

PHCOG MAG.: Research Article

Phytochemical evaluation and validation of a polyherbal formulation using HPTLC

Dhalwal K.¹, Biradar Y.S.², Shinde V.M.^{1*}, Mahadik K. R.¹ and Rajani M²

¹Dept of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth University, Erandwane, Pune- 411038, Maharashtra, India.

²B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Thaltej-Gandhinagar highway, Thaltej, Ahmedabad – 380054, Gujarat, India.

*Author for Correspondence: vaibhavshinde2@rediffmail.com

ABSTRACT

Raktadosh Nasak Vati is a polyherbal formulation used as antioxidant, antiseptic, antiallergic and to eliminate toxins from blood. It includes ten herbal ingredients and the major chemical compounds present are E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid. Simple TLC densitometric methods have been developed for the quantification of these six marker compounds in the formulation using HPTLC. These six compounds were quantified and found to be 0.016 %, 0.015 %, 0.0017 %, 0.17 %, 0.485 % and 0.3 % (w/w) of E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid respectively. The method was validated for instrumental precision, repeatability and accuracy. Accuracy of the methods was checked by recovery study conducted at three different levels and the average percentage recovery was found to be 100.58 % for E-guggulsterone, 100.24 % for Z-guggulsterone, 99.01 % for berberine, 99.57 % for curcumin, 99.9 % for gallic acid and 100.23 % for ellagic acid. The proposed TLC densitometric methods for the quantification of the above marker compounds were found to be simple, precise, specific, sensitive and accurate and can be used for the routine quality control of formulation and herbal raw material containing these marker compounds. They also have the applicability in quantifying any of these marker compounds in other drugs as well.

KEY WORDS: Polyherbal formulation, Quantification, Validation, HPTLC, Markers.

INTRODUCTION

Herbal medicine has been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the ayurvedic medicines is the lack of standard quality control profiles. The quality of herbal medicine i.e. the profile of the constituents in the final product has implication in efficacy and safety. Due to the complex nature and inherent variability of the chemical constituents of plant-based drugs, it is difficult to establish quality control parameters. To overcome these problems modern analytical techniques are expected to help in circumventing this problem (1, 2).

Raktadosh Nasak Vati is a polyherbal formulation, consisting of 13 ingredients of plant origin and it is widely used as antioxidant, antiseptic, antiallergic. The major active components are E-guggulsterone, Z-guggulsterone, Berberine, curcumin, gallic acid and ellagic acid. From such polyherbal formulations separation, identification and estimation of chemical components is very difficult (3). Literature survey

revealed that the above mentioned marker compound have various pharmacological properties. It has been demonstrated that the major active components in the oleogum resin of *Commiphora wightii* are E-guggulsterone and Z-guggulsterone. Guggulsterones were shown to act by blocking bile acid receptors/farnesoid X receptors and consequently decrease blood cholesterol concentration. They also showed marked inhibition of ADP, adrenaline and serotonin induced platelet aggregation and anti-inflammatory activity (4- 6). Extracts and decoctions of Daruharidra have been used in Ayurvedic and Chinese medicine for at least 3000 years. Berberine has been shown to exhibit significant antimicrobial activity. In addition to this, berberine has numerous pharmacological effects like anti-inflammatory, cardiovascular effects, ocular trachoma infections and against intestinal parasites. Berberine (20 mg/kg/d) inhibited platelet aggregation and platelet adhesion induced by ADP, arachidonic acid, and collagen in rats (7- 8). The rhizome and roots of *Curcuma species* are

frequently used in cosmetics and spas for skin nourishment. Pharmacological study reveals its various medical activities such as antioxidant, promotion of blood circulation to remove blood stasis and treatment of cancer. The antioxidants are claimed biological active in protecting the body, the skin collagen and elastic tissue against damaging by reactive oxygen species (9- 11). Gallic acid and ellagic acid are hydrolysable tannins and present in a rich variety of plants like in tea, red wine, fruits, beverages and various medicinal plants. GA is known to have anti-inflammatory, antimutagenic, anticancer and antioxidant activity (12- 14). EA has been found to exhibit antimutagenic, antiviral, anticancer, antitumor and antioxidant properties, along with whitening of the skin (15- 16).

