PHYTOCHEMICAL EVALUATION OF HOMEOPATHIC MOTHER TINCTURES OF DIFFERENT BRANDS: AN APPROACH FOR QUALITY ASSURANCE STANDARDIZATION OF HOMEOPATHIC MOTHER TINCTURES OF DIFFERENT BRANDS

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Abstract:

Context: The high potential variability of chemical composition of the plant material involved in the manufacture of homeopathic mother tinctures (Ǿ) renders quality control and assurance as a significant challenge. In order to assess the quality difference between various brands of homeopathic mother tinctures viz. *Arnica montana (AMǾ)* and *Rhus toxicodendron* (RTǾ) were chosen for their evaluation.

Aims: The present study aims to check whether they are deficient with required drug strength, developing standardized procedure for evaluating the mother tinctures for its accuracy, sensitivity and reproducibility. This research is an attempt to assess variations in drug strength of different brands of mother tincture.

Settings and Design: The Local(L), National(N) and Internationally(I) manufactured AMǾ and RTǾ were standardized by using various physical, chemical and chromatographic parameters and compared with those mentioned in homoeopathic Pharmacopoeia of India (HPI).

Methods and Material: The standardization of mother tinctures were done by using five standard parameters such as pH, alcohol content, total solids, weight per ml and Thin Layer Chromatography (TLC). The aim has been achieved by carrying out physicochemical evaluation, phytochemical screening, quantitative estimation and HPTLC fingerprinting.

Results: The results for each evaluation parameters were found to be significant for the L and N manufactured AMǾ and RTǾ

Conclusions: The experimental findings suggest that L and N manufactured AMǾ and RTǾ, demonstrated a superior quality as compared to the selected IAMǾ and IRTǾ. Thus, the standardization may lead to a solution to the factors which are responsible for variation in the homeopathic tinctures.

Keywords: Standardization, Homeopathy, Mother tincture, HPTLC, Quality.

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INTRODUCTION:

Homeopathy: It is a system of medicine with the history of more than 200 years, due to principle of "*Similia Similibus Curentur*". It is a holistic system of therapy which works at reinforcing the body's own natural capacity to heal and achieve a gentle and lasting cure. It considers the human as a whole not just his individual parts (Pradhan, P.K., 2006). Homeopathic medicines are available in the form of Mother Tinctures, dilutions, biochemics and various patent or combination formulae.

Mother Tincture: The Mother Tinctures are clear liquids ranging in colour from pale straw to dark brown or dark red. They are denoted by the 'Q' or the Greek letter theta (Ø) (Banerjee, D.D., 2006). According to '*Dr. Dewey'*, Mother Tinctures are defined as, "The strongest liquid preparation of drugs used in Homeopathy,and made by maceration or dissolving the drug or portions of it in alcohol and water." (Dewey, W.A., 1998). They contain number of chemical entities (Pathak, S., 2009). Drug strength of a mother tincture is the amount of crude drug contained in it. The maintenance of uniform drug strength of mother tincture is very much required so as to make them therapeutically efficacious. Drug strength i.e. quality of mother tincture can be assessed by different standardization parameters viz., Alcohol content, weight per ml,pH value, total solids and TLC test (Sahani, M.K 2007) .

Standardization and quality control: *Standardization* is a process of having a product in *uniform* standard as mentioned in the pharmacopoeia. It includes a *Quality Control System* with various tests to perform at different stages of manufacture, from raw materials to finished products. It also involves an *Assurance &assessment of quality of products* and *maintenance of purity* with reference to a pharmacopoeial monograph (Mandal, P.P. and Mandal, B 1994). Manufacturing industry rely upon both qualitative and quantitative analysis of raw materials to meet certain specifications and to check final product. Mother tinctures can be subjected to a qualitative and quantitative analysis that evaluates the identity, purity and stability of the preparation (Goel, S., 2007). The parameters to assess the quality of Mother Tinctures are alcohol content determination, Weight per ml, pH value, total solids, Chromatography, Spectro-photometry for λ max, fluorescence Analysis etc (Banerjee, D.D.,2006).

