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Development of HPTLC method for estimation of Wedelolactone, Quercetin and Jatamansone in Polyherbal Formulation

R. M. Thorat*, V. M. Jadhav, V. J. Kadam, S. S. Kamble And K. P. Salaskar

Department of Quality Assurance, Bharati Vidyapeeth's College of Pharmacy, Sector 08, CBD Belapur, Navi-Mumbai – 400 614, India.
***E-mail: drvmjadhav_bvcop@rediffmail.com, rupali.78@rediffmail.com**

ABSTRACT: In the present study an attempt has been made to develope a new, simple, sensitive, precise and robust High-performance thin layer Chromatographic [HPTLC] method for the quantitative estimation of Wedelolactone, Quercetin and Jatamansone in polyherbal formulation. Analysis of Wedelolactone, Quercetin and Jatamansone were performed on TLC aluminium plates pre-coated with silica gel 60F-254 as stationary phase. Linear ascending development were carried out in twin trough glass chamber saturated with mobile phase consisting of Toluene : Acetone : Formic acid [11: 6 : 1 v/v] for Wedelolactone, Toluene : Ethyl Acetate : Methanol [4.4 : 5 : 0.6 v/v] for Quercetin and Petroleum-ether : Acetone [3 : 1 v/v] for Jatamansone at room temperature
[25 ± 2 ° C]. Camag TLC scanner III was used for spectrodensitometric scanning and analysis of the plate in absorbance mode at 366 nm for Wedelolactone, 254 nm for Quercetin, 285 nm for Jatamansone. The system were found to give compact spots for Wedelolactone [Rf = 0.56], Quercetin [Rf = 0.47] and Jatamansone [Rf= 0.34]. The data for calibration plots showed good linear relationship with r^2= 0.9944 for Wedelolactone, r^2 = 0.9985 for Quercetin and r^2 = 0.9975 for Jatamansone in the concentration range of 500-2500 ng/spot, 3000-8000 ng/spot and
2000-6000 ng/spot with respect to peak area. Wedelolactone, Quercetin and Jatamansone were found to be 1.62%, 0.24% and 0.11% respectively in Polyherbal Oil Formulation. The present method were validated for accuracy and recovery. The limits of detection and limits of quantification were determined. Statistical analysis of the data showed that the method is reproducible and selective for estimation of Wedelolactone, Quercetin and Jatamansone.
Key-words : High-performance thin layer chromatography, Wedelolactone, Quercetin, Jatamansone.

INTRODUCTION

Bhavaprakash, an Ayurvedic treatise mentions the use of drug for the treatment of " Indralupta " i. e Drug used in the treatment of hair loss. *Eclipta alba* Hassk [Family Asteraceae], *Hibiscus rosa sinensis* Linn [Family Malvaceae], *Nordostachys jatamansi* DC [Family Valerianaceae] is such herb with traditional claims of hair growth promotion[1] . *Eclipta alba* Hassk [Family Asteraceae], is small much- branched annual herb with white flower heads found in moist situation through-out India ascending upto 600 feet, grows just after the first showers of rainy season. It contain coumestan derivatives: Wedeloactone and demethylwedelolactone[2] . Wedelolactone is responsible for hair growth activity[3] . It is used for hair growth promoter, improving the luster of the hair, treatment of variety of human ailments, particularly liver disorders and wound healing[4] .

The herb *Hibiscus rosa-sinensis* Linn [Family Malvaceae] is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colour of flowers. It contain anthocyanins and flavonoids; cyanidin-3, 5-diglucoside, cyanidin-3-sophoroside-5-glucoside,quercetin-3,7-diglucoside,

***Corres.author:**
Mrs. Rupali M. Thorat.
M.Pharm Student
Bharati Vidyapeeth's College of Pharmacy, Sector 8, CBD Belapur, Navi Mumbai.
E-mail:drvmjadhav_bvcop@rediffmail.com, rupali.78@rediffmail.com
Telephone No. : (022) 27882901, 09766533037

quercetin-3-diglucoside[5] . Quercetin is responsible for hair growth avtivity[6] . The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcers. Flowers have been found to be effective in the treatment of arterial hypertension and to have significant antifertility effect[5] .