The advances in chromatographic techniques made it possible to quantify the chemical constituents in a mixture with comparatively little clean-up using high performance thin layer chromatography [HPTLC] (17). Present study deals with development and validation of methods for quantification of some of the important marker compounds viz. E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid in *Raktadosh Nasak Vati*.

MATERIALS AND METHODS

Chemicals and reagents

The formulation was procured from the local market of Pune. All the chemicals used in the experiments were of analytical grade. E-Guggulsterone, Z-guggulsterone, berberine, curcumin and ellagic acid (purity 97 %, w/w) standards were purchased from Natural Remedies Pvt. Ltd., Bangalore. Gallic acid (purity 99 %, w/w) was a gift sample from Tetrahedron Ltd., India. All the solvent used in the experiments were of analytical grade

Apparatus

Spotting device: Linomat V Automatic Sample Spotter; CAMAG (Muttentz, Switzerland)

Syringe: 100 μ L Hamilton (Bonaduz, Switzerland)

TLC Chamber: Glass twin trough chamber (20 x 10 x 4 cm); CAMAG

Densitometer: TLC Scanner 3 linked to WinCats software; CAMAG

HPTLC plates: 20 x 10 cm, 0.2 mm thickness precoated with silica gel 60 F₂₅₄; E. Merck KgaA, Cat. no. 1.05548; (Darmstadt, Germany)

Experimental conditions: Temperature 25 $\pm$ 2 $^{\circ}$ C, relative humidity 40 %

Preparation of Standard solutions

(a) Standard solution of E-guggulsterone

A stock solution of E-guggulsterone was prepared by dissolving 4 mg of accurately weighed E-guggulsterone in methanol and making up the volume to 25 ml with methanol. From this stock solution standard solutions of 32 μ g/ml to 96 μ g/ml were prepared by transferring aliquots (2 to 6 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

(b) Standard solutions of Z-guggulsterone

A stock solution of Z-guggulsterone was prepared by dissolving 2.5 mg of accurately weighed Z-guggulsterone in methanol and making up the volume to 25 ml with methanol. From this stock solution standard solutions of 10 μ g/ml to 60 μ g/ml were prepared by transferring aliquots (1 to 6 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

(c) Standard solution of berberine

A stock solution of berberine was prepared by dissolving 2 mg of accurately weighed berberine in methanol and making up the volume to 25 ml with methanol. From this stock solution standard solutions of 1.6 μ g/ml to 4.8 μ g/ml were prepared by transferring aliquots (0.2 to 0.5 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

(d) Standard solution of curcumin

A stock solution of curcumin was prepared by dissolving 2 mg of accurately weighed curcumin in methanol and making up the volume to 25 ml with methanol. From this stock solution standard solutions of 4.8 μ g/ml to 11.2 μ g/ml were prepared by transferring aliquots (0.6 to 1.4 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume with methanol.

(e) Standard solution of gallic acid.

A stock solution of gallic acid was prepared by dissolving 5 mg of accurately weighed gallic acid in methanol and making up the volume to 50 ml with methanol. From this stock solution standard solutions of 15 - 75 μ g/ml were prepared by transferring aliquots (1.5 to 7.5 ml) of stock solution to 10 ml volumetric flasks and adjusting the volume to 10 ml with methanol.

(f) Standard solution of ellagic acid

A stock solution of ellagic acid was prepared by dissolving 2 mg of accurately weighed ellagic acid in methanol and making up the volume to 25 ml with methanol. From this stock solution standard solutions of 8 to 24 μ g/ml were prepared by transferring aliquots (1 to 3 ml) of stock solution to 10 ml

volumetric flasks and adjusting the volume with methanol.

Preparation of Sample solutions

Sample solution I:

Twenty Vatis (vati = small round pill) were crushed to fine powder. From this 2.5 gm of the fine powder was extracted exhaustively with methanol (4 x 25 ml). Extract was pooled, filtered and concentrated to 50 ml.

