Short communication

Visible spectro-photometric determination of cerberin in rat plasma

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ABSTRACT

The present paper aims to develop a simple direct colorimetric method for the determination of cerberin in rat plasma without any previous chemical separation of the cerberin from *Cerbera odollam*. The method is based on reaction of the cardenolide group of cerberin with 3,5-dinitro salicylic acid [DNS] in alkaline medium which yields a bright orange-yellow complex that exhibits absorption maxima at 370nm.Beer's law obeyed in the concentration range of 50-250µg/mL. The result of the method was validated statistically and by recovery studies.

INTRODUCTION

Cerbera odollam is a tree belonging to the poisonous Apocynaceae family, which includes the yellow and common oleanders (Anantaswamy, 1940; Pichon., 1948). It is a powerful toxic plant that is currently completely ignored by western physicians, chemists, analysts and even coroners and forensic toxicologists. Cerberin (2-*o*-acetyl neriifolin) is the principal cardiac glycoside present in seeds of *Cerbera odollam* (Fig 1) (Chen *et al.,* 1942) found to be highly toxic (Hien *et a*l., 1991). It is widely used as a suicidal agent in Kerala state (Gaillard *et al*, 2004) of Indian subcontinent. *Cerbera venenifera*, a related species found in Madagascar, has a long history as an ordeal poison, and was responsible for the death of 3000 people per year in previous centuries (Yamauchi *et al*, 1987). The detection of cerberin in human body fluids is very difficult using conventional analytical methods. Only one method is reported so far for the determination of cerberin by UPLC-MS method (Carler *et al.,*

2014). Unfortunately, the assay methods are not handy in smaller medical facilities, as they require sophisticated devices, procedures and highly trained staff.

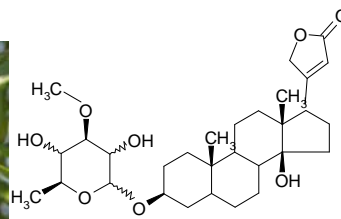

Fig. 1.*Cerbera odollam* **fruit.**

Fig. 2. Structure of cerberin

The structure of Cerberin (Fig 2) makes difficult for their assay in mixtures and even more difficult in complex matrices such as biological fluids or tissues, mainly if low-cost methods are available. Hence a simple colorimetric method is described for the determination of cerberin in rat plasma.

MATERIALS AND METHODS

Instruments

Shimadzu UV Visible spectrophotometer *Pharmaspec* 1700, UV Probe software 2.01 version, Shimadzu electronic balance AW 220 and Centrifuge Kemi 5600 were used for our study.

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Preparation of cerberin standard

One mg of accurately weighed cerberin was transferred in to 10 ml volumetric flask and dissolved in HPLC grade Methanol to get a concentration of 100µg/ml. Further dilutions were made with methanol to get 50-250 µg/ml of cerberin.
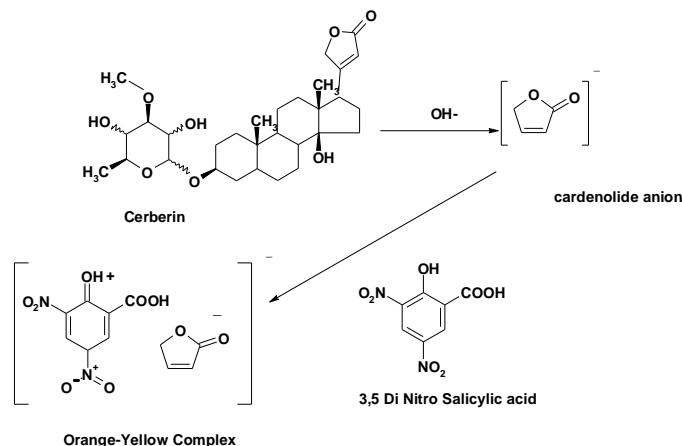
Preparation of sample

The rat plasma was diluted 1:10 with double distilled water. Appropriate aliquots from the stock solution and of the diluted rat plasma to get the desired concentration of cerberin were pipetted in testing tubes and gently vortex-mixed for 7 min. A blank plasma sample was also prepared, containing the amount of methanol used for the samples.

Preparation of calibration curve

Fresh aliquots of cerberin ranging from 0.5 to 2.5 mL (1 ml-1000µg/mL) were transferred into a series of 10 mL volumetric flasks to provide final concentration range of 50 to 250 µg/mL. To each flask 1ml of 3,5-dinitrosalicylic acid (1%) in methanol solution and 1ml of 0.1 N NaOH were added. The solution in each tube were made upto the mark with distilled water. The absorbance of orange-yellow colored chromogen was measured at 370 nm against the blank. The amount of cerberin present in the rat plasma was computed from its Calibration curve (Fig 3).

