Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):812-818

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Differential pulse polarographic study of natural anticancer drug aloe-emodin in natural, biological and industrial samples

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ABSTRACT

Differential pulse polarographic (DPP) methods have been developed and authenticated for the qualitative as well as quantitative analysis of antineuractodermal agent, Aloe-Emodin (AE) in natural origin, human blood sample and industrial samples. 10 mm AE produces a well defined polarographic reduction peak in acetate buffer with E_p = - 1150 mv Vs SCE at pH 6.5 $\pm$ 0.1, however, the E_p value shifts to more electronegative value with increase in AE concentration due to change of the viscosity of the medium. The developed electrochemical methods have been standardized for the analysis of Aloe-Emodin (Antineuroactodermal agent) in pharmaceutical formulations. The electrochemical analysis has been supplemented by FTIR spectroscopic and HPLC methods. An isocratic high performance liquid chromatography (HPLC) method has also been developed and validated to determine AE in human blood plasma. The HPLC separation was carried out on symmetry shield RP18 a mobile phase of methanol –water-acetic acid (65:35:02) and UV detection of λ_{ex} = 410 nm and λ_{em} = 510 nm. The retention time of AE was 4.25 min.

Keywords: Aloe-Emodin, DPP, HPLC, antineuractodermal agent, chemopreventative agent.

INTRODUCTION

Aloe-Emodin (AE) 1, 8 dihydroxy 9, 10 anthracenadone is a known antineuractodermal agent [1, 2]. It is present in aloe-vera leaves [3, 4]. AE is the tumor cell-specific drug. It shows toxicity for malignant tumor such as neuroblastoma cells, Ewing's sarcoma cells etc. It is believed to be a good inhibitor for the proliferation of different types of cancers [4-6] without any damage to the normal cells [7-10]. These specific properties of the AE make opportunities for its use as a novel anticancer agent.

The goal of the present study is to authenticate differential pulse polarographic (DPP) methods for an indigenous analysis of the Aloe-Emodin in phytochemicals and industrial samples. Organic compounds having ketonic groups produce well-defined polarographic responses and can be accurately determined [11] qualitative as well as quantatively. A survey of literature reveals the use of chromatographic and spectral methods for the analysis of AE in samples of different origin [12]. However, a little work has been done on AE analysis using electrochemical DPP methods. This paper reports an inclusive procedure for the analysis of AE by electrochemical and some other physico-chemical methods in samples of different origin. HPLC technique has been applied to explore the identification of AE in blood plasma.

EXPERIMENTAL SECTION

Material and Reagents:

The chemicals used were of analytical reagent grade. However, Aloe-Emodin was of Sigma-Aldrich grade, Aloe-Vera leaves were purchased from local market. Double distilled water and ethanol were used for preparing solutions. HPLC grade methanol and water was obtained from Fluka Riedel-detlaen.

Instrumentation and Conditions:

(1) For Polarography: For DPP studies µp-polarographic analyser (Elico, India) model CL –362 is used. An Elico digital pH meter model 335 was used for pH measurements. The polarographic cell consisted of three electrode assembly with a saturated calomel electrode (reference electrode) and platinum electrode (auxiliary electrode). The working electrode was a dropping Mercury electrode.

(2) For HPLC Analysis: A Thermo finnigan HPLC, spectral system vaccum membrane designer SCM 1000, gradient pump P400, autosampler AS3000, a UV detector uv6000 hp, SN 4000, Software version 2.51, symmetry shield RP 18 (3.5 µm) was used.

(3) For Spectroscopy: Perkin Elmer spectrum one FTIR spectrometer was used for FTIR spectroscopic measurement equipped with an universed ATR Accessory.

Sample Preparation for polarography analysis :

(i) Extraction of Aloe-Emodin from aloe-vera plant leaves - The Aloe-vera leaves were cut into 6" – 9" pieces. The upper green skin of leaves was scratched with a sterilized knife. These scratched pieces were cut into the small pieces and mashed with water and filtered. The filtrate was mixed with a known volume of ethanol.