Dried rhizome of *Nordostachys jatamansi* DC [Family Valerianaceae] is a small herbaceous Himalayan genus, common in Garhwal, Kumaun and Sikkim Himalaya between

3000 and 5000 m[4] . It contain eight monoterpenes [2.0%] and 25 sesquiterpenes [66.0%]. Various sesquiterpenes such as Jatamansone and Jatamansic acid have been reported to be present in the roots of the plant[7] . It is used for hair growth, convulsions, sedative, treatment of hypertension, dysuria, cystitis and uterine inflammation[8].

EXPERIMENTAL

Materials and Methods

Chemicals

All chemicals including solvents [analytical grade] were purchased from S. D. Fine chemicals, Mumbai. The HPTLC plates Silica 60 F 254 [20 cm x 20 cm] were purchased from E. Merk

[Darmstadt, Germany].

Collection and authentication of plant

The leaves of *Eclipta alba*, flowers of *Hibiscus rosa sinensis*, rhizomes of *Nardostachys jatamansi* were purchased from local market and authenticated by Agharkar Research Institute, Pune. The various parts of plant drugs are crushed in mixed and passed through the sieve number 80. The various powder drugs were subjected to pharmacognostic studies for confirmation.

Preparation of Polyherbal Oil Formulation

Polyherbal hair oil was prepared by using *Eclipta alba* Hassk [Asteraceae] 10% w/v, *Hibiscus rosa sinensis* Linn [Malvaceae] 10 % w/v, *Nardostachys jatamansi* [Valerianceae] 5 % w/v respectively in 1 lit. of coconut oil.

Plain coconut oil [commercial product] was used as control.

Isolation of Markers [Wedelolactone and Jatamansone]

Dried powder of *Eclipta alba* [75 gm] and dried rhizome of *Nardostachys jatamansi* [75 gm] were exhaustively extracted with 500 ml methanol respectively in soxhlet apparatus for 24 hrs. After complete extraction is achieved dark green extract of *Eclipta alba* and dark brown extract of *Nardostachys jatamansi* were concentrated via rota vacum drier. Concentrated methanolic extract were subjected to preparative TLC. Preparative TLC were performed on 20 cm × 10 cm TLC aluminium coated with 200 µm layer thickness of silica gel 60 F 254 using

Toluene : Acetone : Formic acid [11: 6 : 1 v/v] for Wedelolactone and Petroleum-ether : Acetone [3 : 1 v/v] for Jatamansone as mobile phase. The developed plates observed under UV lamp at long wavelength for Wedelolactone [Rf = 0.56] and short wavelength for Jatamansone

[Rf= 0.34] with the help of a cutting blade and a ruler, the area on the plate at the same Rf position for Wedelolactone and Jatamansone were marked. The silica in the resepective marked area were scrapped off and collected carefully in a test tube. The scrapped silica, which contained the marker i.e Wedelolactone and Jatamansone then extracted in methanol and filtered individually. The filtrates containing Wedelolactone and Jatamansone were evaporated under reduced pressure to obtain Wedelolactone and Jatamansone marker respectively.