Thus, the objective of this study is to evaluate the accuracy and genuineness of quality standards of commercial homoeopathic mother tinctures manufactured in India. Current study is very much needed as it is a known fact that, the final product i.e. Mother Tinctures ensures the essential components present in prepared drug and the Mother Tinctures are responsible for efficacy of Homeopathic potencies prepared from it. The problems of quality control with the homoeopathic potencies serve one of the areas of greatest challenge. Till date, no standardization parameters have been set for evaluation of drug strength and high potencies of homoeopathic medicines. As the homoeopathic medicinal agents consist of highly diluted and potentized medicines, care at each step of manufacture adds on to the quality and reliability of the final product. Thus, the present research work has been planned to compare the Homeopathic Mother Tinctures of various brands for its quality. In this study, the mother tinctures of *Arnica montana*and *Rhus toxicodendron*are selected to evaluate.

***Arnica Montana* L.:** *Arnica montana*, also known as wolf's bane, leopard's bane, mountain tobacco and mountain arnica, is a moderately toxic ethnobotanicalEuropean flowering plant in the sunflower family (Asteraceae) (H.P.I. 1971). Arnica flower contains mainly flavonoids such asarnisterol, thymol, inulin, luteolin, tricin, kaempferol, quercetin, choline, coumarin, scopoletin, umbelliferone, arnicin, caffeic acid, α- and βcarotene, cryptoxanthin, lutein, isorhamnetin etc. (Varma, P.N. and Vaid,I2017). Traditionally used for the external treatment of sprains and bruises, typically as the tincture, as an antiseptic, anti-inflammatory, antibacterial, decongestive andantifungal properties. In homeopathic preparations it is also used for internal and external injuries (Thakur, T., 2017).

***Rhus toxicodendron*:** *Rhus toxicodendron* is produced from the plant commonly known as poison ivy which grows as a shrub or a woody vine, spreading all over the countryside as a weed in the Eastern USA and Canada. It is a member of the Anacardiaceae family of plants. *Rhus toxicodendron*contains Urushiol, Cardol, Phenolic acid, Resin, Fiestin, Tannins. It is mainly used in Homeopathic preparations used to treat pain, rheumatoid arthritis, menstrual period problems, swelling, and itchy skin disorders. It is also used as a restlessness&stiffness(Jadhav, H.P.etal. 2017).

SUBJECTS AND METHODS:

Procurement of different brands of mother tinctures: The different brands of tinctures are selected for the study such as local brand, national brand and international brand. The Local brand was procured from the Homeopathic Pharmacy Jhansi Rani square, Burdi, and Nagpur. The National brand and International brand were procured from Aster Homeopathic Pharmacy Burdi Nagpur. Therefore, the National brand is Schwabe and International is Dr. Reckeweg. The abbrevations for all the samples of different brands of homeopathic mother tinctures are as follows the *Arnica montana* local brand – LAMǾ, for national brand – NAMǾ & for international brand – IAMǾ. Whereas *Rhus toxicodendron* local brand- LRTǾ,for national brand – NRTǾ & for international brand – IRTǾ.

Standardization of mother tincture: Standardization of mother tincture was conducted to compare the organoleptic properties such as color, odor, taste and physicochemical properties of mother tincture such as weight per ml, total solid content, alcohol content, pH value, λ max (H.P.I. 1971).

Physicochemical evaluations:
The physicochemical evaluations are done for determination of weight per milliliter, total solids, alcohol content, pH value, λ max for the tinctures. All the procedures were carried out in triplicates by following standard procedures. (Skoog, D.A., Holler, F.J. and Crouch 2004).

Qualitative evaluations by preliminary phytochemical screening:
The plants are considered as biosynthetic laboratory for group of compounds like alkaloid, glycoside, volatile oils, tannins, saponins, flavanoids etc. These compounds are termed as secondary metabolites and are responsible for therapeutic effects. To check the presence or absence of primary and secondary metabolites, all the mother tinctures were subjected to series of chemical test (Khandelwal, K.R., 2008)

Quantitative estimations:
The quantitative estimation is carried out for the determination of amount of secondary metabolites present in the sample. The various estimations were performed on all the samples of tinctures by the standard procedures such astotal flavonoid content by aluminium chloride and 2,4-dinitrophenyl hydrazine using Quercetin as an internal standard (Khadabadi, S.S., Deore, S.L. and Baviskar, B.A 2011), total phenolic contents by Folin- ciocalteau method using Gallic acid as an internal standard (Sadasivam, S. and Manickam 1996) , estimation of total carbohydrate content by phenol sulphuric acid method using dextrose as standard (Sadasivam, S. and Manickam 1996) and DPPH radical scavenging activity by usingascorbic acid as an internal standard (Parejo, I.,eta 2002).