Reaction Mechanism

Application of the method for quantification of the Cerberin

The absorbance of the sample solution was measured keeping all the parameters same as that for the standard. The value obtained was compared with that of the values in calibration curve and concentration of cerberin in rat plasma was found out.

Sensitivity

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated to express the sensitivity of the developed method. LOD was calculated using the expressions, LOD=3.3 x SD/S, and LOQ =10 x SD/S, where SD stands for the standard deviation and S for the slope of the line in calibration curve. The LOD and LOQ were found to be 4.25 and 404 µg/ml respectively.

Precision

The method proved to be precise with respect to both repeatability and reproducibility. The method showed the same λ_{max} on repeated trials for different concentrations (Fig 4) in intra-day and inter-days.

RESULTS AND DISCUSSION

The optical characteristics such as Beers law limit, Sandell's sensitivity, molar extinction coefficient, percent relative standard deviation (calculation from eight measurements containing ¾th of the amount of the upper Beers law limit) were calculated and summarized in Table 1. Regression Characteristics like slope, intercept, correlation coefficient and percentage range of errors (0.05 and 0.01 confidence limits), LOD, LOQ, standard error of estimation were calculated and are shown in Table 1. Accuracy of the method was carried out in rat plasma by adding known amount of cerberin in to plasma and percentage recovery were calculated (Table2). Reagent strength used was also optimized. The stability of the chromogen developed were also studied (Fig 4). The result shows the absorbance was stable for more than ten minutes. The percentage recovery shows there is no interference of plasma with Cerberin. So this simple, sensitive, accurate and precise method can be used for routine analysis of Cerberin in human plasma also in the case of accidental or poisoning cases.

Table 1: Optical characteristics and statistics.

Parameters	
λmax (nm)	370 nm
Beer's law limits (mµ/mL)	50-250
Color	Orange yellow
Molar absorptivity (lit/mol-1 cm-1)	0.00426
Limit of Detection (LOD/ mcgml-1)	4.25
Limit of Quantification (LOQ/ mcgml-1)	404
Sandell's sensitivity (mcg/ml/0.001 abs units)	0.0015
Regression equation (Y*)	0.00094x+0.50120
Slope (b)	0.094 x 10-2
Intercept (a)	0.50120
Standard error of estimation	2.22x10-3
Correlation coefficient (r)	0.99932
% RSD	0.00192
Confidence limits with 0.05 level	0.0017*
Confidence limits with 0.01 level	0.0025*
**Average of eight determination,	

Table 2: Recovery data of Cerberin from Rat plasma

Amount of Sample (µg)	Quantity of Standard added(µg)	Amount of Cerberin recovered(µg)	Percentage of Cerberin	Average %
50	50	42.52	85	
50	150	139.74	93	92.33
50	200	248.71	99	

*Average of three determinations.

Standard Curve

$y = 0.00094 x + 0.50120$
r2 Correlation Coefficient = 0.99932
Standard Error of Estimate = 0.00222
Residual Standard Deviation = 0.00192