Instrumentation

Melting point of Wedelolactone and boiling point of Jatamansone were determined by open capillary method using Veego's [VMP-D]melting point and boiling point apparatus. Structural confirmation of the isolated compounds i.e. Wedelolactone and Jatamansone were done by using I.R. Spectroscopy. FTIR spectra of the isolated compounds were recorded using KBr on a Shimadzu FTIR 8400S and characteristic absorption signals are reported.The isolated compounds i.e. Wedelolactone and Jatamansone were dissolved in methanol at 10 µg/ml respectively and solution were subjected to scanning

Chromatographic Specifications

HPTLC was performed on 20 cm × 10 cm TLC aluminium plates coated with 200-µm layer thickness of silica gel 60F 254 (E. Merck, Germany). Samples were applied as 8 mm width bands using Camag 100 microlitre sample syringe (Hamilton, Switzerland) with a Camag Linomat 5 applicator (Camag, Switzerland). Linear ascending development with Toluene : Acetone : Formic acid [11: 6 : 1 v/v] for Wedelolactone, Toluene : Ethyl Acetate : Methanol [4.4 : 5 : 0.6 v/v] for Quercetin and Petroleum-ether : Acetone [3 : 1 v/v] for Jatamansone respectively as mobile phase were carried out in a twin trough glass chamber [Camag] (20 x 10 cm) previously saturated with mobile phase vapour for 20 mins (optimized chamber saturation time) at room temperature (25 ± 2°C). The development distance was 80 mm. After development plates were air- dried. Scanning were performed using Camag TLC Scanner 3 at 366 nm through fluorescence mode for Wedelolactone, 254 nm in the absorbance mode for Quercetin and 285 nm in the absorbance mode for Jatamansone and operated by Win CATS Software [Version 1.4.1]. The slit dimensions were 5 mm × 0.45 mm and the scanning speed were100 mm/ s.

HPTLC method for estimation of Wedelolactone, Quercetin, Jatamansone

Preparation of Calibration Curve of Wedelolactone

A stock solution of 1000 µg/ml of Wedelolactone was prepared in methanol and dilution was done to obtain a solution of 100 µg/ml which was used for further analysis. Different volumes of the diluted solution [5, 10, 15, 20 and 25 µl] were applied in duplicate on plate to furnish

500-2500 ng/spot of Wedelolactone. Peak area data and the corresponding amounts were treated by linear least-square regression analysis.

Preparation of Calibration Curve of Quercetin

A stock solution of 1000 µg/ml of Quercetin was prepared in methanol and was used for analysis. Different volumes of this solution [3, 4, 5, 6, 7, and 8 µl] were applied in duplicate on plate to obtain concentration from 3000-8000 ng/spot of Quercetin. Peak area data and the corresponding amounts were treated by linear least-square regression analysis.

Preparation of Calibration Curve of Jatamansone

A stock solution of 1000 µg/ml of Jatamansone was prepared in methanol and was used for analysis. Different volumes of this solution [2, 3, 4, 5 and 6 µl] were applied in duplicate on plate to obtain concentration from 2000-6000 ng/spot of Jatamansone. Peak area data and the corresponding amounts were treated by linear least-square regression analysis.

Sample preparation for estimation of Wedelolactone, Quercetin, Jatamansone from Polyherbal Oil Formulation

The prepared Polyherbal Oil Formulation and methanol were taken in ratio 1:3 w/v and homogenized at 200 rpm at 50°C for 20 min. This mixture was centrifuged at 3000 x g for 20 min at 4°C. The supernatants were collected and concentrated under vacuum at < 45°C, then analyzed for the content of Wedelolactone, Quercetin and Jatamansone[9] .

Method Validation

The HPTLC methods developed for Wedelolactone, Quercetin and Jatamansone were validated and parameters like precision, accuracy [recovery], robustness, LOD and LOQ were checked as per ICH guidelines[10] .

RESULTS AND DISCUSSION

1. Structural Elucidation of Wedelolactone and Jatamansone

Melting point of isolated compounds was found to be 299°C for Wedelolactone and boiling point of isolated compounds was found to be 155°C for Jatamansone respectively. While scanning isolated compounds i.e. Wedelolactone and Jatamansone under UV spectrophotometer it observed that maximum absorption take place for Wedelolactone at 366nm and for Jatamansone at 285 nm.[Fig 7,9] I.R. Spectrum of Wedelolactone and Jatamansone showed in fig 10, 11. This matches the reported values [11-12].

