Homeopathy (2016) 105, 48–54

http://dx.doi.org/10.1016/j.homp.2015.08.003, available online at http://www.sciencedirect.com

ORIGINAL PAPER

Standardization of homeopathic mother tincture of *Toxicodendron pubescens* and correlation of its flavonoid markers with the biological activity

Hemant P Jadhav[1], Ghanshyam G Chaudhari[1], Dipak D Patil[2], Ramchandra B Jadhav[3], Navya M Reddy[1], Atul A Shirkhedkar[4], Sameer N Goyal[1] and Chandragouda R Patil[1,*]

[1]*Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist.: Dhule, MS, 425405, India*
[2]*Department of Pharmaceutical Chemistry, H. R. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist.: Dhule, MS, 425405, India*
[3]*Department of Pharmacognosy, Arvind Gavali College of Pharmacy, Jaitapur, Dhangarwadi, Satara, MS, 415004, India*
[4]*Department of Pharmaceutical Chemistry, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist.: Dhule, MS, 425405, India*

Background: **Standardization and quality control of homeopathic drugs is very challenging. As mother tinctures are derived from complex natural resources, there is a need of systematic evaluation of chemical markers which correlate with the proposed biological activities of mother tinctures.**
Methods: **In present study, High-Performance Thin-Layer Chromatography (HPTLC) standardization method of homeopathic mother tinctures of *Toxicodendron pubescens* using quercitrin and rutin as chemical markers is validated and correlations of content of these markers with its anti-inflammatory effects are established.**
For HPTLC analysis, precoated silica gel plates were used as stationary phase. Two flavonoids, namely quercitrin and rutin were used as markers. Separation was achieved using methylene chloride:methanol:water:glacial acetic acid (15:1.5:1:8 v/v/v) as mobile phase. The developed plates were scanned at 365 nm.
Results: **It was observed that quercitrin (Rf value 0.63) and Rutin (Rf value 0.41) are well resolved. The minimum detectable concentrations for quercitrin and rutin were 5 ng/spot. The linearity range was between 100 and 2000 ng/spot for both the markers. Subsequently, anti-inflammatory activity of these formulations was determined against carrageenan-induced paw edema in rats, pain threshold determined by electronic Von-Frey apparatus and paw withdrawal latency (PWL) on hot-plate. All the tested formulations of *Rhus Tox* showed anti-inflammatory and analgesic activity against carrageenan induced paw edema in rats. Quantitative correlation between the content of markers and anti-inflammatory activity of mother tinctures was established. Results: Anti-inflammatory effect as well as effect on paw withdrawal and pain threshold, at third hour after carrageenan injection, correlated with quercitrin and rutin content in the respective formulations.**
Conclusions: **This study validates a quantitative HPTLC method for standardization of homeopathic mother tincture of *Rhus Tox* and establishes quercitrin and rutin as**

*Correspondence: Chandragouda R Patil, R. C. Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist.: Dhule, MS, 425405, India.
E-mail: pchandragouda@yahoo.com
Received 16 October 2014; revised 16 July 2015; accepted 12 August 2015

markers corresponding its biological activity. Contents of quercitrin and rutin in *T. pubescens* mother tincture correlates with its anti-inflammatory and analgesic actions and the validated HPTLC method can be used in standardization of homeopathic mother tincture of *T. pubescens*. *Homeopathy* (2016) **105**, 48–54.

Keywords: HPTLC; Homeopathic drug standardization; Quercitrin; Rutin; *Toxicodendron Pubescens*; Mother tincture

Introduction

Homeopathic medicines are criticized for inconsistent efficacy and lack of stringent quality control parameters. However, lower dilutions of homeopathic medicines containing detectable amount of active ingredients can be subjected to chemical profiling and quantification of active ingredients or chemical markers.[1] A vast number of homeopathic drugs contain hydro-alcoholic extracts of medicinal plants. Though, the issue of standardization of ultra-high homeopathic dilutions can't be effectively resolved due to practical absence of active ingredients, the quality of lower dilutions based on the concentrations of the active principles needs urgent attention. Homeopathic pharmacopoeias of different nations are continuously revising the monographs of homeopathic drugs through inclusion of sophisticated analytical techniques.[1] Use of Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) fingerprint profiles are routinely used for deciding the identity, purity and strength of the homeopathic drugs.[2]

In India the market of homeopathic medicines is growing at 25% a year, and more than 100 million people depend on homeopathic remedies for their routine health care. The popularity of these remedies is continuously escalating in India.[3] Global market for the homeopathic drugs is of multibillion US dollars. Considering such enormous market volume and widespread consumption of homeopathic drug, implementation of validated, accurate and sensitive analytical methods in standardization of homeopathic drugs is an urgent need.