Sample solution II:

1 gm of the formulation powder was hydrolyzed with 40 ml of 2 N HCl for 2 hrs under reflux. It was cooled and filtered through whatmann's filter paper. The marc was washed with water three times and basified with 10 % sodium carbonate solution. The marc was washed with distilled water until filtrate tested neutral. The marc was dried in oven at 60 °C. The dried marc was extracted with chloroform (3 x 25 ml) under reflux for 2 hrs in a water bath. The extract was filtered, pooled and concentrated to 10 ml.

Calibration curve for berberine, curcumin, gallic acid, and ellagic acid

10 µl of each of the standard solutions of berberine, curcumin, gallic acid, and ellagic acid were applied in triplicate on TLC plates. The plates were developed in a solvent system of toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8) at 25 ± 2 °C temperature and 40 % relative humidity up to a distance of 8 cm. After development, the plates were dried in air and scanned densitometrically at 429 nm for curcumin, at 366 nm for berberine and at 280 nm for gallic acid and ellagic acid. The peak areas were recorded. Calibration curves of berberine, curcumin, gallic acid, and ellagic acid were prepared by plotting peak areas vs concentration.

Calibration curve of Z-guggulsterone and E-guggulsterone

10 µl each of the standard solutions of Z-guggulsterone and E-guggulsterone were applied in triplicate on TLC plates. The plates were developed in a solvent system of petroleum ether: Ethyl acetate: Methanol (6: 2: 0.5 v/v) in a twin trough chamber at 25 ± 2 °C temperature and 40 % relative humidity up to a distance of 8 cm. The plate was scanned at 254 nm. The peak areas were recorded and calibration curve was prepared by plotting peak areas vs concentration applied.

Quantification of berberine, curcumin, gallic acid and ellagic acid

10 µl of sample solution I was applied in triplicate on a precoated silica gel 60 F₂₅₄ TLC plate (E. Merck) (0.2

mm thickness) with the CAMAG Linomat V Automatic Sample Spotter. Plate was developed in the solvent system of toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8) and scanned at 429 nm for curcumin, at 366 nm for berberine and at 280 nm for gallic acid and ellagic acid. The peak areas and absorption spectra were recorded. To check the identity of the bands UV absorption spectrum of each standard was overlayed with the corresponding band in the sample track. To check the purity of the bands in the sample extract the absorption spectra were recorded by overlaying at start, middle and end position of the bands. The amount of berberine, curcumin, gallic acid and ellagic acid in the sample was calculated using the respective calibration curves.

Simultaneous quantification of E-guggulsterone and Z-guggulsterone

10 µl of sample solution II was applied in triplicate on a precoated silica gel 60 F₂₅₄ TLC plate (E. Merck) (0.2 mm thickness) with the CAMAG Linomat V Automatic Sample Spotter. Plate was developed in the solvent system of petroleum ether: Ethyl acetate: Methanol (6: 2: 0.5 v/v) and scanned at 254 nm. The peak areas and absorption spectra were recorded. To check the identity of the bands UV absorption spectrum of each standard was overlayed with the corresponding band in the sample track. To check the purity of the bands in the sample extract the absorption spectra were recorded by overlaying at start, middle and end position of the bands. The amount of E-guggulsterone and Z-guggulsterone in the sample was calculated using the respective calibration curves.

Validation of the Method

ICH guidelines were (CPMP/ICH/381/95; CPMP/ICH/281/95) followed for the validation of the analytical procedure. The method was validated for precision, repeatability and accuracy. Instrumental precision was checked by repeated scanning of the same spot of E-guggulsterone (560 ng), Z-guggulsterone (300 ng), berberine (24 ng), curcumin (80 ng), gallic acid (450 ng), and ellagic acid (160 ng) seven times and was expressed as coefficient of variance (% CV). The repeatability of the method was affirmed by analyzing 560 ng/spot of standard solution of E-guggulsterone, 300 ng/spot of standard solution of Z-guggulsterone, 24 ng/spot of standard solution of berberine, 80 ng/spot of standard solution of curcumin, 450 ng/spot of standard solution of gallic acid, and 160 ng/spot of standard solution of ellagic acid after application on the TLC plate (n = 6) and was expressed as % CV. Variability of the method was

studied by analyzing aliquots of standard solution of E-guggulsterone (480, 640, 800 ng/spot), Z-guggulsterone (200, 300, 400 ng/spot), berberine (24, 32, 40 ng/spot), curcumin (48, 64, 80 ng/spot), gallic acid (150, 300, 450 ng/spot) and ellagic acid (120, 160, 200 ng/spot) on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % CV.