2. Development of the Optimum Mobile Phase

The HPTLC methods developed for Wedelolactone, Quercetin and Jatamansone were optimised. For Wedelolactone, Quercetin and Jatamansone various ratios of mobile phases were tried and optimum mobile phase was selected. i. e Toluene : Acetone : Formic acid [11: 6 : 1 v/v] for Wedelolactone, Toluene : Ethyl Acetate : Methanol [4.4 : 5 : 0.6 v/v] for Quercetin and Petroleum-ether : Acetone [3 : 1 v/v] for Jatamansone. This mobile phases gave good resolution, dense, compact and well- separated spots of Wedelolactone, Quercetin and Jatamansone as well as a well- defined peak at Rf value of 0.56, 0.47, 0.34 respectively. [fig. 1, 2, 3] These optimised HPTLC methods were used for quantification of Wedelolactone, Quercetin and Jatamansone from the prepared Polyherbal oil formulation.

3. Validation of HPTLC method

A. Linearity

A representative calibration curve of Wedelolactone, Quercetin and Jatamansone were obtained by plotting peak area of Wedelolactone, Quercetin and Jatamansone against the concentration range of 500-2500 ng/spot, 3000-8000 ng/spot and 2000-6000 ng/spot respectively. The coeffient of determination for Wedelolactone, Quercetin and Jatamansone were found to 0.9944, 0.9985 and 0.9975 respectively. [Fig. 4,5,6]

B. Accuracy [% Recovery]

The % Recovery of Wedelolactone, Quercetin and Jatamansone given in Table 1, 2, 3 were found to be 100.30, 99 and 100.02 which is satisfactory.

C. Limit of Detection

The minimum detectable limit were found to be 5 ng/spot, 32 ng/spot and 27 ng/spot for Wedelolactone, Quercetin and Jatamansone respectively. [Table 4]

CONCLUSION

The proposed HPTLC method were found to be rapid, simple and accurate for quantitative estimation of Wedelolactone, Quercetin and Jatamansone in Polyherbal Oil Formulation. The recovery values of Wedelolactone, Quercetin and Jatamansone were found to be about 100.30, 99 and 100.02 respectively. For Wedelolactone, Quercetin and Jatamansone which shows the reliability and suitability of the method. The lowest detectable limit of Wedelolactone, Quercetin and Jatamansone were found to be 5 ng/spot, 32 ng/spot and 27 ng/spot respectively.

Wedelolactone, Quercetin and Jatamansone were found to be 1.62%, 0.24% and 0.11% respectively in Polyherbal Oil Formulation.

Table 1. Recovery studies for Wedelolactone (n = 3)

Amount of Wedelolactone added (ng)	Amount of Wedelolactone found (ng)	Recovery (%)	Average Recovery (%)
500	499.25	99.85	100.30
1000	1011.47	101.147	
1500	1498.66	99.91	

Table 2. Recovery Studies for Quercetin (n = 3)

Amount of Quercetin added (ng)	Amount of Quercetin found (ng)	Recovery (%)	Average Recovery (%)
2500	2480.96	99.00	99.00
5000	5065.65	101.00	
7500	7455.59	99.40	

Table 3. Recovery studies for Jatamansone (n = 3)

Amount of Jatamansone added (ng)	Amount of Jatamansone found (ng)	Recovery (%)	Average Recovery (%)
1500	1499.25	99.95	100.02
3000	3011.47	100.38	
4500	4498.66	99.97	

Table 4. Results of LOD and LOQ

Standards	LOD	LOQ
Wedelolactone	5	15
Quercetin	32	97
Jatamansone	27	83

Table 5. Content of Wedelolactone, Quercetin and Jatamansone in Polyherbal Oil Formulation

Sample	Content (%)		
	Wedelolactone	Quercetin	Jatamansone
Polyherbal Oil Formulation	1.62	0.24	0.11

