERENCES

1. Sharma M, Chopra S, Prasad SB. Guazuma tomentosa: A Valuable Medicinal Plant.. *Int J Pharmacog Phytochem Res.* 2015, 7(1),197-200.

2. Verma H, Prasad SB, Yashwant SH. Herbal drug delivery system: A modern era prospective. *Int J Current Pharma Rev Res.* 2013;4:88-101.
3. Sharma M, Prasad SB. Evaluation of Anthelmintic Activity of Leaves Extracts of Guazuma Tomentosa. *International Journal of Pharmacology and Pharmaceutical Sciences.* 2014;1:1-5.
4. Sharma M, Prasad SB. Standardisation of Guazuma tomentosa leaf. *International journal of pharmacognosy and phytochemical research.* 2014 Dec;15(6):4.1010-1014.
5. Singh B, Santhakumar R, Bala I, Prasad SB, Verma S. Development and Validation of RP-HPLC Method for the Dissolution and Assay of Etoricoxib in Pharmaceutical Dosage Forms. *International Journal of Pharmaceutical Quality Assurance* 2014; 6(1); 1-7.
6. Sharma A, Verma S, Prasad SB., 2014. Evaluation of Anti-Obesity Activity of Convolvulus pluricaulis Extract. *International Journal of Toxicological and Pharmacological Research.* 6(4):148-152.
7. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy.* 47 edition, Nirali Prakashan, Pune.
8. Sharma M, Yashwant, Prasad SB., 2013. Hepatoprotective activity of Guazoma tomentosa leaf extracts against CCl₄ induced liver damage in rats. *International Journal of current pharmaceutical review and research.* 4 (4) 128-138.