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Standardization of Emodin-An Bioactive Molecule, Using Spectral Methods

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Abstract

A simple, accurate, sensitive, precise & reproducible UV spectroscopic method has been developed for the standardization of emodin in pure form and in indifferent extracts of medicinal plant. Emodin was estimated at 437 nm in methanol medium. Beer's law was obeyed in the concentration range of 10-100 µg/ml (r2= 0.973). The method was tested and validated for various parameters according to the ICH (International Conference on Harmonization) guidelines. Emodin content in extracts of various plant parts carried out at λmax. The detection and quantification limits were found to beo.41µg/ml and1.25µg/ml, respectively. The present study deals with stability of Emodin, studied for futher using spectral reagents like NaOMe,NaOAc,$AlCl_3$ for their spectral shifts studied by UV-VIS spectroscopic and Infrared spectral method. Emodin content in extracts of various plant parts of *Cassia auriculata* L was found using same method. The results demonstrated that the procedure is accurate, precise and reproducible being simple, cheap and less time consuming, and hence can be suitably applied for the estimation of emodin in different dosage forms.

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Key words:
Spectrophotometric Method, standerdization, Accuracy, Emodin.

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Introduction

Antibiotic resitance from pathogenic microorganism renders drug ineffective and calls for improved designing and development of new drugs.Various medicinal plants contain 1,8-Dihydroxyanthraquinone and its derivatives that, have been isolated from various botanical families

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such as Rhamnaceae (buckthorn,cascara), liliaceae(aloe), polygonaceae(rhubarbs) and Caesalpiniaceae(senna)[1]. Emodin isolated from cassia family such as *Cassia fistula, Cassia auriculata* L , posses potent antimicrobial activity against skin infecting pathogenic organisms[2,3].Emodin is a biologically active, naturally occurring anthraquinone derivative[4]. From ancient times, herbal extracts of *c. auriculata* have been used in medical treatment[5]. Several scientific studies of its biological activity have been performed[5]. More recently, investigations are stressed towards spectral methods. The aim of the present study is to validate the potency of the molecule by UV-VIS and Infrared spectral methods. Various methods have been reported in the literature for the analysis of emodin[6] . The objective of the present study is to develop simple, precise, accurate and economic analytical method for the standardization of emodin. The developed analytical method is validated as per the ICH (International Conference on Harmonisation) guidelines[7]. Present study is carried out from various extracts of various parts of *C. auriculata* L along with biomarker like emodin.

Materials and Methods

All chemicals and solvents used were of Spectroscopic grade. Emodin, standard compound was procured from Sisco Research Laboratory. UV-VIS was recorded on Schimadzu UV-1700 Spectrophotometer. The IR Spectra (4000-350cm^{-1}) was recorded on Shimadzu FTIR84005 spectrophotometer.

Preparation of Standard Solution

Accurately weighed emodin (1mg) was, transferred to 10 ml volumetric flask and dissolved with methanol to get a standard solution of 100µg/ml.

Preparation of reagents

1. Unhydrous Sodium acetate reagent was used as it is.
2. Aluminium chloride solution (5%)
3. Sodium methoxide (2.5%)
4. NaOH (5%)

Collection of Plant materials and preparation of extracts

Cassia auriculata L was collected from Western Pune, Maharashtra, India. The taxonomic identification was carried out with the help of Flora of Botany Presidency and Flora of Maharashtra (Singh et al.2000) and herbaria were prepared by following standard methods. The specimen was authenticated at, Botanical survey of India, Maharashtra, India .Its voucher specimen number is BSI/WC/Tech/2009/95.

Air shade dried and pulverized parts of plant material was used. Extracts were prepared using exact weighed sample powder by refluxing in solvents such as acetone,ethanol,methanol. The solvents were recovered under reduced pressure .The dried extracts were used for the experiments.

Determination of λ max

Standard solution (100µg/ml) was scanned in UV-VIS range (200-800nm) for maximum absorbance after enabling blank correction for methanol in the above region. The maximum absorbance(437nm) was observed.

Results and Discussion :

Calibration curve

Various concentrations (10-100 µg/ml) of standard solution were prepared using methanol. The absorbance was measured at 437nm against a solvent blank and from it validation parameters were calculated (**Table 1).**

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Table 1: Validation parameters.