Fig.1.Chromatogram of Wedelolactone standard

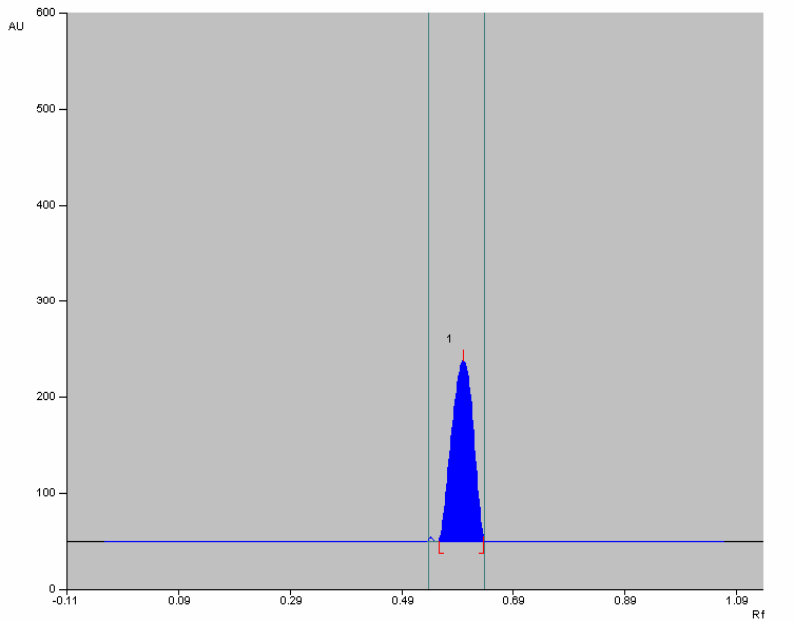


Fig.2.Chromatogram of Quercetin standard

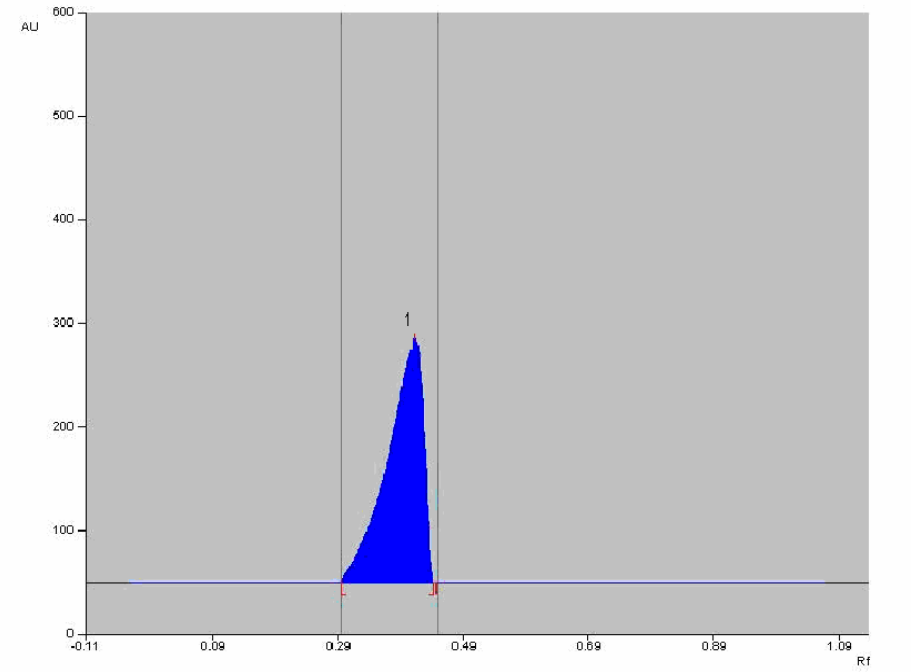


Fig.3.Chromatogram of Jatamansone standard