Rhus Tox is also known as '*Poison Ivy*' and belongs to the family: Anacardiaceae and information regarding experimental proving of the biological activities of this homeopathic drug is available in scientific literature.[4] The fresh leaves of *Rhus Tox* contain a volatile principle called toxicodendrol which contains a complex active principle-urushiol.[5] In homeopathy, the mother tincture of *Rhus Tox* is not used in treatment but all higher dilutions are manufactured using it.

The homeopathic pharmacopoeias belong to the particular country and the regulations are laid by respective Governments. For the quality control of homoeopathic drugs, Government of India published Homoeopathic Pharmacopoeia of India. As per Drugs and Cosmetics Act 1940, homoeopathic medicine should contain compounds and drugs which are mentioned in any official pharmacopoeia and authentic reference.[6] The Indian Homeopathic Pharmacopoeia, European and French pharmacopeias prescribe qualitative TLC analysis of quercitrin and rutin in the *Rhus Tox* formulations. However, there is no data to substantiate the correlation of marker content with the anti-inflammatory activity of *Rhus Tox*. Further, qualitative determination of these flavonoids may not be true representatives of quality and biological activity of the *Rhus Tox* formulations. In present piece of work, five different mother tincture formulations of *Rhus Tox* – a prototype, anti-inflammatory homeopathic formulation – from different manufacturers were quantified using validated HPTLC method for quercitrin and rutin as the chemical markers. Correlation of the flavonoid marker content with anti-inflammatory efficacy was established through evaluation of anti-inflammatory activity of mother tinctures in the Carrageenan-induced rat paw edema method as described by Patil et al.[7]

Materials and method

Mother tinctures, standards and reagents

Four marketed mother tincture preparations were obtained from Indian manufacturers named SBL Pvt. Ltd., Delhi (India); Sintex International Pvt. Ltd., Kalol, Gujarat (India); HAPCO (HAPCO publishing Co. Pvt. Ltd.), Kolkata (India); and a German manufacturer, Reckweg and company, Germany. A mother tincture prepared according to Homeopathic Pharmacopoeia of India using the authentic gift sample of *Rhus Tox* from Homeopathic Pharmacopoeia laboratory, Ghaziabad, U.P. (India); this is mentioned as 'self prepared' formulation. Carrageenan (λ), rutin, quercitrin hydrate and Diphenylboric acid aminoethylester were obtained from Sigma–Aldrich, USA. Macrogol-400, Methanol, glacial acetic acid, dichloromethane of analytical grade was purchased from Loba chemie, India.

Chromatographic study was performed on 20 cm × 10 cm aluminum-backed HPTLC plates coated with 200 μm layers of silica gel 60 F_{254}, purchased from E Merck. Densitometry detection was performed with a Camag TLC Scanner 3 (Camag, Muttenz, Switzerland) fitted with win CATS software (version 1.3.0.). Chemical marker and samples were applied on the HPTLC plates using Linomat 5 (Camag) applicator under nitrogen gas flow.

Animals

Male Wistar rats of 8–9 weeks age weighing 180–200 g were used in the study were procured from institutional animal house facility. All the animal study procedures were approved by Institutional Animal Ethical Committee (Registration No. 651/02/C/CPCSEA with

resolution number RCPIPER/IAEC/2013-14/02, approved 15/04/2013). The animal experimentation was approved by the Institutional ethical committee after due consideration that the exact mechanism of anti-inflammatory action of *Rhus Tox* mother tincture is not known and hence, *in-vitro* alternative methods to test anti-inflammatory activity could not be used for present study. Rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22°C under lighting controls (12-h-light/12-h-dark cycle). Standard rat feed pellets (Amrut rat feed, manufactured by Nav Maharashtra Chakan, India) and tap water was given *ad libitum*. Each kilogram of rat feed contained 212 g of casein, 3 g of L-cystine, 439 g of starch, 50 g of cellulose, 100 g of sucrose, 100 g of maltodextrin, 50 g of soya bean oil, 35 g of salt mix and 10 g vitamin mix.