(ii) Authentic AE sample - 242.2 mg AE was transferred to a 100 ml volumetric flask and dissolved in 100 ml ethanol, sets of solution containing varying concentration of AE were prepared in acetate buffer of pH 6.5 $\pm$ 0.1 for polarographic study.

(iii) Preparation of solution of pharmaceutical formulations- Pharmaceutical formulation kumaryasava (A) and green gel (B) contain Aloe-Emodin. 2 ml of kumaryasava with 4 ml of ethanol was transferred to a polarographic cell containing acetate buffer of pH 2.3 $\pm$ 0.1, similar procedure was followed to convert green gel 50 mg in to analyte of acetate buffer at pH 4.7$\pm$ 0.1 for polarographic study.

Determination of Aloe-Emodin in different samples:

(i) In Authentic Sample - Aloe-Emodin produces a well defined polarographic wave/peak (fig. 1 DPP) in acetate buffer of pH 6.5 $\pm$ 0.1 with E_p = - 1150 mv vs SCE. Its peak height (ip) was found to be proportional to AE concentration.

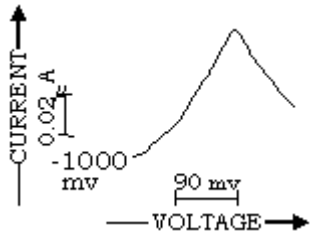

Fig. 1: Authentic sample at pH 6.5 ± 0.1 (DPP)

(ii) In Aloe-vera leaves and pharmaceutical formulations :

The Aloe-vera leave extract when subjected toDPP study also produced a well defined peak similar to that observed with authentic AE sample with E_p value. The concentration of Aloe-Emodin in the extract was determined by external spiking method. The results have been depicted in table 1.

Table 1: Polarographic analysis (DPP) of AE in Extracted sample

S.No.	Sample	Amount of AE (mg/ml)		Percentage recovery	% S.D.
		Added	Found*		
1	Extracted sample 15 ml	— 25.0	23.57 48.4	99.8	0.45
2	30 ml	— 50	47.17 97.0	99.89	0.38

** Average of four determination*

Similar procedure was used for its determination in pharmaceutical formulations, but pH was adjusted to 2.3 $\pm$ 0.1 and 4.7 $\pm$ 0.1 for its analysis in Kumaryasava and green gel samples respectively. The results have been tabulated in table 2.

Table 2: Polarographic analysis (DPP) of AE in Pharmacological sample (A) Kumarayasava and (B) Green gel

S.No.	Sample	Amount of AE (mg/ml)		Percentage recovery	% S.D.
		Added	Found*		
1.	Kumaraysava (A) 1 ml	— 0.242	1.85 2.095	99.2	0.38
2.	2 ml	— 3.5	3.70 7.15	99.30	0.35
3.	Green gel (B) 4 ml	— 2.42	4.03 6.42	99.98	0.42
4.	8 ml	— 8.00	8.06 16.00	99.7	0.32

** Average of four determination*

Effect of pH:

The pH of test solution is a key factor to get better polarographic waves. In the present experiment the pH 6.5 ± 0.1 of the test solutions has been used for the determination of AE in authentic and natural origin samples.

At this pH of test solution the observed peak, were well defined and i_d/E_p of the polarogram was found to be proportional to the AE concentration. However, in the analysis of pharmaceutical formulations the matrix effect was much stronger at pH 6.5 ± 0.1. As such the determination of AE in pharmaceutical formulation was done at pH 2.3 ± 0.1 in Kumaryasava and green gel at 4.7 ± 0.1 under this above pH values the matrix effect is minimized and very well defined. Polarographic signals are observed.

3. Sample preparation for HPLC:

(i) Preparation of standard solutions:

For HPLC analysis standard solution was prepared weighing 5 mg of AE on a microbalance and transferred to 50 ml volumetric flask and dissolved in appropriate amount of methanol to yield 100 μg/ml solution and the solution was further diluted with methanol to obtain working Aloe-Emodin at conc. of 1, 5,10 μg/ml (wAE) standard solution (wAE).