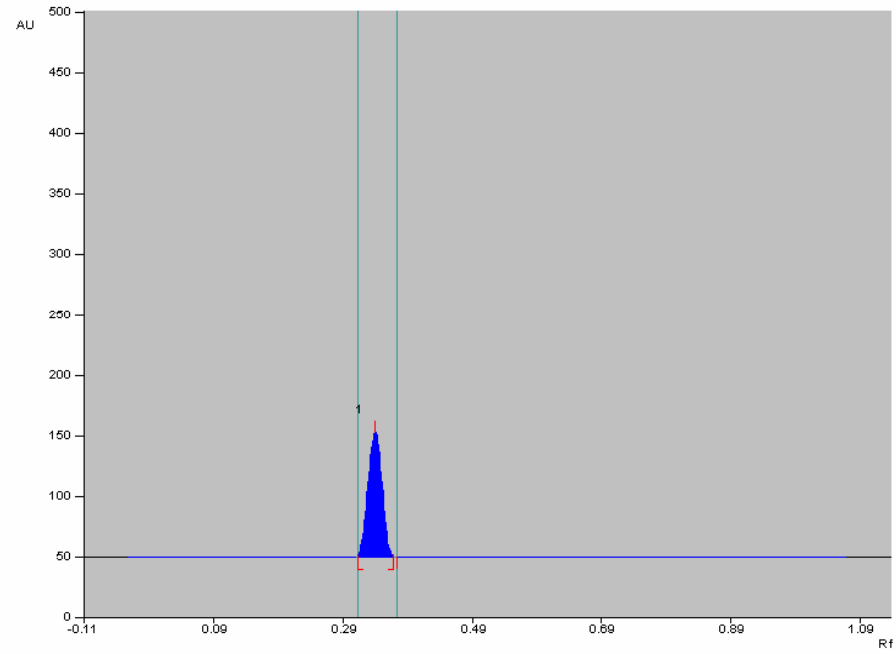
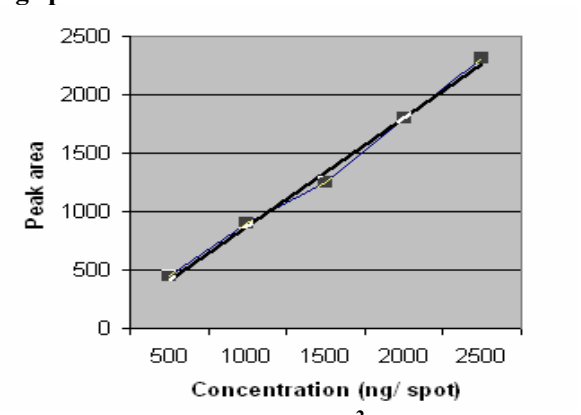
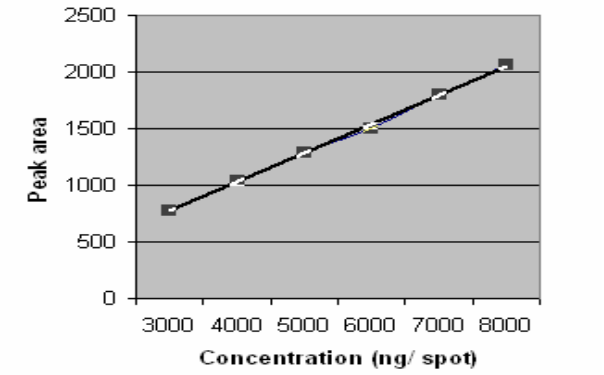


Fig. 4. Calibration curve of peak area versus standard Wedelolactone concentration ranging from 500-2500 ng/spot.