Preparation of mother tincture

Dried coarse powder of leaves of *Rhus Tox* was pulverized and 10 g of powder was mixed with 100 ml of 70 % ethanol and kept in a glass jar for 7 days with intermittent shaking every day.[8,9] On 8th day, the mixture was filtered through Whatman filter paper and the filtrate was used as mother tincture.

Preparation of stock standards

Stock standard solutions of quercitrin and rutin were prepared separately by dissolving 5 mg each in 20 ml of 96% v/v ethanol. It was then further diluted with the same solvent to obtain concentrations of 10, 20, 50, 100 and 200 μg/ml each.[10]

HPTLC for standardization of mother tincture

The samples and standards were applied on HPTLC plates as bands of 8 mm and methylene chloride:methanol:water:glacial acetic acid (15:1.5:1:8 v/v/v) was used as mobile phase. Mobile phase components were mixed prior to use and the development chamber (20 × 10) was left for saturation with mobile phase vapor for 20 min before each run at room temperature (25°C ± 2). Densitometry scanning was done in absorbance-reflectance mode at 365 nm using a deuterium lamp with slit dimensions at 6 mm.

Linearity studies

To study linearity for quercitrin and rutin, fixed volume of 10 μl each stock standards solution were separately applied on HPTLC plate to obtain 100, 200, 500, 1000, and 2000 ng/band concentration of quercitrin and rutin, respectively. The plates were developed and scanned as stated above. Each sample was applied in triplicates. The plates were developed and scanned as described above. The amounts of quercitrin and rutin present in the samples were calculated by interpolation.[11–13]

Quantification of quercitrin and rutin in marketed mother tincture

Standard and test samples were applied on HPTLC plates. The plates were developed and scanned at 365 nm for quantitative evaluation. Contents of quercitrin and rutin in standard and marketed mother tincture were estimated using linearity equation.

Validation of HPTLC method

The HPTLC method was validated as per ICH guideline Q2 (R1).[14] For determining precision, quercitrin and rutin solutions having appropriate concentration were applied in six replicates (n = 6) and quantified. For determination of intra-day and inter-day precision, freshly prepared quercitrin and rutin standard solutions were applied in triplicates on the same day and on three different days. The specificity of the method was ascertained by analyzing standard quercitrin and rutin and samples of mother tincture. The spots for quercitrin and rutin in the samples were confirmed by comparing the Rf and peak purity spectra of the spots. The robustness of method was estimated by making deliberate changes in the composition of mobile phase (±5%), mobile phase volume (5% v/v), development distance and chamber saturation time. Two mobile phases as dichloromethane:methanol:water:glacial acetic acid (15:3:2:8 v/v/v) and of dichloromethane:methanol:water:-glacial acetic acid (15:1.5:1:8 v/v/v) were used. The saturation time of developing chamber with mobile phase was 20 min ± 2 min (±10%). The results were indicated by the standard deviation between the data for each variable condition. The accuracy of the method was determined by performing recovery experiments at three different levels (80%, 100% and 120% addition of quercitrin and rutin) using the standard addition method. The known amounts of quercitrin and rutin standards (50 μg/ml) were added by spiking. The values of % recovery and average value of % recovery for quercitrin and rutin were calculated.

Biological activity

Carrageenan-induced paw edema: Anti-inflammatory and analgesic activity in carrageenan-induced paw edema in rats For determination of the anti-inflammatory and analgesic activity, rats fasted for twelve hours were orally administered with 0.1 ml of mother tincture mixed with 0.9 ml of distilled water 30 min prior to subplantar injection of 0.1 ml of 1.2% carrageenan solution (n = 7 per group per formulation). Oral Diclofenac sodium at 5 mg/kg dose (in 1 ml water) administered 30 min prior to carrageenan served as the standard drug. The negative control group received 1 ml water. The paw volumes were measured at 0 min (immediately after carrageenan injection) and up to 6 h at an interval of one hour. Paw volume was determined using digital Plethysmometer (Ugo Basile, Italy; model 7140).

Percentage rise in the paw volume at third hour post carrageenan injection, calculated by following formula[15]

was considered to compare the anti-inflammatory activity of mother tinctures.

$$\% \ Rise = Vt - \frac{V0}{V0} \times 100 \qquad (1)$$

Where, Vt = Paw volume at third
V_0 = Paw volume immediately after carrageenan injection

Carrageenan-induced mechanical hyperalgesia: It was estimated as paw withdrawal threshold (PWT) using electronic Von Frey analgesiometer (IITC life science). Pressure was applied on the carrageenan injected paw using semi rigid probe tips and the pressure (in grams) at which paw withdrawal occurred was recorded as PWT.[15]

Carrageenan-induced thermal hyperalgesia: It was measured by determining the paw withdrawal latency (PWL) for the carrageenan injected paw of rats freely moving on the Eddy's hot plate maintained at 51°C.[16–18] The rise in PWL was taken as an indication of the analgesic effect.