Accuracy of the method was tested by performing recovery studies at three levels (50 %, 100 % and 125 % addition). The percent recovery as well as average percent recovery was calculated. For the determination of limit of detection and limit of quantification different dilutions of the standard solutions of E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid were applied along with methanol as the blank and determined on the basis of signal to noise ratio.

RESULTS AND DISCUSSION

Raktadosh Nasak Vati is a polyherbal formulation, consisting of 13 ingredients of plant origin (Table 1) and it is widely used as antioxidant, antiseptic, antiallergic. The major active components in the oleogum resin of *Balsamodendron mukul* are E-guggulsterone and Z-guggulsterone that are responsible for its several activities. *Guggul* is the major ingredient of the formulation and responsible for the activity of the preparation. E-guggulsterone and Z-guggulsterone content reflects the quality of *guggul* used in the formulation. Berberine from berberis species (18), curcumin from *Curcuma longa*, gallic acid and ellagic acid from Triphala. Gallic acid and ellagic acid are present in many ingredients including the fruit pulp of *Terminalia chebula*, *T. belerica* and *Emblica*

officinalis. TLC densitometric methods were developed using HPTLC for the quantification of six marker compounds from the polyherbal formulation *Raktadosh Nasak Vati*. Solvent systems were optimized to achieve best resolution of the marker compounds from the other components of the sample extracts. Of the various solvent system tried, the one containing toluene: ethyl acetate: methanol: formic acid (3: 3: 0.2: 0.8) gave best resolution of berberine ($R_f = 0.27$), curcumin ($R_f = 0.74$), gallic acid ($R_f = 0.63$) and ellagic acid ($R_f = 0.51$) in the presence of other compounds in the sample extract and enabled the quantification of marker compounds. It was not possible to resolve Z-guggulsterone and E-guggulsterone from methanolic extract of sample formulation due to interference of other compounds. Hence to resolve these two compounds sample preparation was modified. Solvent system of petroleum ether: ethyl acetate: methanol (6: 2: 0.5) gave best resolution of E-guggulsterone ($R_f = 0.64$) and Z-guggulsterone ($R_f = 0.71$). In these two solvent systems the marker compounds were well resolved from the other components of the formulation. The identity of the bands in the sample extracts were confirmed by comparing the R_f and the absorption spectra by overlaying their UV absorption spectra with those of their respective standard using CAMAG TLC Scanner 3 (Figure 1). The purity of the bands due to E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid bands in the sample extract was confirmed by overlaying the absorption spectra recorded at start, middle and end position of the band in the sample tracks.

The methods were validated in terms of precision, repeatability and accuracy. (Table 2 and 3)

Table 1: Composition of the formulation.

Sr. No.	List of Ingredients	Common name	Weight per Tablet (mg)
1	<i>Rubia cordifolia</i>	Manjishtha	20
2	<i>Berberis species</i>	Daruharidra	20
3	<i>Curcuma aromatica</i>	Haridra	20
4	<i>Tinospora cordifolia</i>	Amruta	20
5	<i>Tribulus terrestris</i>	Gokshur	20
6	Shuddha Gandhak	Shuddha Gandhak	20
7	<i>Azadirachta indica</i>	Kadu Nimba	30
8	<i>Hemidesmus indicus</i>	Sariva	30
9	<i>Balsamodendron mukul</i>	Shuddha Guggal	30
10	Triphala	Triphala	120

Table 2: Method validation parameters for the estimation of marker compounds by HPTLC.

