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Original Research Article

Spectrometric Method Development and Validation of Catechin and Quercetin in Khadirarista

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Abstract: Catechin and quercetin are the major bioactive phenolic compound of *Acacia catechu,* was estimated and validated by RP- HPLC in khadirarista. The analytical method validation was carried out according to ICH method validation guidelines. The development was carried out in Lichrosper 100 C-18(250*4.6*5) column and gradient elution. The determining wavelength was confirmed as 280nm and 360nm for catechin and quercetin. The detection and quantification limits of catechin were 0.57mcg/ml & 1.74mcg/ml and for quercetin 0.52mcg/ml & 1.51mcg/ml respectively. The developed HPLC method is very accurate, precise and cost effective and it can be successfully applied to the assay of marketed formulation.
Keywords: Catechin, quercetin, *Acacia catechu*, khadirarista, RP-HPLC, validation.

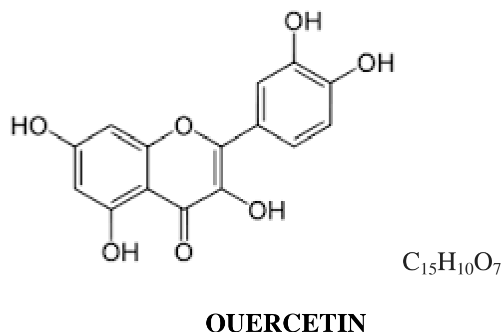
INTRODUCTION

About 2% of the materials formed by photosynthesis in plant body are transformed to flavonoids [1]. The role of flavanoids in the plant body is to protect itself from ultraviolet lights and bacteria [2]. A flavanoid called chalcone is formed first in the plant body and is transformed to various flavanoids such as flavones, catechin, epicatechin, quercetin etc [3]. Flavanoids are a group of naturally occurring polyphenolic compounds. Polyphenols have also been recently recognized as functionally active molecule possessing antioxidant, anticancer, antimutagenic properties as well as exerting protective effects against cardiovascular and other disease (Michael, 1999; John 2008).

Ayurveda is one of the best known ancient disciplines throughout the world and poly herbal formulation. Polyherbalism, dispensed water decoction or ethanolic extracts, therefore medicinal parts should be authentic and free from microbial contamination. WHO has set specific guidelines for the assessment of safety, efficacy and quality of the herbal medicines [4]. Arista has been used as medicine for over 3000 years to treat various disorders. Arista contains self-generated alcohol which has tremendous medicinal value, sweet taste and easy availability. The development of authentic analytical methods and quantitative analysis of bioactive compounds and other major constituents, is a major challenge to scientists. Hence standardization and development of reliable quality protocols for the formulation containing alcohol using sophisticated techniques of analysis is extremely important. Department of AYUSH has given preliminary guidelines for standardizing these conventional formulations.

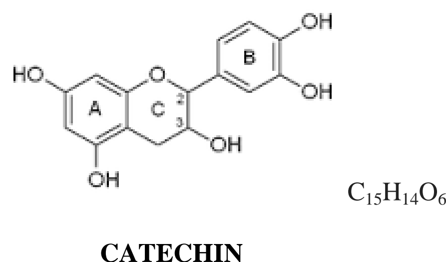
Khadirariista is one of the most important arista and considered as excellent anti lipid peroxidation activity with human erythrocytes. Khadirarista which shows presence of multi antioxidant compounds which might have synergistically contributed to restrain lipid peroxidation of human [5]. Khadirarista is mainly made out of the *Acacia catechu* along with some other herbs and spice plants. Quercetin and catechin is the major bioactive phenolic compound of Acacia catechu. Studies show that catechins and quercetin have antioxidant, antimutagenic and antiinflamatory [6, 7]. The purpose of this study is to determine the amount of quercetin and catechin in khadirarista by HPLC, and to validate as per ICH [8] guidelines.

CHEMICAL STUCTURE OF QUERCETIN AND CATECHIN



$C_{15}H_{10}O_7$

QUERCETIN



$C_{15}H_{14}O_6$

CATECHIN

MATERIALS AND METHODS

CHEMICALS AND SOLVENTS

Quercetin and catechin were procured from Natural Remedies, Bangalore, India. All chemicals and reagents used in the study were of analytical grade and purchased from Merck Specialist Pvt, Ltd, Mumbai. Commercial herbal formulation was purchased from the local market which contains *Acacia Catechu.*

INSTRUMENTATION AND CHROMATOGRAPHIC CONDITION:

Chromatographic separation analysis of quercetin and catechin were done using a prepacked column, Lichrosper 100, RP –e, a binary LC-20 AD prominance pump, an auto sampler (SIL-HTc) and a solvent degasser was used for study. A gradient mobile phase (**Table No: 01**) consisting of acetonitrile and buffer was to separate the analytes, at ambient temperature, and delivered at a flow rate1.5µl with column an effluent being monitored at 360nm and 280nm.