Chromatographic studies:
TLC is one of the effective techniques for the identification and separation of chemical constituents in an extract. TLC profile developed for sample could be used as fingerprints in comparative qualitative evaluation of herbal drugs. The inclination of evaluation by this method is becoming prevalent in view of its easiness and reproducibility.

Thin layer chromatography (Stahl, E. 1969 & Wagner, H. and Bladt, S 1996): The drug samples taken were LAMǾ,NAMǾ,IAMǾ,LRTǾ, NRTǾ and IRTǾ. The reference compounds were Rutin& Quercetin. All these were loaded on the Silica gel G coated glass plate (10cm X 10 cm) in the solvent system Chloroform: Methanol (8:2 v/v). The chromatogram was developed and for detection,Aniline - Sulphuric acid reagent was used.

Quantifcation of Quercetin by (HPTLC) High Performance Thin Layer Chromatographic studies (Dwivedi, B.K., ETAL 2017) **:**Quantification of Quercetin was done by HPTLC [CAMAG Linomat5. CAMAG Twin Trough Chamber, Camag TLC Scanner and integration software (winCATS)] by using standard Quercetin (1mg/ml). The samples and standards were loaded onSilica gel GF254 (Merck) 10 × 10 cman developed in the solvent systemChloroform: Methanol (8:2,v/v). The chromatogram developed was scanned at wavelength: 254 nm and linear response was taken.

Linearity response
The volume of the mother tincture was optimised to 2 µl for quantification. It was then simultaneously applied with different concentrations of standard Quercetin i.e., 4, 6, 8, 10 and 12 µl.The plates were developed and scanned as described above. The amounts of quercetin present in samples were calculated by interpolation.

RESULTS:

Organoleptic properties:

The colour variations of homeopathic mother tinctures are dependent on various factors including the time of harvest, the amount of rainfall and even small differences in the manufacturing process can cause a change in the colour of the mother tinctures. A high chlorophyll concentration in the plant at the time of harvest may render a tincture showing a more greencolouring whereas a high flavonoid glycoside concentration will show a more yellowish colour of the mother tincture. The colourcompliance according to GHP for standard*Arnica Montana* tincture isGreenish to yellow brown and for*Rhus toxicodendron*is Yellowish to red brown . In each case the tincture was greenish brown to yellowish brown in colourand also clear liquid and characteristic in odour to astringent to sweet in taste.

Physicochemical properties

Weight per ml

The results of weight per ml of *Arnica montana* and *Rhus toxicodendron*mother tinctures are presented in Table 1. When compared to the standard parameter of HPI it was observed that the LAMǾ was close enough to the standard HPI value and it was good in quality while NAMǾ is acceptable and IAMǾ is poor in quality. Similarly LRTǾ is near to the standard values of HPI,
NRTǾ is acceptable and IRTǾ was poor in quality.

Total solid content

The total solid content in *Arnica montana* and *Rhus toxicodendron*mother tinctures are reported in Table 1. It was observed that the NAMǾ was close enough to the standard HPI value and found to be of good quality while LAMǾ is acceptable and IAM is poor in quality. Similarly NRTǾ is close enough to the standard parameter of HPI, LRTǾ is acceptable and IRTǾ was poor in quality.

Alcohol Content

The alcohol content of *Arnica Montana & Rhus toxicodendron* mother tinctures of different brands are presented in Table 1.When compared to the standard parameter of HPI it was observed that the LAM Ǿwas close enough to the standard value it reveals its good quality and IAMǾ is exact matched to the standard value of HPI. While in IRTǾ it was also observed that it exactly matches to standard value of HPI. So it can be concluded that there is slight difference in all the values of different brands of mother tinctures when compared to standard HPI.

pH

The results of pH of *Arnica Montana &Rhus toxicodendron*mother tinctures of different brands are reported in Table 1. It shows that there is a slight deviation in pH of all the mother tinctures.