Validation Parameters	Observations
lamda max (λmax)	437nm
Regression equation(y=a+bx)	y=0.0034x
Slope.(S.E.) a	2.40(0.0312)
Y- intercept.(S.E.)	a 0.034(0.0012)
Range(µg/ml)	10-100
Correlation coefficient (r)	0.989
Correlation coefficient (*r*2)	0.973
Limit of detection (µg/ml)	0.41
Limit of quantification (µg/ml)	1.25

a=Standard error of mean

Stability Profile

Stability of absorbance is of major importance in Spectrophotometric measurements. The period over which absorbance at 437 nm of emodin in methanol remain stable was investigated using concentration range(10-100µg/ml). The absorbance were measured for above solutions at 15 min intervals for a period of 1 hour.

Table 2: Stability profile(n=3)

No.	Concentration (µg/ml)	Absorbance at 437 nm at time intervals in minutes Mean ± SD				
		0	15	30	45	60
1	10	0.076±0.001	0.076±0.001	0.076±0.001	0.076±0.001	0.085±0.001
2	20	0.094±0.001	0.099±0.002	0.101±0.001	0.104±0.004	0.106±0.005
3	30	0.127±0.002	0.129±0.001	0.131±0.003	0.131±0.001	0.135±0.001
4	40	0.137±0.003	0.138±0.001	0.140±0.002	0.142±0.002	0.144±0.004
5	50	0.172±0.001	0.174±0.002	0.176±0.004	0.177±0.003	0.178±0.003
6	60	0.203±0.002	0.205±0.001	0.206±0.003	0.208±0.002	0.210±0.002
7	70	0.267±0.001	0.269±0.002	0.271±0.002	0.274±0.001	0.277±0.004
8	80	0.299±0.002	0.302±0.003	0.306±0.002	0.308±0.003	0.310±0.005
9	90	0.316±0.003	0.318±0.002	0.320±0.001	0.321±0.001	0.322±0.004
10	100	0.332±0.001	0.333±0.004	0.334±0.001	0.336±0.002	0.337±0.003

Spectral shift study:

Emodin was verified with spectra shift reagents like NaOMe, NaOAc, $AlCl_3$.The stock solution of emodin was used along with the above mentioned of reagents.The shifts in absorption with respect to λ max of emodin and comparitive spectral peak scans for the different spectral reagents were recorded. (fig 2).The analytical parameters such as Molar extinction coefficient, P^H, conductivity are recorded under same set of condition (Table 3).

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Table3: Absorption maxima, Molar extinction coefficient, P^H, Conductivity with various reagents:

No.	Reagents	Absorption Maxima(λmax)	Molar Extinction Coefficient (ε)	P^H	Conductivity $m\Omega^{-1}$
A	Emodin	437	821.35	6.4	0.54
B.	Emodin+ NaOAc	495,467	547.83,555.94	8.8	1.13
C.	Emodin+NaOMe	526	368.37	13.0	7.75
D.	Emodin+$AlCl_3$	482	290.54	6.5	0.47
E	Emodin+NaOH	529	587.29	10.4	0.34

IR spectral study

IR spectra were recorded in Nujol under same set of condition for the test solutions A,B,C,D over a range of 4000-400cm^{-1} to study the change in stretching and bending frequency of carbonyl and the hydroxyl group region of emodin(Table 4).

Table4: Change in frequency of-O-H and -C=o with various reagents.

Sl. No	Reagent	-O-H frequency cm^{-1}	-C=o frequency cm^{-1}
A	Emodin	3390.97	1626.
B	Emodin+NaAc	3417.98	1647.26
C	Emodin+NaOMe	3412.19	1651.12
D	Emodin+$AlCl_3$	3419.90	1647.26

Emodin content in different extracts of plant parts

Weighed amounts of dried extracts were dissolved in known volume of methanol. The absorbance of the resultant solution was scanned in UV-VIS range (200-800nm) for maximum absorbance after enabling blank correction for methanol in the above region(Table 5).

Table5: Emodin content in different extracts of plant parts at λmax:

Plant parts	Extracts	Emodin content in µg/gm
Leaves	Acetone	0.117
	Ethanol	0.101
	Methanol	0.054
Stem	Acetone	0.006
	Ethanol	0.004
	Methanol	0.003
Fruits	Acetone	0.092
	Ethanol	0.048
	Methanol	0.116

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Fig.1:UV-VIS spectral scan of Emodin