Results

Present study establishes a quantitative correlation between flavonoid markers with the anti-inflammatory activity of *Rhus Tox* mother tinctures. The quercitrin and rutin concentration was quantified with validated HPTLC method.

The mothers tincture were investigated for quercitrin and rutin content using silica gel 60 F 254 as stationary phase and methylene chloride: methanol: water: glacial acetic acid (15:1.5:1:8 v/v/v) as the mobile phase. The chamber saturation time was optimized 20 min whereas quantitative estimations were performed at 365 nm. The rutin (Rf = 0.41, Figure 1) and quercitrin (Rf = 0.63, Figure 2) were well resolved without any interference. The peaks of flavonoids were quantified before and after derivatization with diphenylboric acid aminoethylester and macrogol-400 in 96% ethanol. Figure 3.

The linear regression data were obtained for calibration curves showed an excellent linear relationship over wide range of concentration 100–2000 ng/spot for both standards quercitrin and rutin with reference to their peak area. Linearity curve for quercitrin was found to be y = 3767.8x − 2489.7 with regression value 0.9901 and for rutin was found to be y = 4056.3x − 2582.4 with regression value 0.9912.

HPTLC analysis

Quantification of quercitrin and rutin in marketed mother tincture: The concentration of rutin, quercitrin within marketed formulation was quantitated by the developed HPTLC method. The results are shown in Table 1. In all the marketed formulations along with self-prepared preparation, the presence of rutin and quercitrin was found. The quantitative analysis shows the variation in rutin and quercitrin in marked formulation (Table 1). The concentration of quercitrin decreases in order of Sintex, Reckweg, self-prepared, SBL and HAPCO whereas, for rutin the order was Sintex, SBL, Reckweg, HAPCO and self-prepared.

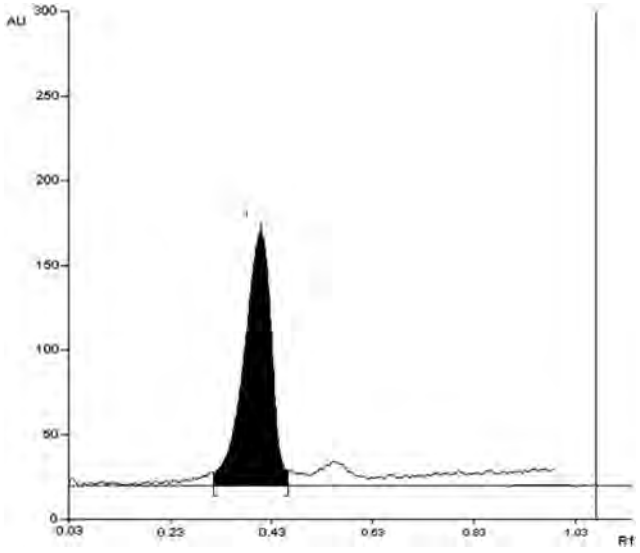

Figure 1 Revealed Rf value of Rutin.

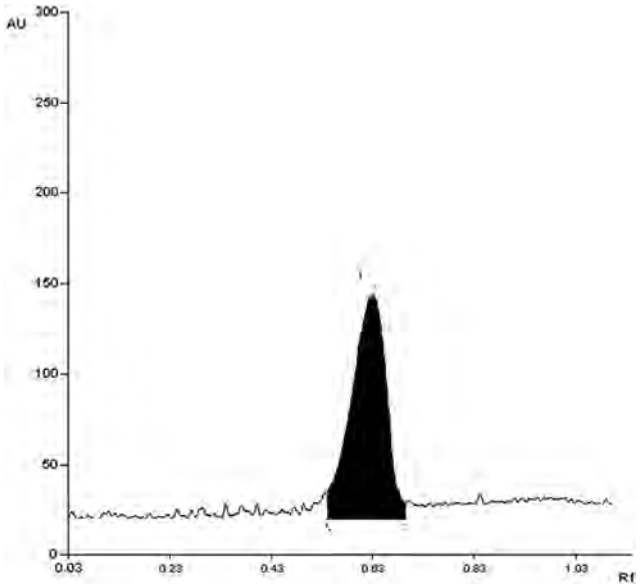

Figure 2 Revealed Rf value of Quercitrin.