Table 1 - Physicochemical properties of AM & RT mother tinctures of different brands

SN	Physicochemical properties	Different Brands of Homeopathic Mother Tinctures							
		Values as per theHPI		LAMǾ	NAMǾ	IAMǾ	LRTǾ	NRTǾ	IRTǾ
		AM	RT						
1	Weight per ml (g/mL)	0.9173	0.8810	0.8926 ± 0.0035	0.8842 ± 0.0042	0.809 ± 0.0033	0.8624 ± 0.0052	0.8842 ± 0.0042	0.8519 ± 0.0047
2	Total solid content (%)	2.96 %	3.02 %	1.2 ± 0.26	1.6 ± 0.20	0.41 ± 0.030	2.60 ± 0.020	4.42 ± 0.48	0.18 ± 0.020
3	Alcohol content (%)	57.87 %	78.57%	60.22 ± 0.61	55.50 ± 0.58	57.20 ± 0.37	76.20 ± 0.31	76.83 ± 0.12	78.23 ± 0.77
4	pH	5.70	5.76	5.5 ± 0.100	5.03 ± 0.052	5.55 ± 0.117	5.73 ± 0.205	5.51 ± 0.060	5.93 ± 0.045

(The values are expressed as Mean of three studies, Mean ± SD, N = 3)

UV spectral analysis:

a. λ max of AM mother tinctures

The UV spectral analysis of *Arnica montana* (AM) mother tinctures of different brands are presented in a Fig no.1 & Table 2. While figure a) shows the UV- VIS absorption spectrum of LAMǾ & this was characterized by a large peak around 321 nm, followed by one shoulder at 289 nm, fig b) shows the UV-VIS absorption spectrum of NAMǾ and it was characterized by a large peak around 320 nm followed by 294 nm. And fig c) shows the UV-VIS spectrum of IAMǾ or it shows the large peak around 323 nm followed by 296 nm.

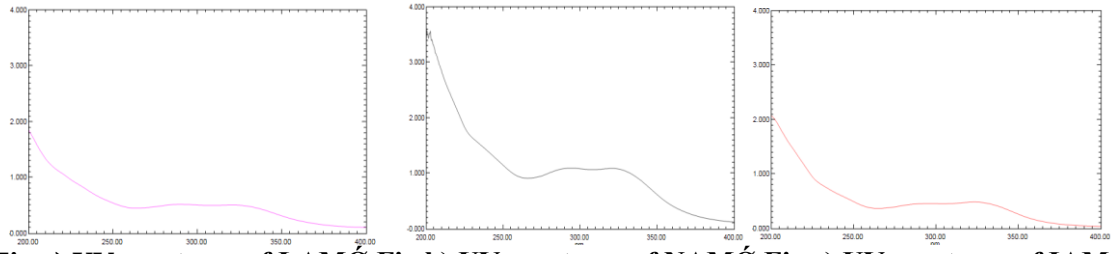


Fig a) UV spectrum of LAMǾ Fig b) UV spectrum of NAMǾ Fig c) UV spectrum of IAMǾ
Fig 1 – UV spectrum of *Arnica montana* mother tinctures of different brands

Table 2 - Absorbance & wavelength of AM mother tinctures

S.N	Different brands of HMT	Wavelength	Absorbance
1.	LAMǾ	321	0.506
		289	0.517
2.	NAMǾ	320	1.089
		294	1.096
3.	IAMǾ	323	0.484
		296	0.456

b. λ max of RT mother tinctures:
The UV spectral analysis of *Rhus toxicodendron* mother tinctures of different brands are presented in a Fig no.2 & Table 3. The fig a) shows the UV-VIS absorption spectrum of LRTǾ & this was characterized by a large peak around 268 nm, followed by one shoulder at 209 nm. fig b) shows the UV-VIS absorption spectrum of NRTǾ and it was characterized by a large peak around 339 nm followed by 265 nm.And fig c) shows the UV-VIS spectrum of IRTǾ or it shows the large peak around 339 nm followed by 264 nm.

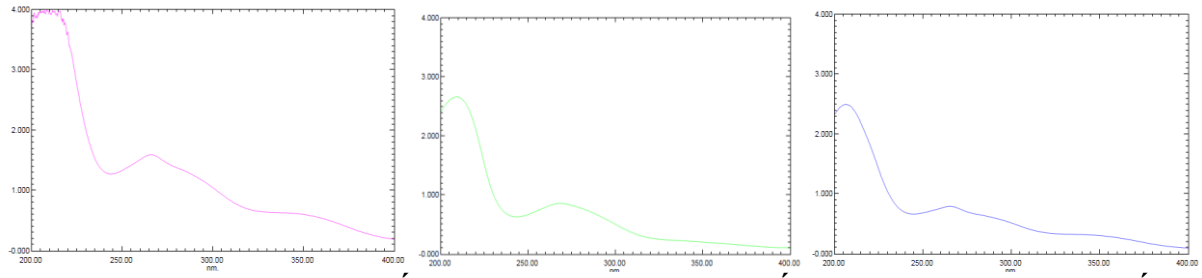


Fig a) UV spectrum of LRTǾ Fig b) UV spectrum of NRTǾFig c) UV spectrum of IRTǾ
Fig 2 – UV spectrum of *Rhus toxicodendron* mother tinctures of different brands