Parameters	E-Guggulsterone	Z-Guggulsterone	Berberine	Curcumin	Gallic acid	Ellagic acid
Instrumental Precision (% CV) (n=7)	1.04	0.19	0.97	0.99	0.083	0.78
Repeatability	0.93	1.17	1.14	1.42	1.07	1.50
Limit of detection (ng/spot)	12	10	8	24	50	40
Limit of quantification (ng/spot)	24	20	16	36	150	80
Specificity	Specific	Specific	Specific	Specific	Specific	Specific
Linearity (Correlation coefficient)	0.992	0.997	0.998	0.999	0.997	0.999
Range (ng/spot)	320-960	100-600	16- 48	48- 112	150-750	80-240

Table 3. Intra- and inter-day precision study.

Marker compound	Concentration (ng spot ⁻¹)	Intra-day precision*	Inter-day precision*
E-Guggulsterone	480	1.54	1.23
	640	1.06	1.58
	800	1.80	1.29
Z-Guggulsterone	200	0.90	0.98
	300	1.71	1.74
	400	1.32	1.03
Berberine	24	1.23	1.02
	32	0.90	1.54
	40	0.99	1.76
Curcumin	48	1.34	1.33
	64	1.22	1.56
	80	1.50	1.41
Gallic acid	150	1.60	1.81
	300	1.12	1.05
	450	1.40	1.75
Ellagic acid	120	0.92	1.18
	160	1.50	1.31
	200	0.77	1.06

* Relative standard deviation (% CV, n = 3).

Table 4: Recovery study of marker compound by proposed HPTLC method.

Marker compound	Amount present in the sample, (µg)	Amount added, (µg)	Amount found*, (µg)	Recovery*, (%)	Average Recovery, (%)
E-Guggulsterone	80	40	123 ± 0.08	102.51 ± 0.02	100.58
	80	80	157 ± 0.23	98.13 ± 0.09	
	80	100	182 ± 0.10	101.11 ± 0.08	
Z-Guggulsterone	75	37.5	114 ± 0.09	101.33 ± 0.30	100.24
	75	75	148 ± 0.03	98.66 ± 0.12	
	75	93.75	170 ± 0.08	100.74 ± 0.10	
Berberine	10	5	14.6 ± 0.09	97.33 ± 0.11	99.01
	10	10	19.5 ± 0.08	97.50 ± 0.20	
	10	12.5	23.0 ± 0.06	102.20 ± 0.11	
Curcumin	165	82.5	243 ± 0.12	98.2 ± 0.09	99.57
	165	165.0	332 ± 0.13	100.6 ± 0.16	
	165	206.3	369 ± 0.09	99.9 ± 0.06	
Gallic acid	485	242.5	725 ± 0.15	99.6 ± 0.12	99.9
	485	485.0	973 ± 0.08	100.3 ± 0.13	
	485	606.3	1089 ± 0.12	99.8 ± 0.06	
Ellagic acid	300	150.0	453 ± 0.09	100.6 ± 0.05	100.23
	300	300.0	598 ± 0.07	99.8 ± 0.09	
	300	450.0	752 ± 0.05	100.3 ± 0.16	

* Mean ± standard deviation (SD, n = 3).

Table 5: Marker compounds quantified by HPTLC method from Raktadosh Nasak Vati

Sr. No.	Marker compound	Content of marker compound % (w/w) *
1	E-Guggulsterone	0.016 ± 0.021
2	Z-Guggulsterone	0.015 ± 0.011
3	Berberine	0.002 ± 0.015
4	Curcumin	0.165 ± 0.040
5	Gallic acid	0.485 ± 0.031
6	Ellagic acid	0.300 ± 0.022

*Mean ± SD (n = 3)