Table–1 : gradient condition

TIME	SOLVENT A	SOLVENT B
0.01	95.0	5.0
12.0	80.0	20.0
18.0	55.0	45.0
25.0	20.0	80.0
30.0	20.0	80.0
35.0	95.0	5.0
40.0	95.0	5.0

PREPARATION OF STANDARD SOLUTION

For the determination of quercetin and catechin, 10mg of standard was weighed and dissolved in 30ml of HPLC grade water by sonicating for 5min and then cooled made up to volume 100ml with water.

PREPARATION OF SAMPLE SOLUTION:

QUERCETIN: 5ml of sample was transferred to 50ml volumetric flask and dissolved in 20ml hot methanol. The flask was sonicated for 10mins and the volume was made up to 50ml with hot methanol. The solution was filtered through PES filter paper and injected for quantification.

CATECHIN: 2.0ml of sample (formulation) was taken to 50ml volumetric flask. 20ml of hot water was added to it and sonicated for 10mins. Supernatant was passed through PES filter and was injected for quantification.

HPLC METHOD DEVLOPMENT

The instrument was set as per the chromatographic condition that has been prescribed above.20µl of standard and sample was injected and the resultant chromatogram was recorded.

HPLC METHOD VALIDATION

The developed RP-HPLC method was validated as per ICH guidelines.

System suitability studies

System suitability test were carried out on freshly prepared standard quercetin and catechin by injecting six replicate and % relative standard deviation (%RSD) of peak area, were determined.

Calibration curve (Linearity)

To determine the linear relationship between peak areas and concentration of quercetin & catechin, six solutions with 10-50 mcg/ml were analyzed in triplicate and all the solution were injected three times. The linearity was calculated by linear regression analysis.

Precision

Interday and Intraday studies were performed in three concentration of the standard was repeated on three consecutive day.

Accuracy

Accuracy of the analytical method was studied by recovery experiments. Different amounts of standard were added (80, 100, and 120 %) to qualify. The solutions of different concentration levels are prepared in triplicate and each solution was injected once.

LOD & LOQ

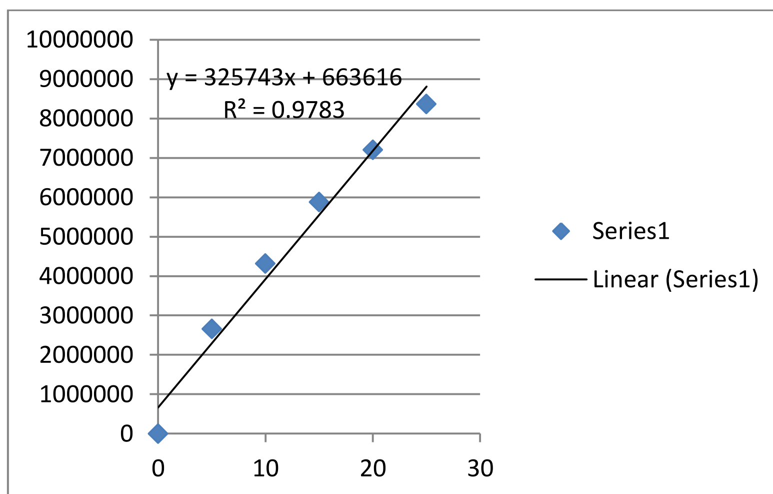
Both LOD & LOQ were calculated on the basis of calibration curve. The LOD was calculated as 3.3σ/s and LOQ was 10σ/s respectively, where s= slope of calibration curve and σ= standard deviation of the response.