Table 3 - Absorbance & wavelength of RT mother tinctures

S.N	Different brands of HMT	Wavelength	Absorbance
1.	LAMǾ	268	0.856
		209	2.666
2.	NAMǾ	339	0.631
		265	1.532
3.	IAMǾ	339	0.323
		264	0.792

Qualitative evaluations by preliminary phytochemical screening:
The preliminary phytochemical screening shows the presence of carbohydrates, steroids, flavanoids and tannins / phenolic compounds in *Arnica montana*mother tincture . Whereas, in *Rhus toxicodendron* mother tincture of different brands the steroids, flavanoids and tannins/ phenolic compounds were found to be present.

Quantitative estimations:
i. Estimation of Total Flavonoid content
The results of total flavonoid content of *Arnica Montana* &*Rhus toxicodendron* mother tinctures of different brands are presented in table 3. The

standard curve ofQuercetin from 10-100 µg/ ml was prepared. The absorbance of all samples was measured at 415 nm using UV/VIS spectrophotometer.The total flavonoid content in LAMǾ and LRTǾare found to be in higher amount as compared to national and international brands of both the tinctures .

ii. Estimation of Total Phenolic content

The results of total phenolic content of *Arnica Montana* &*Rhus toxicodendron* mother tinctures of different brands were presented in table 4. The standard curve of Gallic acid from 10-100µg/ml was prepared. The absorbance of all samples was measured at 725 nm using UV/VIS spectrophotometer. In AMǾ of different brands the total phenolic content in LAMǾ shows the higher amount of phenols as compared to NAMǾ & IAM. When compared with the standard curve of gallic acid. Similarly in RTǾ, LRTǾ shows the higher amount of phenols as compared to NRTǾ & IRTǾ.

iii.Estimation of Total Carbohydrate content

The results of total carbohydrate content of *Arnica Montana* &*Rhus toxicodendron*mother tinctures of different brands were presented in table 4.The standard curve of dextrose from 20 – 200 µg/ ml was taken. The absorbance of all samples was measured at 490 nm using UV/VIS spectrophotometer.From all of the mother tinctures of different brands it shows that there is a not much significant differences in total carbohydrate content.

iv. Free radical scavenging activity (DPPH)

The demonstrated modified spectrophotometric method makes use of the 2,2-diphenyl-picryl hydrazyl (DPPH) radical and its specific absorbance properties. The absorbance decreases when the radical is reduced by antioxidants. The absorbance was measured at a wavelength of 517 nm which enabled the measurements of the stable free DPPH radical. Usually, DPPH absorbance is measured at a wavelength of 515-520 nm. And the IC50 is the concentration of an inhibitor where the response (or binding) is reduced by half. The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function.

The degree of discoloration of violet color of DPPH, as it gets reduced, indicated the radical scavenging potential of the antioxidant. Results of the DPPH scavenging activity of the studied samples, expressed as IC_{50} value that represent the concentration of the sample required to scavenge 50% of DPPH radical. The results of free radical scavenging activity DPPH of AMǾ & RTǾ of different brands were presented in table 4. The antioxidant properties of AM mother tincture of different brands such as LAMǾ it was found that the IC_{50} value is 64.01 µg/ml and in NAMǾ & IAMǾ it was found that the IC_{50} value is 68.125 µg/ml & 80.26 µ/ml respectively. From this it was concluded that LAMǾ & NAMǾ matched the IC_{50} value. And in RT mother tinctures of different brands such as LRTǾ it was found that the IC_{50} value is 31.69 µg/ml and in NRTǾ & IRTǾ it was found that the IC_{50} value is 56.875 µg/ml & 61.51 µg/ml respectively. Thus, it is concluded that the NRTǾ matches the IC_{50} value of the standard.