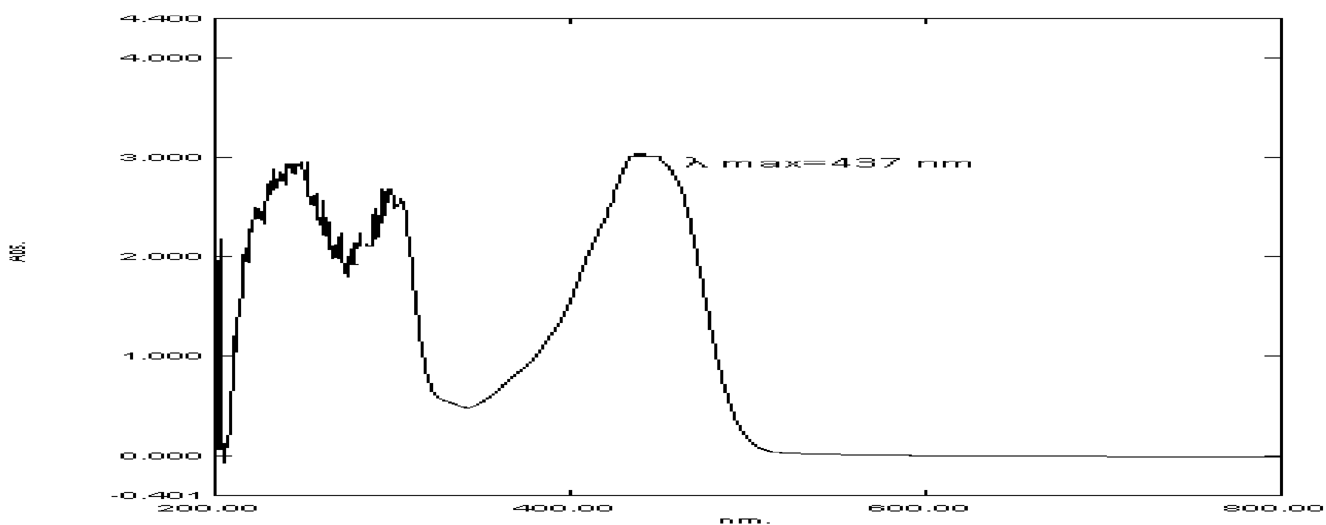


Fig2. Comparitive spectral peak scans

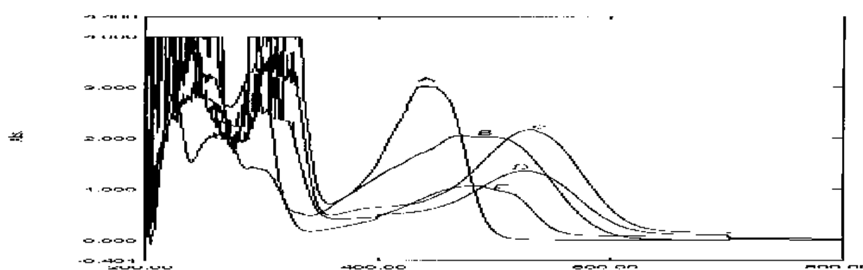
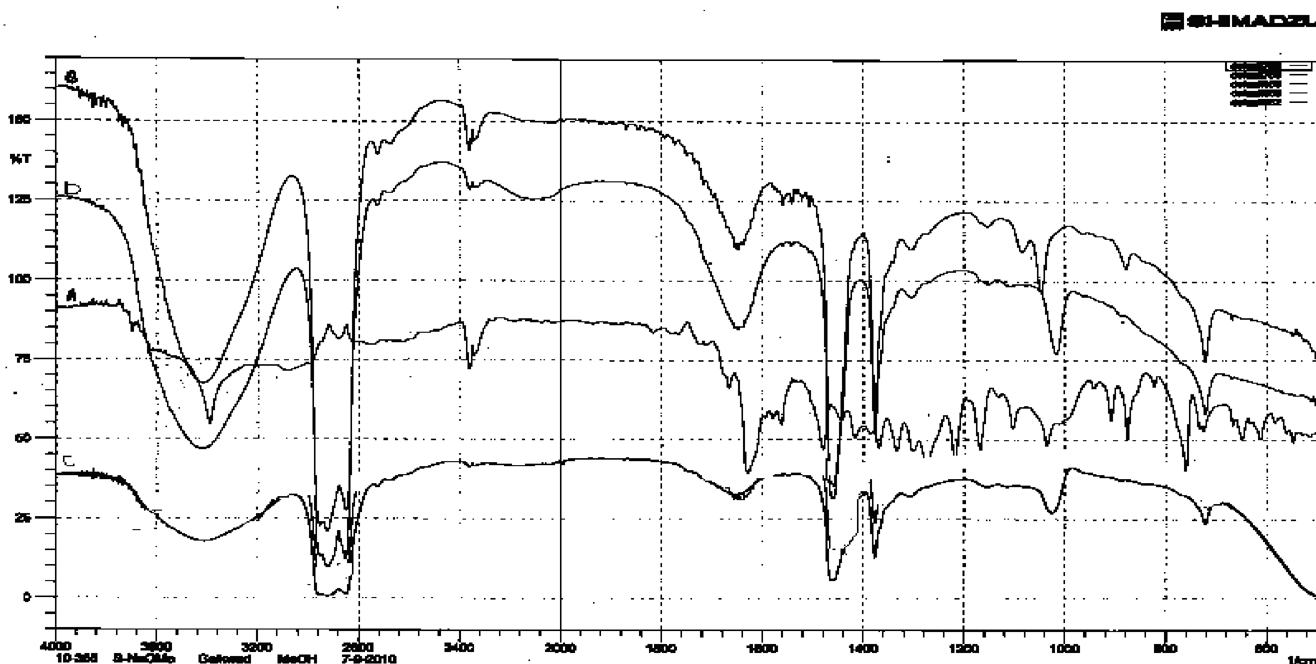