RESULTS AND DISCUSSION

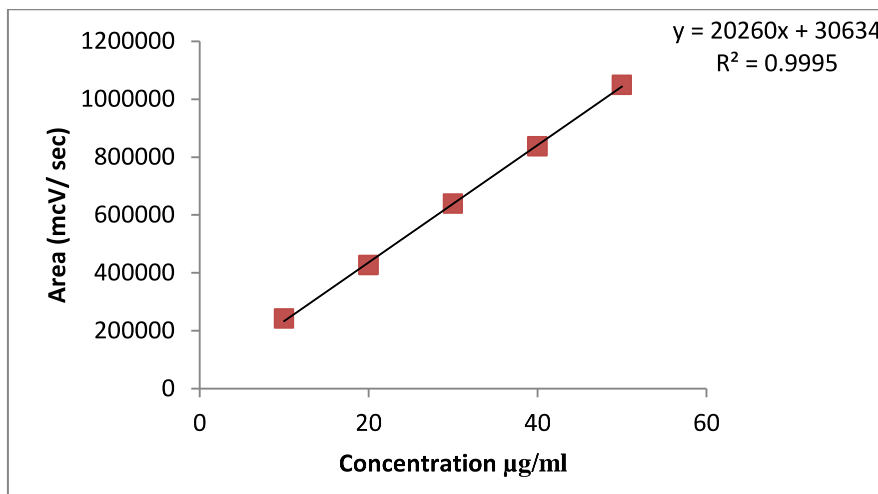
The chromatographic conditions were optimized to perform a good performance. The absorption spectra of catechin and quercetin were measured at 280nm and 360nm respectively (**Fig 03 & 05**). Linearity regression data show a good linear relationship between concentration and peak area over a concentration range of 10-50µl for quercetin and 5-25 µl catechin **(Fig 01&02)**. The correlation coefficient was found to be 0.978 and 0.999.Three replicate injection containing known amount of marker at 80%, 100% and 120% with respect to assay concentration. The developed method satisfies the acceptance criteria and ensures accuracy of method. The low coefficient of variation values of interday and intraday precision showed the developed method is precise and RSD% values were within the acceptance limit. LOD and LOQ were found to be 0.57µl & 1.74µl for catechin and 0.52µl & 1.51µl for quercetin respectively.

Table−2 : Validation Parameter Of catechin and Quercetin

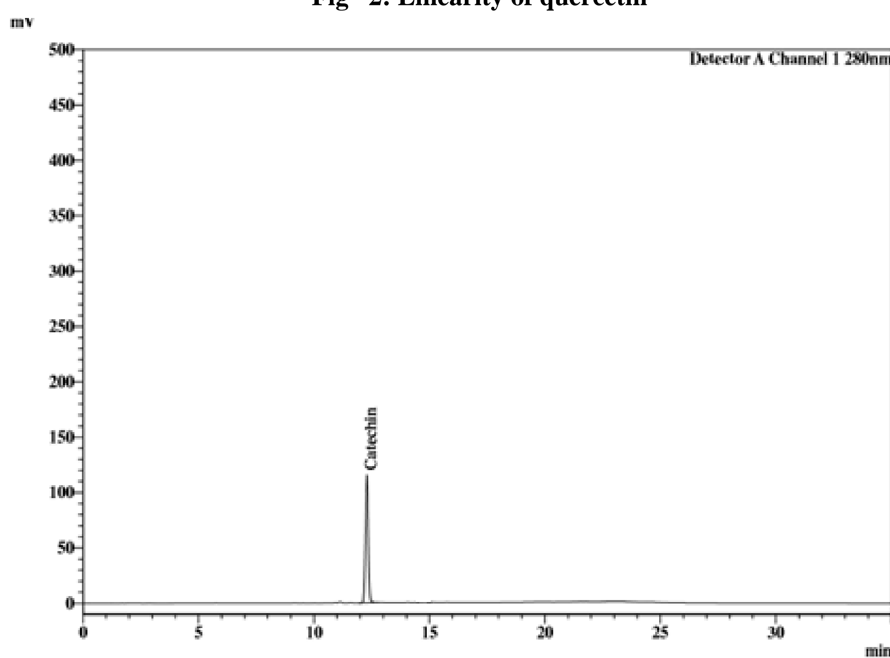
	Catechin	**Quercetin**
Retention time	12.24min	15.96min
Beer's law limit	5-25 µg/ml	10-50µg/ml
Wavelength	280nm	360nm
Regression equation	y =32574x-66361	y=20260x+30634
Correlation coefficient(r^2)	0.978	0.999
Precision(%RSD)	0.13	0.26
Accuracy (%)	99%	98%
Limit of detection(LOD)	0.57 µg/ml	0.52 µg/ml
Limit of quantification(LOQ)	1.74 µg/ml	1.51 µg/ml