Table 4 - Quantitative estimations of AM & RT mother tinctures of different brands

SN	Quantitative Estimations	Different Brands of Homeopathic Mother Tinctures					
		LAMǾ	**NAMǾ**	**IAMǾ**	**LRTǾ**	**NRTǾ**	**IRTǾ**
1	**Total Flavonoid content (µg/ ml) Eqv. Quercetin**	7.095 ± 0.044	6.873 ± 0.033	6.825 ± 0.031	5.158 ± 0.027	5.079 ± 0.005	4.952 ± 0.010
2	**Total Phenolic content (µg/ ml) Eqv. Gallic acid**	9.28 ± 0.011	7.714 ± 0.011	7.0 ± 0.006	4.0 ± 0.019	2.714 ± 0.009	2.285 ± 0.010
3	**Total Carbohydrate content(µg/ ml) Eqv. Dextrose**	0.319 ± 0.008	0.314 ± 0.006	0.312 ± 0.011	0.180 ± 0.014	0.176 ± 0.011	0.133 ± 0.010
4	**Antioxidant activity (IC 50 µg/ml) Eqv. Ascorbic acid**	64.01 ± 0.010	68.125 ± 0.014	80.26 ± 0.012	31.69 ± 0.022	58.875 ± 0.015	61.51 ± 0.011

(The values are expressed as Mean of three studies, Mean ± SD, N = 3)

Chromatography

i. Thin layer chromatography

For thin layer chromatography the different brands of AM & RT mother tinctures (4µl) were applied to 10 × 10 cm plate coated with silica gel G. Tincture samples were spotted as shown in fig. no. 3. The several ratio of solvents were tried and finally, mobile phase selected was Chloroform : Methanol (8:2). The compounds were visualized under UV chamber at 254 nm & 365 nm as shown in fig no. 4, 5 and by spraying with aniline-sulphuric acid reagent as shown in fig no. 6.

The mother tinctures of different brands of AM & RT are difficult to distinguish but often it is necessary in practice. The different- brands of mother tinctures of AM & RT have shown a similar pattern of flavonoid glycosides so they cannot be distinguished by analysis of these components. The Rf values of different brands of AM & RT mother tinctures are presented in table 5.

The mother tinctures of different brands of AM were detected by chromatography with authentic marker Rutin. While the different brands of AM tinctures may contain chlorogenic acid & caffeic acid with bluish brown zone. In RT mother tinctures of different brands were also detected with authentic marker Quercetin. In sample no. 6, 7 & 8 the sequence of fluorescent zones were found to be present in the chromatograms. Further other light fluorescent zones are also observed. As per literature the standard Rf value of Quercetin 0.63 and Rutin is 0.41. So, the RT mother tinctures of different brands may contain flavonoids glycoside.

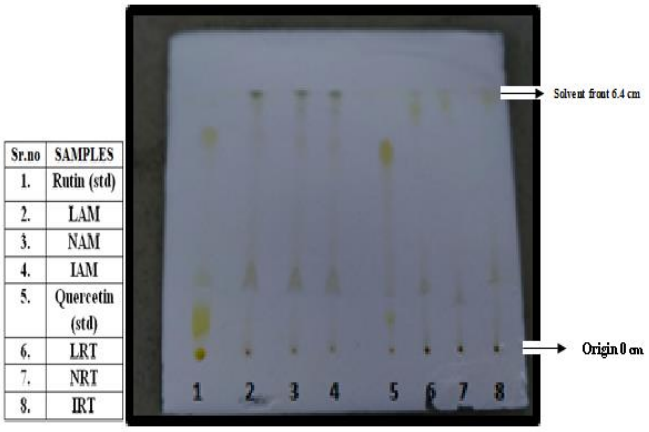


Fig. 3 - TLC Profile of AMǾ & RTǾ of different brands

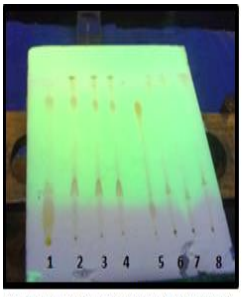


Fig 4 – TLC profile of AM & RT mother tinctures of different brands at short wavelength 254 nm

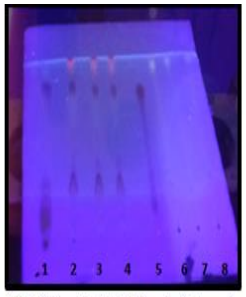


Fig 5 – TLC profile of AM & RT mother tinctures of different brands at long wavelength 365 nm