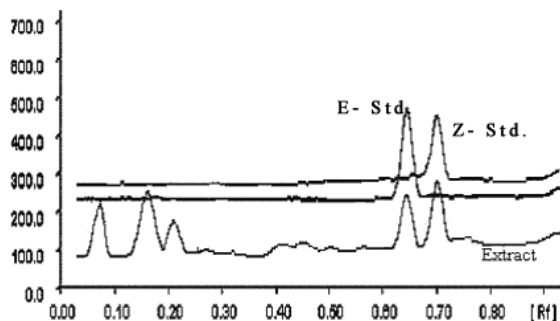


Figure 1 A

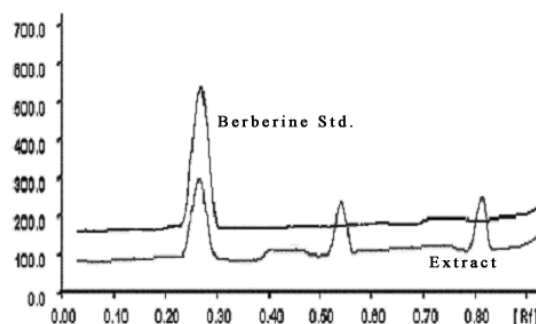


Figure 1 B

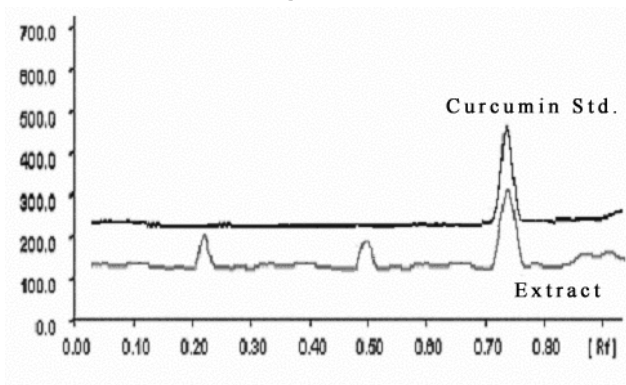


Figure 1 C

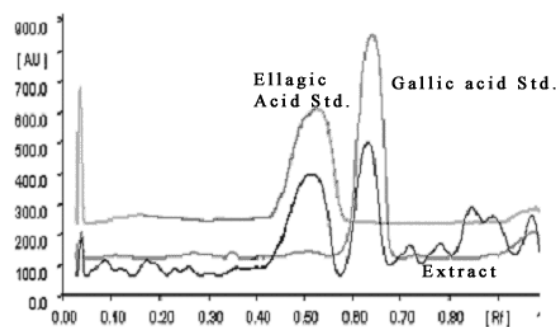


Figure 1 D

Figure 1: TLC densitograms of sample solutions of Raktadosh Nasak Vati with their respective standard scanned at different wavelength. A – E- Guggulsterone and Z-Guggulsterone scanned at 254, B– Berberine at 366, C– Curcumin at 429 and D– Gallic acid and Ellagic acid at 280.

The relationship between the concentration of standard solutions and the peak response was linear within the concentration range of 320-960 ng/spot with a correlation coefficient of 0.997 for E-guggulsterone, 100-600 ng/spot with a correlation coefficient of 0.997 for Z-guggulsterone, 16- 48 ng/spot with a correlation coefficient of 0.998 for berberine, 48- 112 ng/spot with a correlation coefficient of 0.999 for curcumin, 150-750 ng/spot with a correlation coefficient of 0.997 for gallic acid

and 80-240 ng/spot with a correlation coefficient of 0.999 for ellagic acid. The average percent recovery at three different levels was found and the results are presented in Table 4.

E-guggulsterone, Z-guggulsterone, berberine, curcumin, gallic acid and ellagic acid content in a polyherbal formulation *Raktadosh Nasak Vati* was estimated by the proposed method (Table 5). The method developed was found to be suitable for the quantification of these marker compounds in the

herbal raw materials.

CONCLUSION

We established TLC densitometric methods for the quantification of four bioactive compounds viz., berberine, curcumin, gallic acid and ellagic acid and simultaneous quantification of two bioactive compounds viz., E-guggulsterone and Z-guggulsterone from *Raktadosh Nasak Vati*, a polyherbal formulation, using HPTLC. The methods were found to be simple, precise, specific, sensitive and accurate and can be used for their quantification in the plant materials and also in routine quality control of the raw materials as well as formulations containing any or all of these compounds.

ACKNOWLEDGEMENTS

We thank Prof. Harish Padh, Director, B. V. Patel Pharmaceutical Education and Research Development (PERD) Centre for providing instrumentation facilities.