Method validation of HPTLC: The developed HPTLC method was validated for different parameters as per ICH guidelines like linearity, precision, robustness, specificity, accuracy which helped for quantitative analysis of quercitrin and rutin in *Rhus Tox*. The peaks of quercitrin and rutin also well resolved. The compounds were confirmed by comparing the Rf and spectra with that of standards peak at start, mid and end position. Absence of any interfering peak indicated that the method was specific. The intra-day and inter-day precision analysis (n = 3) results showed % RSD less than 2, indicates that the method is precise. Robustness of method was studied by conscious change in mobile phase composition and by altering chamber

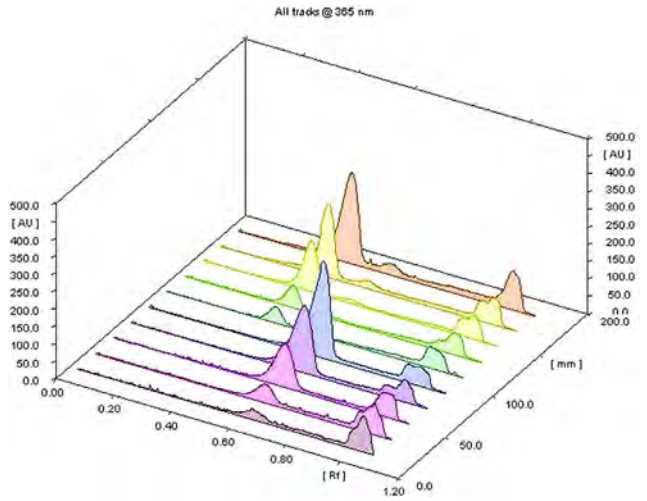

Figure 3 Revealed densitometric analysis of Quercitrin and Rutin (From bottom to top: Peak-1: Que. – 10 μg/ml, Peak-2: Que. – 20 μg/ml, Peak-3: Que. – 50 μg/ml, Peak-4: Que. – 100 μg/ml, Peak-5: Que. – 200 μg/ml, Peak-6: rutin – 10 μg/ml, Peak-7: Rutin – 20 μg/ml, Peak-8: Rutin – 50 μg/ml, Peak-9: Rutin – 100 μg/ml, Peak-10: Rutin – 200 μg/ml).

Table 1 Amount of quercitrin and rutin found in *Rhus Tox*

Rhus Tox	*Quercitrin*		*Rutin*	
	Amt. (ng/spot)	*S.D.*	*Amt. (ng/spot)*	*S.D.*
A (Self prepared)	2.91	0.88	1.22	0.31
B (SBL)	2.82	1.21	2.46	0.0015
C (Reckweg)	4.80	1.44	2.40	0.0011
D (Sintex)	5.09	0.92	3.46	1.26
E (HAPCO)	1.98	0.85	1.58	0.50

Amt. = Amount, S.D. = Standard Deviation.

saturation time. Results indicated % RSD less than 2 shows the developed method was robust one. The summary of results of validation parameters is shown in Table 2.

Biological activity

Anti-inflammatory and analgesic effects of Rhus Tox *mother tinctures*: All the tested mother tincture formulations showed anti-inflammatory effects as the rise in paw volume is inversely proportional to the anti-inflammatory activity. In the rats treated with Diclofenac (5 mg/kg) the rise in paw volume was $26 \pm 2.9\%$ as compared to $73 \pm 4.3\%$ in negative control group. There was no statistically significant difference in the anti-inflammatory activity of Diclofenac and mother tinctures of *Rhus Tox*. The intensity of anti-inflammatory activity was found to descend in the order of the mother tincture formulation from Sintex, Reckweg, self-prepared, SBL and HAPCO.

Carrageenan-induced mechanical hyperalgesia: Effect of drug treatments on the hyperalgesia induced by sub plantar injection of carrageenan was estimated using electronic Von-Frey apparatus. The pressure (in grams) exerted by the semi rigid Von-Frey filament at which rat shows paw withdrawal was taken as a parameter of hyperalgesia. The values in second column of Table 3 indicate the mean PWT of three readings recorded at interval of one minute at the third hour post carrageenan injection. The analgesic effect of Diclofenac was most potent (PWT = 22.23 ± 3.9). The effects of mother tinctures were more than the control group values however this rise in the PWT was not statistically significant.