Standard Table

	Sample ID	Type	Ex	Conc	WL370.0	Wgt.Factor	Comments
1	1	Standard		50.000	0.550	1.000	
2	2	Standard		100.000	0.594	1.000	
3	3	Standard		150.000	0.639	1.000	
4	4	Standard		200.000	0.688	1.000	
5	5	Standard		250.000	0.737	1.000	
6							
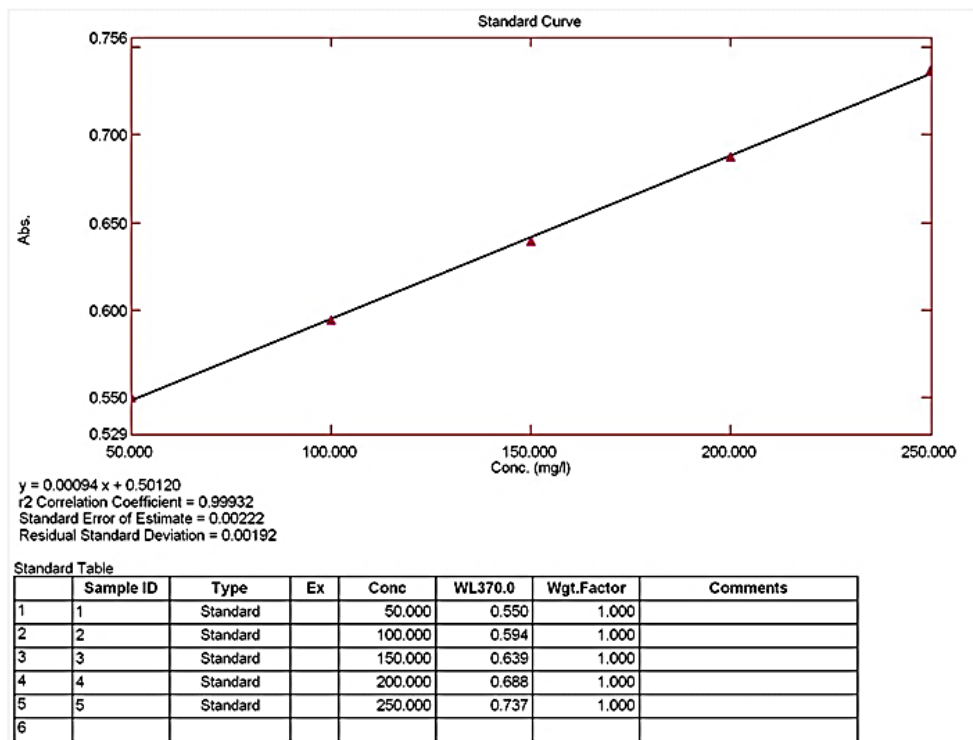
Fig. 3: Calibration curve of cerberin.

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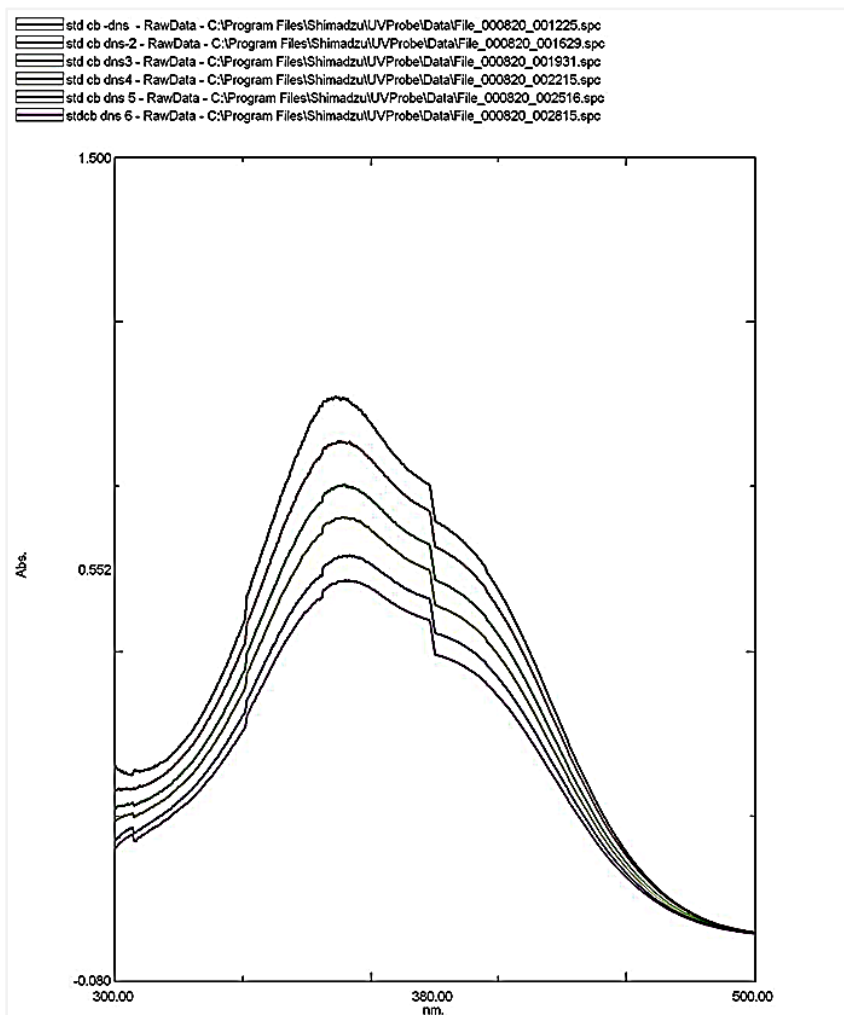
Fig. 4: Overlay spectrum of various concentrations of cerberin.

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