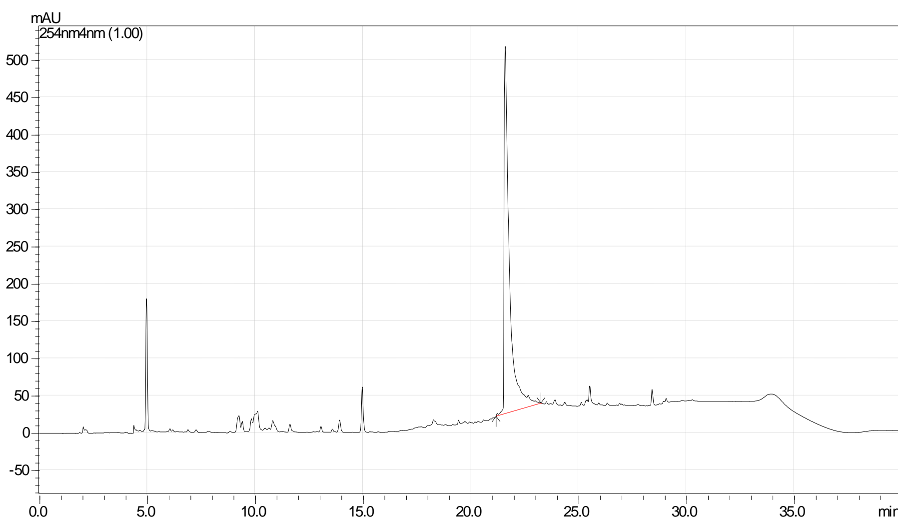
Fig−1: Linearity of Catechin



Fig−2: Linearity of quercetin



Fig−3: HPLC Chromatogram for Catechin (standard)



Fig−4: HPLC Chromatogram for Catechin (Sample)

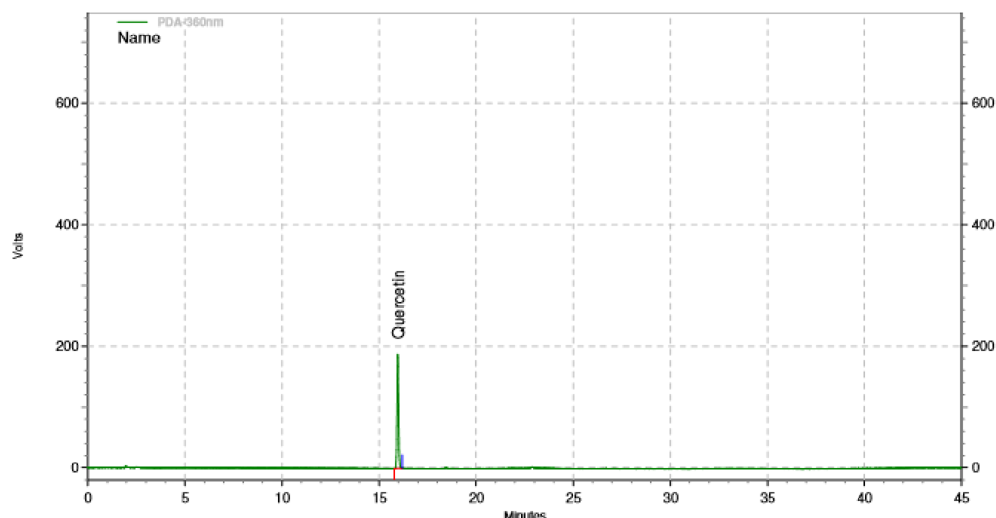
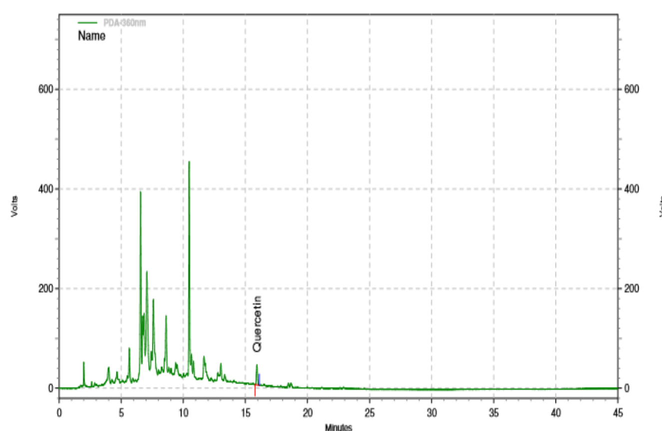


Fig –5: HPLC Chromatogram for Quercetin (standard)



Fig–6: HPLC Chromatogram for Quercetin (sample)

CONCLUSION

The proposed method is simple, rapid, specific, accurate and precise for determination of catechin and quercetin in khadirarista. Because of the short chromatographic run time, the developed method can be adopted for the routine quantification and quality control.

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