Preparation of plasma solutions:

Every run analysis of 3 plasma concentration standards at different AE concentrations in the range of 250–1000 ng/ml were prepared containing 270 or 280 l0 μl blood plasma with different amount of AE working solution according to the following scheme

Standard (ng/ml)	Preparation
250	15 ml wAEg 5.0μg/ml + 285 μl plasma + 15 μl methanol
500	20 ml wAE 5.0 μg/ml + 270 l μl plasma
1000	30 ml wAE 10.0 μg/ml + 270 l μl plasma

Extraction Procedure :

Plasma sample (0.3) ml was dilutd 1:1 (v/v) with water and extracted with 5ml dichloromethane. After 20 min of shaking samples were centrifuged at 4,000 rpm for 10 min at 4ºC, 3.2 ml of organic phase were accurately measured separated into tubes. Then vortexed for 1 min and centrifuged again at 14,000 rpm for 5 min. A volume of 50 ML of the superanatent was put in to the sample tubes of HPLC for qualitative as well as quantitative analysis.

Chromatographic Conditions:

HPLC analysis were carried out using a symmetry shield RP 18 (3.5 μm x 4.6 mm x 150 mm) column equipped with a guard column of the same material and mobile phase of methanol-water-acetic acid glacial (65:35:0.2). The flow rate 1.4 ml/min and peak was detected with ultraviolet detector at λ_{ex} = 410 nm and 51λ_{em} 0 nm and HPLC analysis of only 15 min. run time.

RESULTS AND DISCUSSION

In acetate buffer of pH 6.5 ± 0.1 the AE present in the extract of Aloe – vera showed one DPP signal (fig 2) but pH was adjusted to 2.3 ± 0.1 and 4.7 ± 0.1 for the analysis of pharmaceutical formulation Kumaryasava (A) (fig. 3) and Green gel (fig. 4). The observed E_p values were – 1150 mv that varied with the increased concentration of the extract/Pharmaceutical formulation as is the case with authentic AE sample. The shifts in E_p values may be explained on the basis of changed viscosity of the medium which causes a change in diffusion coefficient [13, 14].

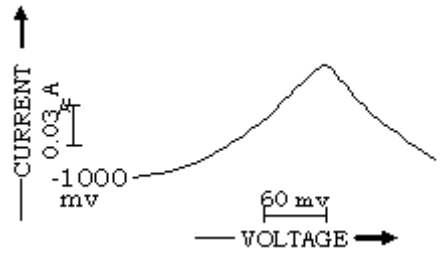

Fig. 2: Extracted sample at pH 6.5 ± 0.1 (DPP)

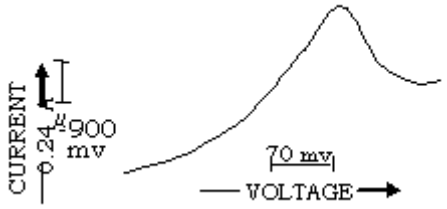

Fig. 3 : Pharmaceutical formulation Kumaryasava (A) at pH 2.3 ± 0.1 (DPP)

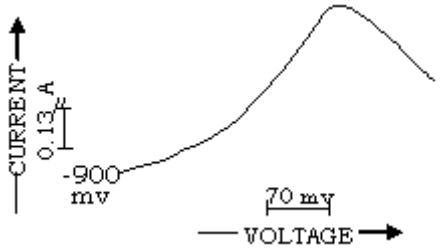

Fig. 4: Pharmaceutical formulation Green Gel (B) at pH 4.7 ± 0.1 (DPP)

Aloe-Emodin containing keto group produces well defined polarographic wave with its wave height, proportional to its concentration. Method of standard addition was used for quantitative analysis. The results have been depicted in table i. The percentage recovery is found to be more than 98% in each case. The standard deviation and coefficient of variance never exceeded 0.5% and 0.9% respectively. The statistical data in the table speaks the reliability of the observed results. The recovery was over 99.9% for Aloe-Emodin in natural sample and pharmaceutical formulation.