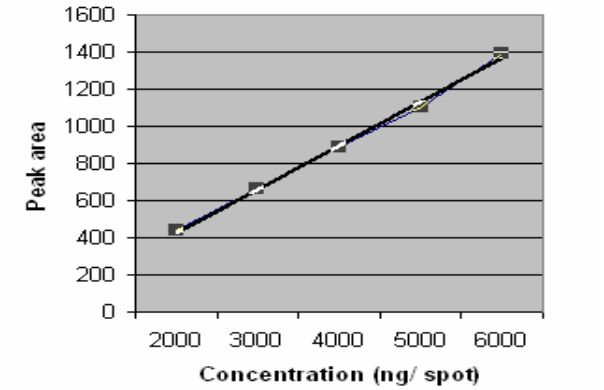
y =462.35x-44.469; r^2 = 0.9944

Fig. 5. Calibration curve of peak area versus standard Quercetin concentration ranging from 3000-8000 ng/spot.



y =255.62x+514.75; r^2 = 0.9985

Fig. 6. Calibration curve of peak area versus standard Jatamansone concentration ranging from 2000-6000 ng/spot.



y =234.33x+193.59; r^2 = 0.9975

Fig. 7 Spectra of Wedelolactone standard

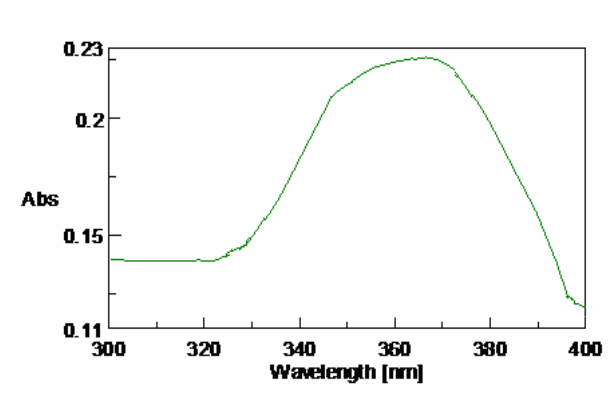


Fig. 8 Spectra of Quercetin standard

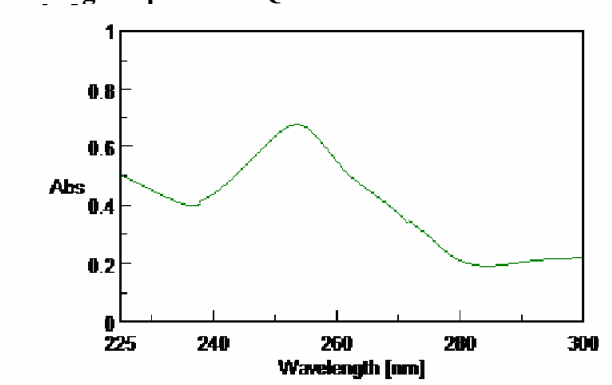


Fig. 9 Spectra of Jatamansone standard

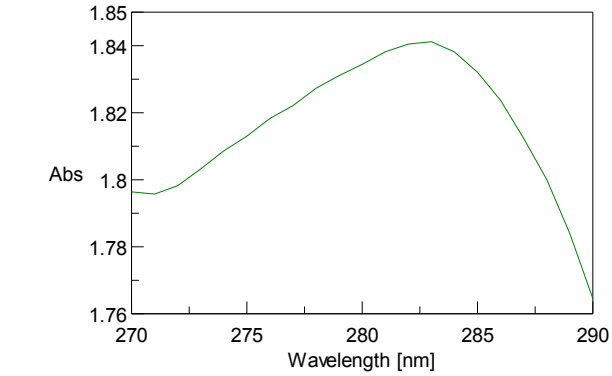