REFERENCES

1. L.V. Asokar, K.K. Kakkar and O.J. Chakra, *Glossary of Indian medicinal plants with active principles*, (Publication and Information Directorate, New Delhi, 1992) pp. 122.
2. M.N. Ravishankara, N. Shrivastava, H. Padh and M. Rajani. HPTLC method for the estimation of alkaloids of *Cinchona officinalis* stems bark and its marketed formulations. *Planta Med.* **67**: 294-296 (2001).
3. K. Dhalwal, Y.S. Biradar and M. Rajani. TLC densitometric method for simultaneous quantification of phyllanthin, hypophyllanthin, gallic acid and ellagic acid in *Phyllanthus amarus* using HPTLC. *J. AOAC. Inter.* **89**: 619- 623 (2006).
4. N.L. Urizar, A.B. Liverman, D.T. Dodds, F.V. Silva, P. Ordentlich, Y. Yan, F.J. Gonzalez, R.A. Heyman, D.J. Mangelsdorf and D.D. Moore. A natural product that lowers cholesterol as an antagonist ligand for FXR. *Science.* **296**: 1703-6 (2002).
5. J. Wu, C. Xia, J. Meier, S. Li, X. Hu and D.S. Lala. The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor. *Mol. Endocrinol.* **16**(7):1590-7 (2002).
6. N.L. Urizar and D.D. Moore. Guggulipid: A natural cholesterol-lowering agent. *Annu. Rev. Nutr.* **23**: 303-13 (2003).
7. Z. Xu, H.Y. Cao and Q. Li. Protective effects of berberine on spontaneous ventricular fibrillation in dogs after myocardial infarction. *Chung Kuo. Yao. Li. Hsueh Pao.* **10**: 320-324 (1989).
8. J.F. Wu and T.P. Liu. Effects of berberine on platelet aggregation and plasma levels of TXB2 and 6-keto-PGF1 alpha in rats with reversible middle cerebral artery occlusion. *Yao. Hsueh Hsueh Pao.* **30**: 98-102 (1995).
9. J.H. Shi, C.Z. Li and D.L. Liu. Experimental research on the pharmacology of *Curcuma aromatica* volatile oil. *Zhong Yao Tong Bao.* **6**: 36-38 (1981).
10. X. Li and X. Liu. Curcumin: The story so far. *Eur. J. Cancer.* **41**(13): 1955-68 (2005)
11. M. Notarbartolo, P. Poma, D. Perri, L. Dusonchet, M. Cervello and N. D'Alessandro. Curcumin ameliorates oxidative stress during nicotine-induced lung toxicity in Wistar rats. *Ital. J. Biochem.* **53**(2): 82-6 (2004).
12. T. Okuda. Tannins, a new family of bioactive natural organic compounds. *Yakugaku Zasshi.* **115**: 81 -100 (1995).
13. T. Okuda, T. Yoshida and T. Hatano. Phenolic compounds in food and their effects on health. In M.T. Huang, C.T. Ho and C.Y. Lee. ACS Symposium Series, Vol.507, 2 Ed. Washington, DC:American Chemical Society, 87 (1992).
14. T. Tanaka. Structure, property and function of plant polyphenols Foods. *Food Inged. J. Jpn.* **64**: 180 (1999).
15. U.C. Bhargava, and B.A. Westfall. Antitumor activity of *Juglans nigra* (black walnut) extractives. *J. Pharm. Sci.* **57**: 1674 -1677 (1968).
16. S. Mandal and G.D. Stoner. Inhibition of N -nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats by ellagic acid. *Carcinogenesis.* **11**: 55 -61 (1990).
17. Anonymous: *Quality Standards of Indian Medicinal Plants*, Vol-1, (Indian Council of Medical Research, New Delhi, 2003) pp. 10- 50.
18. C.W. Lau, X.Q. Yao, Z.Y.Chen, W.H. Ko and Y. Huang. Study on the anti-inflammatory action of *Berberis vulgaris* root extract, alkaloid fractions and pure alkaloids. *Int. J. Immunopharmacol.* **18**(10): 553-61 (1996).