Fig 3: Comparative IR spectras



The UV spectra of emodin was measured in the range 200-400 nm against methanol as blank solution (Fig. 1). The standard solution shows a broad band of absorption ranging from 200 - 400 nm was observed with λmax at at 437nm,299nm,260nm (Fig.1). From the UV spectra it is clarified that the 437 nm was the maximum UV absorption wavelength for emodin.

System precision study: Calibration curve obtained from various concentrations for emodin guides the linear regression equation with a regression coefficient. Standard deviation (SD=0.0012) of Beer's law is obeyed in the concentration range of 10–100 μg/ml (r^2 = 0.973) in methanol.

Linearity-The linearity range for emodin is found to be 10–100 μg/ml(r^2 = 0.973) in methanol.(Table 1). The lower values of the standard error (SE) of slope and intercept (Table1) indicates high precision of the proposed method.

Accuracy: The accuracy ranges from 10 to100 μg/ml (Table 2). The excellent mean percentage recovery values are close to 100 %.The standard deviation values (SD <1.0) indicate high accuracy of the analytical methods.

Detection limit: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as an exact value. The limit of detection of emodin by the proposed method was found to be 0.41μg/ml(Table 2).

Quantification Limit (LOQ): The quantification limit of an individual analytical procedure is the lowest amount of analyte in the sample which, can be quantitatively determined with suitable level of precision and accuracy. The limit of quantification of emodin by the proposed method was found to be 1.25μg/ml(Table 2).

Stability : Stability of absorbance is of major importance in Spectrophotometric measurements. The period over which absorbance value at 437nm of emodin in methanol remains stable is investigated using concentration range (10-100μg/ml). The absorbance values are measured at 15 min intervals over a period of 1 hour. Results reveals absorption stability(Table2).

Emodin(A) shows absorption maxima at437nm,299nm,260nm. Maximun absorption wavelength at 437nm is selected and further changes in absortion maxima with reagents are studied. The test sample B shows the bathochromic shift in absorption maxima having broad region at 495 and 467nm. The test solution C shows longer bathochromic shift at 526 nm as sodium methoxide being a weaker base ,it takes acidic hydroxyl proton. The test solution D indicates bathochromic shift having absorption maxima at 482nm by forming stable complex between ortho dihydroxyl groups and keto function. (Fig.2). Although addition of all reagents show bathochromic shift but intensity of the absorption has been reduced as compared to emodin in all solutions causing hypochromic shift.It is maximum with aluminium chloride and minimum with sodium hydroxide causing distortion in geometry of the formed molecule. (Table 3).

Infrared spectra of of emodin-A shows frequency at 3391 cm^{-1} that assigned to hydroxyl groups. Characteristic bands for anthraquinones are 1666 cm^{-1} and 1620 cm^{-1} assigned to the free carbonyl group and the conjugated carbonyl group stretching frequencies respectively. The observed frequency at 1475 cm^{-1} is assigned to a skeletal ring stretching frequency. The change in characteristic bands for B, C and D for hydroxyl as well as for carbonyl groups have been shifted to higher frequency indicate complex formation with the used reagents. (Fig.3). The change in pH and conductivity is also confirms the complex formation. (Table 4).

Emodin content measured by developed method for various part of *Cassia auriculata* L in various extracts shows leaves and fruits extracts shows comparatively higher content than stem. Acetone

extract of leaves and methanol extract of fruits shows nearly same content of emodin (Table 5).

The proposed method stability, new approach to isolate and formulate content of emodin will be definitely helpful for the quantification of drug formulations and in various plant part extracts thus it will be useful for the validation study in plant parts as well as drugs.

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