The HPLC method described was used to assess for the separation and determination of the Aloe-Emodin in blood plasma. It should be noted that the chromatogram were recorded using UV detector at at λ_{ex} = 410 m and λ_{ex} = 510 nm shown in fig. (5) and fig. (6). the chromatographic separation and resolution from plasma matrix appears very good. The method is very rapid and simple.

FTIR spectroscopy has been used for the characterization of the sample of Aloe-Emodin. The FTIR spectra of authentic AE, using ATR accessory clearly shows three characteristic signals at (A) 1268.85 cm^{-1} (B) 740 cm^{-1} (c) 1623 cm^{-1} respectively, corresponding to the presence of AE extracted from Aloe-vera also showed three signals at the same wave numbers. Thus, confirming the presence of 1, 8 dihydroxy anthraquinone in the extracted sample.

Validation study:

DPP analysis depends on the physical environment and chemical behavior of the AE. The external spiking method has been used to validate the DPP methods applying for the analysis of natural (AE) extract and pharmaceutical formulation samples. The polarographic results on the spiked samples showed good percentage recovery of electroactive compound from the analytes. Thus confirming the utility of the developed procedure for an accurate analysis of AE in the samples.

Chromatograms for AE (fig VI, Vii) were evaluated for the peak area of AE. A linear relationship from the plot of peak area for each signal of the chromatogram vs AE concentration (authentic sample) was observed. The peak area as observed by AE in extracted sample was compared with the above calibration plot to evaluate this value of AE present in blood plasma. AE chromatogram in blood plasma and blank blood plasma (fig 5, 6) give satisfactory results.