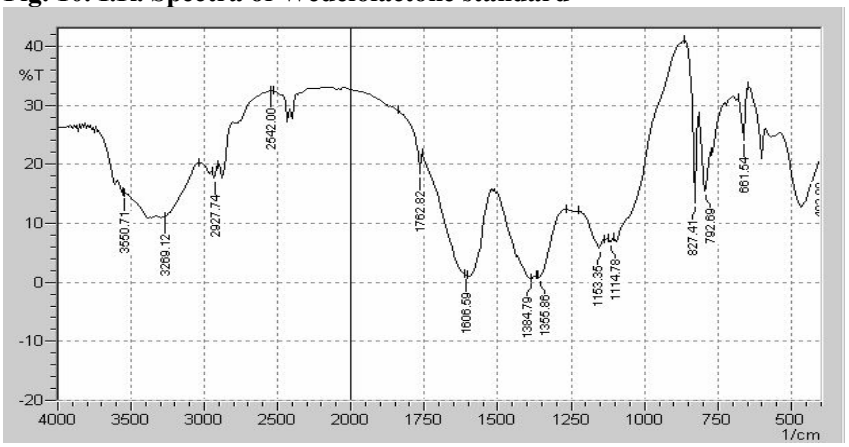
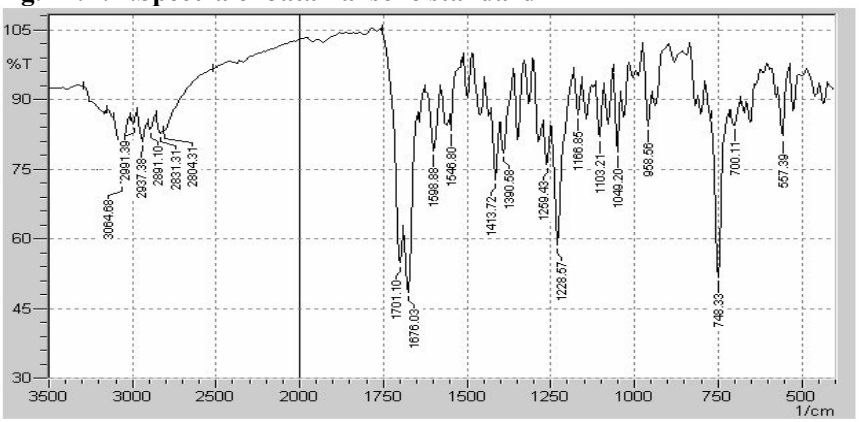


Fig. 10. I.R. Spectra of Wedelolactone standard



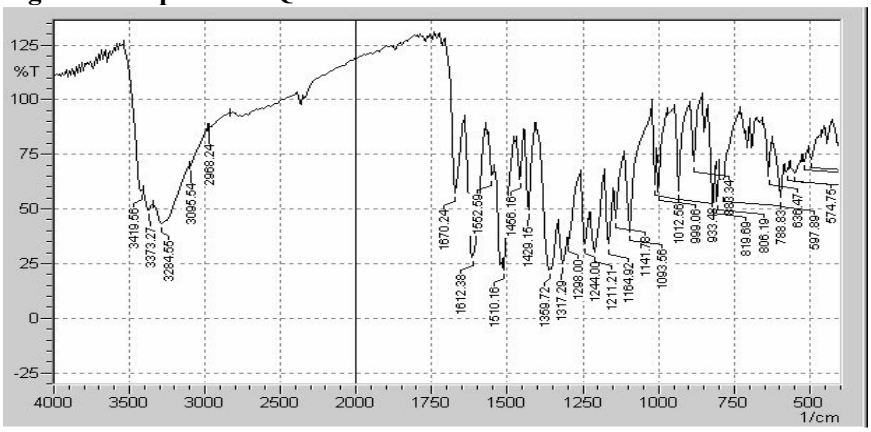
O-H stretch- 3550, O-H bend- 1384, C=O stretch –1762, C-H stretch – 2927, C-H bend- 792, 827.

Fig. 11. I.R.Spectra of Jatamansone standard



C=O stretch – 1701, 1676, C-H stretch – 3064, 2991, C-H bend- 748, 958.

Fig. 12. I.R.Spectra of Quercetin standard



O-H stretch- 3419, 3373, O-H bend- 1429, 1456, C=O stretch –1670, C-H stretch – 3095, 2908, C-H bend- 788, 806, 933.

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