Diclofenac treatment increased the PWL in carrageenan injected animals. The mother tincture increased the PWL but in statistically insignificant manner.

As shown in the Figure 4, the content of quercitrin and rutin showed correlation with the anti-inflammatory effects of mother tinctures. Such correlation was not evident between the flavonoid content parameters related analgesic activity.

The biological effects of *Rhus Tox* mother tinctures including anti-inflammatory activity, PWL and PWT were plotted against the quercitrin and rutin contents. It was observed that anti-inflammatory activity of the mother tinctures was proportional to the content of both rutin and quercitrin. However, such correlation was not evident when the concentrations of these flavonoids were plotted against PWL and PWT. Figure 4 depicts the correlation of quercitrin and rutin contents (Table 3) with the anti-inflammatory activity of mother tinctures. The formulations obtained from Sintex Pvt. Ltd. Showed highest contents of quercitrin and rutin and the percentage rise in paw edema in the rats receiving this mother tincture was least amongst the tested samples.

Table 2 Method validation parameters for quercitrin and rutin

Validation parameters		*Quercitrin*	*Rutin*	*Maximum acceptance*
R_f		0.63	0.41	–
Linearity				
Linearity range		100–2000 ng/spot	100–2000 ng/spot	
Correlation coefficient (r^2)		0.9901	0.9912	Within 0.9–1.1
Linear regression equation, (Y = mX + c)		y = 3767.8x – 2489.7	y = 4056.3x – 2582.4	–
Selectivity		Selective	Selective	–
Specificity		Specific	Specific	No interference observed
Robustness		Robust	Robust	–
Repeatability (n = 6)		0.94	1.73	% RSD ≤2%
Precision	Intraday (n = 3)	1.03–1.31	0.7–1.2	% RSD ≤2%
	Interday (n = 3)	0.5–2.2	0.74–2.74	
Accuracy (% Recovery)		97.24%	97.29%	

% RSD = % Relative Standard Deviation.

Table 3 Correlation of biological activity with present chemical ingredients

Sr. No.	*Formulation*	*Amount found in formulations (ng/spot)*		*Biological activity (% Rise in inflammation)*	*Mechanical hyperalgesia PWT (g)*	*Thermal hyperalgesia PWL seconds*
		Quercitrin	*Rutin*			
1.	Negative control			72.64 ± 4.3	9.4 ± 1.1	7.5 ± 1.15
2.	Diclofenac			25.82 ± 2.9*	22.23 ± 3.9*	15.66 ± 1.4*
3.	Sintex	5.09	3.46	35.40 ± 1.9*	13.76 ± 2.6	13.66 ± 1.1
4.	Reckweg	4.80	2.40	36.03 ± 3.6*	11.02 ± 1.4	10.83 ± 1.7
5.	Self-prepared	2.91	1.22	36.92 ± 3.8*	15.16 ± 3.6	11.33 ± 1.7
6.	SBL	2.82	2.46	39.98 ± 6.9*	12.61 ± 2.2	11.33 ± 2.5
7.	HAPCO	1.98	1.58	50.25 ± 4.6*	13.24 ± 2.4	13.33 ± 1.4
	ANOVA			P = ***0.0001 [F = 12; df = 6; n = 7/group]	P = *0.0359 [F = 2.4; df = 6; n = 7/group]	P = *0.048 [F = 2.4; df = 6; n = 7/group]

(ANOVA = Analysis Of Variance, PWT = Paw Withdrawal Threshold, PWL = Paw Withdrawal Latency, df = degree of freedom).
(For Bonferroni multiple comparison post-test *p < 0.05).
Figures indicate % rise in inflammation and increased hyperalgesia. n = 7 animals per group.
***P < 0.001 compared with control.
*P < 0.01 compared with control.
Statistical analysis was done by one way ANOVA test.
Bonferroni multiple comparison test < *0.05.