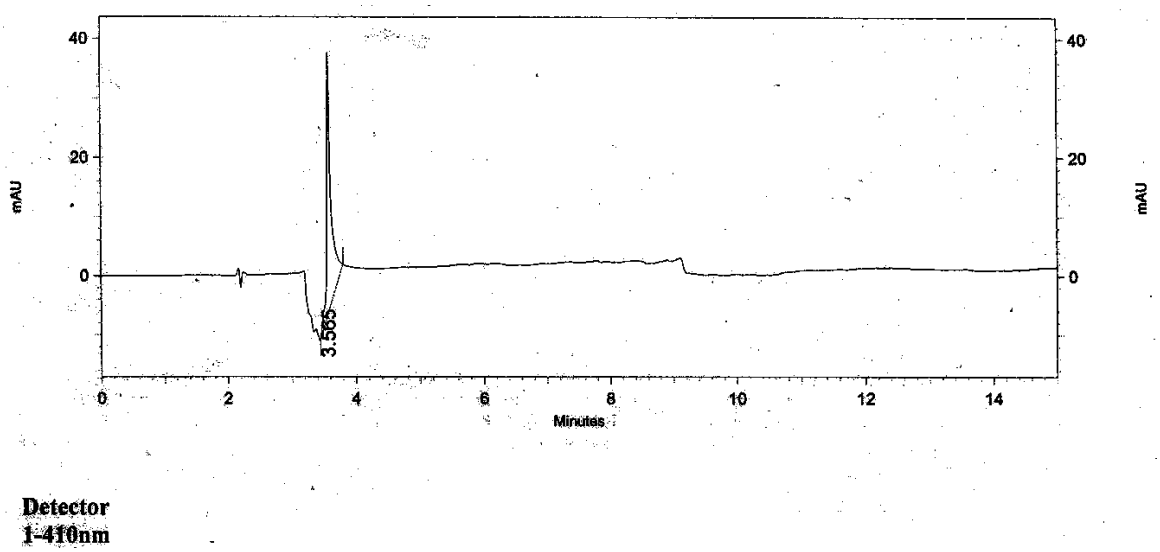

Fig. 5: HPLC Chromatogram of blank blood plasma

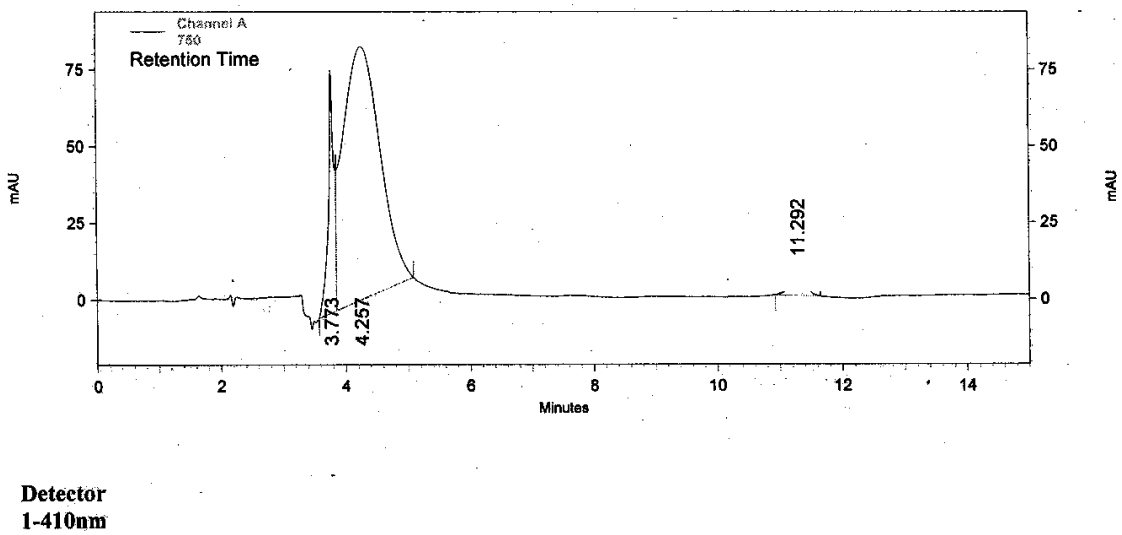

Fig. 6 HPLC Chromatogram for AE in human blood plasma (1000 ng/ml)

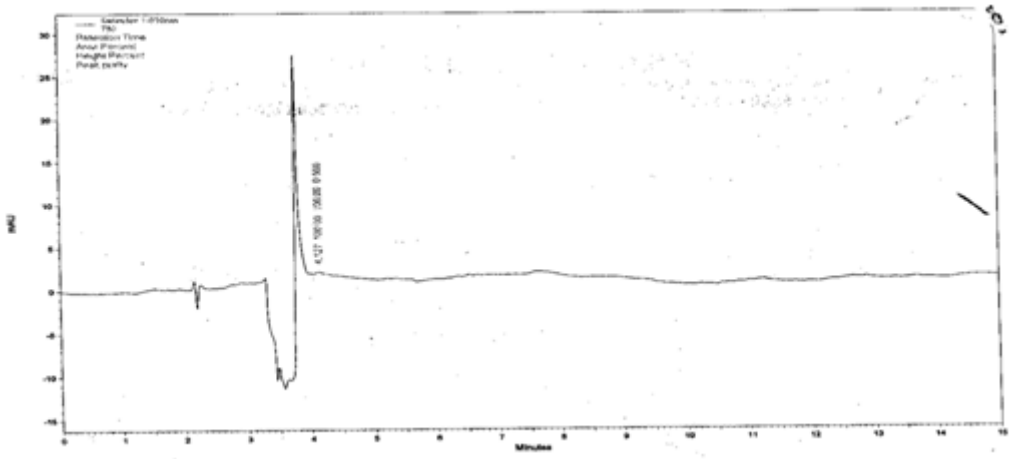

Fig. 7: HPLC Chromatogram for AE in human blood plasma(1000 ng/ml)

CONCLUSION

Although DPP, HPLC, FTIR spectroscopy could be successfully used for the chemical analysis of Aloe-Emodin in authentic and samples obtained from different origin but, DPP being species sensitive methods and following the developed procedures it has been possible to scrutinize. Samples of different origin i.e. phytochemical, pharmaceutical formulation, blood samples either without extensive extraction of pure AE from its source and it could scrutinize AE directly in pharmaceutical formulation. Besides, the use of electrochemical methods is highly economic.

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