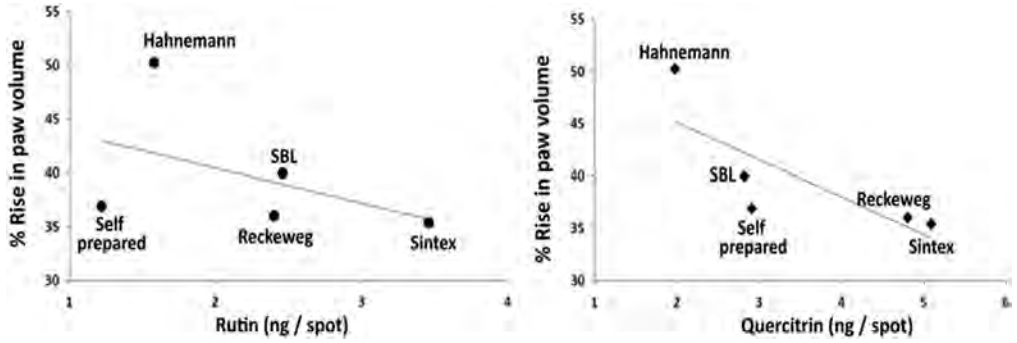

Figure 4 Revealed correlation of Quercitrin and Rutin of *Rhus Tox* mother tincture with its biological activity.

Discussion

Homeopathic medicines are prepared as hydro alcoholic extracts of source material including plants, animals, microbes, mineral and nosodes. Obviously, maintenance of the quality and consistency of such medicines developed from such diverse origins is a challenge. With the advent of sophisticated, highly sensitive analytical methods, at least the crude drug material and lower dilutions like homeopathic mother tinctures can be subjected to standardization. Recent editions of homeopathic pharmacopoeias of different countries include TLC, High Performance Liquid Chromatography (HPLC) and HPTLC methods of standardization for the mother tinctures used in homeopathy. However, the markers specified in such monographs are not validated in terms of their relevance with the biological activity.

HPTLC is an effective analytical method for detection and quantification of analytes at micro and nanogram levels. With minimum operating cost, it has been very prominent method with rich sample throughput and normal sample clean-up for detection. HPTLC supersedes HPLC method because of its less analysis time, economy and conceited power for detection of complex mixture of phytoconstituents with higher resolution.[10] HPTLC analysis offers chromatographic fingerprinting of compounds, which helps to detect new compounds from complex herbal extract.[10]

TLC is mainly principled on simple steps: it is an open system, dependent on environmental factors (temperature, light, fumes, humidity), that can influence the resulting data which are not fully controlled, reproducible and reliable. The problems associated with conventional TLC methods like dependence of environmental factors, openness of the system etc. are overcome by HPTLC.[19] Recently, the HPTLC methods have been employed for standardization of the homeopathic mother tincture of Nux Vomica.[20]

Results of present study on mother tincture of *Rhus Tox* reveal that HPTLC method for quantitative estimation of quercitrin and rutin has advantages over the qualitative method prescribed in current editions of Homeopathic Pharmacopoeias. This quantitative method is rapid, simple, precise, accurate and specific. Major contribution of this study is an effort to correlate the contents of the markers with the biological activity of mother tinctures. It was observed that the content of quercitrin and rutin were in proportion to the biological activity of the *Rhus Tox* mother

tinctures from different sources. Further, consolidation of such correlations through *in vivo* and *in vitro* experimental models of inflammatory process can substantiate the relevant marker for standardization of *Rhus Tox* mother tincture.

In present context, the standardization of high and ultra high dilutions of homeopathic medicines is difficult to achieve through chemical or instrumental analysis. However, these methods in association with the biological standardization satisfactorily resolve the issue of standardization of lower dilution like mother tinctures used in homeopathy. The mother tinctures of *Rhus Tox* can be standardized according to the validated HPTLC method using quercitrin and rutin as a valid markers related to anti-inflammatory activity. Further studies to substantiate the correlation between marker content with the biological activity through *in vivo* and *in vitro* assays is warranted to consolidate the validity of chemical markers in standardization of the mother tinctures of other homeopathic medicines.

Conflict of interest

The authors have no conflict of interest.

Acknowledgment

The authors are thankful to Jadhav RB, Principal, Arvind Gavali college of Pharmacy, Satara, Maharashtra (India) for supply markers used in study, Shivang Swaminarayan, Head – Healthcare division, Sintex International Pvt. Ltd., Kalol, Gujarat (India), and Hahnemann Publishing Co. Pvt. Ltd. (HAPCO), Kolkata, West Bengal (India) for generous supply of the crude form and mother tinctures of *Rhus Tox*. Thanks to the Director, Homeopathic Pharmacopoeia Laboratory, Ghaziabad UP, (India) for supply crude drug and authenticate the drug.

References

1 Satti J. Homeopathic drug standardization. *Semin Integr Med* 2005; **3**: 113–122.
2 Choudhary N, Sekhon BS. An overview of advances in the standardization of herbal drugs. *J Pharm Educ Res* 2011; **2**: 55–58.
3 Prasad R. Homoeopathy booming in India. *Lancet* 2007; **370**: 1679–1680.
4 Huh YH, Kim MJ, Yeo MG. Homeopathic Rhus toxicodendron treatment increased the expression of cyclooxygenase-2 in primary cultured mouse chondrocytes. *Homeopathy* 2013; **102**(4): 248–253.
5 Dos Santos AL, Perazzo FF, Cardoso LG, Carvalho JC. *In vivo* study of the anti-inflammatory effect of Rhus toxicodendron. *Homeopathy* 2007; **96**(2): 95–101.
6 Tiwari L, Rai N, Sharma RK. Regulatory standards on homoeopathic drugs: Indian perspective. *Int J Adv Pharma Sci Tech* 2013; **1**(1): 1–20.
7 Patil CR, Gadekar AR, Patel PN, Rambhade A, Surana SJ, Gaushal MH. Dual effect of *Toxicodendron pubescens* on Carrageenan induced paw edema in rats. *Homeopathy* 2009; **98**(2): 88–91.
8 Shanbhag DA, Khandagale AN. Screening and standardisation of *Terminalia Arjuna* used as medicine in homoeopathy using HPTLC method. *Int J Anal Bioanal Chem* 2011; **1**(3): 57–60.
9 Milind P, Pathak A. HPTLC standardization of homeopathic mother tincture of *chenopodium album (bathua sag)*. *J Glob Pharma Tech* 2009;54–57.
10 Tomczyk M, Bazylko A, Bonarewicz J. Method development and validation for optimized separation of quercetin derivatives in selected Potentilla species using high-performance thin-layer chromatography photodensitometry method. *J Pharm Biomed Anal* 2012; **61**: 265–270.
11 Shanbhag DA, Khandagale AN. Application of HPTLC in the standardization of a homoeopathic mother tincture of *Syzygium Jambolanum*. *J Chem Pharm Res* 2011; **3**(1): 395–401.
12 Jain R, Rajput SJ. Development of pharmacognostical parameters and estimation of quercetin using HPTLC in leaves of *Nelumbo nucifera* Gaertn. *Pharmacogn J* 2012; **4**(34): 31–37.
13 Shanbhag DA, Jayaraman S. Application of HPTLC in the standardization of a homoeopathic mother tincture of *Nux Vomica*. *Indian J Res Homeopathy* 2008; **2**: 1–7.
14 International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). *Validation of analytical procedures: text and methodology Q2(R1)*. Available on, http://www.ich.org/products/guidelines/quality/article/quality-guidelines.html; 2005. accessed on 15/07/2015.
15 Orlandi L, Vilela FC, Santa-Cecília FV, Dias DF, Alves-da-Silva G, Giusti-Paiva A. Anti-inflammatory and antinociceptive effects of the stem bark of Byrsonima intermedia A. Juss. *J Ethnopharmacol* 2011; **137**(3): 1469–1476.
16 Albayrak A, Halici Z, Cadirci E, *et al*. Inflammation and peripheral 5-HT7 receptors: the role of 5-HT7 receptors in carrageenan induced inflammation in rats. *Eur J Pharmacol* 2013; **715**(1–3): 270–279.
17 Bonet IJ, Fischer L, Parada CA, Tambeli CH. The role of transient receptor potential A 1 (TRPA1) in the development and maintenance of carrageenan-induced hyperalgesia. *Neuropharmacology* 2013; **65**: 206–212.
18 Chakrabarty A, McCarson KE, Smith PG. Hypersensitivity and hyperinnervation of the rat hind paw following carrageenan-induced inflammation. *Neurosci Lett* 2011; **495**(1): 67–71.
19 Nicoletti M. HPTLC fingerprint: a modern approach for the analytical determination of Botanicals. *Braz J Pharmacogn* 2011; **21**(5): 818–823.
20 Patel DK, Patel K, Duraiswamy B, Dhanabal SP. Phytochemical analysis and standardization of *Strychnos Nux-vomica extract* through HPTLC techniques. *Asian Pac J Trop Med* 2012; **2**(1): S56–S57.