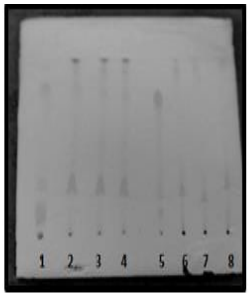


Fig 6- TLC Profile of AM & RT mother tinctures of different brands after spraying with aniline sulphuric acid reagent

Table 5 - TLC data of AMǾ &RTǾ of different brands

S.n	Different brands of HMT	Rf value values
1.	Rutin (std)	0.85
2.	LAMǾ	0.87
3.	NAMǾ	0.81
4.	IAMǾ	0.84
5.	Quercetin (std)	0.82
6.	LRTǾ	0.51
7.	NRTǾ	0.42
8.	IRTǾ	0.50

ii. Quantification of quercetin by HPTLC

Quantification of quercetin is done by HPTLC method in different brands of *Rhus toxicodendron* mother tinctures.

The Camag HPTLC system comprising Linomat 5 is used as sample applicator and TLC scanner controlled by Wincats software is used for quantitative evaluation. The stationary phase is silica gel 60 F254 and the mobile phase is chloroform: methanol (8:2 v/v). samples and standard is applied with 10 mm spots distance between the tracks. The chamber saturation and plate equilibrium are given with filter paper for 15 min. ascending development for a distance of 80 mm in a twin trough chamber was completed in approximately 15 min. volume of different brands of *Rhus toxicodendron* mother tinctures (ϕ) were first optimised 2µl for quantification. The λ max of quercetin was found to be 254 nm after taking the spectra of the standardof quercetin. Quantitative measurement in the absorbance mode was done at 254 nm using a slit dimension of 4.00 mm × 0.30 mm.

Linearity response: The volume of the mother tincture was optimised to 2 µl for quantification. It was then simultaneously applied with different concentrations of standard Quercetin, i.e. 4, 6, 8 and 10 & 12 µl. The method was found to be linear with a regression of 0.99983, and a standard deviation of 1.67% and the amount of Quercetin was calculated in the mother tinctures.

The mother tinctures were chromatographed simultaneously along standard Quercetin respectively, on the same plate for comparison [Figures 7 – 9]. Multiwavelength (MWL) scan was done for finding the optimum wavelength for scanning. The optimum wavelength was found to be 336 nm. The entire plate was further scanned at this wavelength for quantification and spectral match [Figures 10 and 11]. Individual λ max of each fraction was also found with the help of spectral scanning, and then the plate was scanned with these selected wavelengths in MWL mode. The pattern of the peaks was compared for the standards & different brands of *Rhus toxicodendron* mother tinctures. It was observed that the response for various concentrations of standard Quercetin was linear in the range of 100–500 ng with a coefficient of variation of 0.99983 and a standard deviation of 1.67%. Quercetin was quantified and the amount was calculated in individual mother tinctures. With this method, all available mother tinctures were compared and the active principle was quantified. The Quercetin content was calculated from the calibration graph & it was found to be 337.56 ng or 0.33756 µg. From that the Quercetin content in different brands of *Rhus toxicodendron*mother tinctures was calculated. While sample 1 represents the LAMǾ sample 2 represents the NRTǾ & sample 3 represents the IRTǾ.

Repeatability of the method was checked by scanning 9 tracks of standard & different brands of *Rhus toxicodendron*mother tinctures. The percentage recovery was calculated LRTǾ was found to be 0.5381 % of quercetin while NRT has not shown the any peak of quercetin& in IRT the percentage of quercetin was found to be 0.0290 %. Hence it was concluded that the LRTǾ shows the higher amount of Quercetin as compared to NRTǾ & IRTǾ.

IRTǾ showing a lesser amount of Quercetin hence may not be up to the standard level. This quantification may lead to the better quality of checking of samples which in turn will be responsible for better therapeutic efficacy.

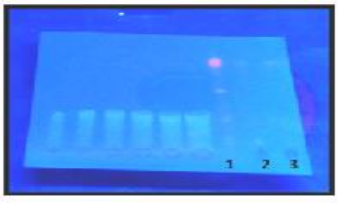


Fig 7- HPTLC fingerprints of different brands of *Rhus toxicodendron* mother tinctures (samples 1,2 &3) under UV 254 nm

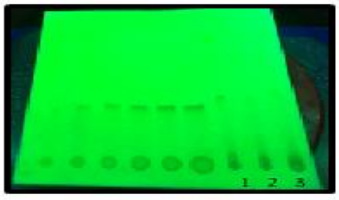
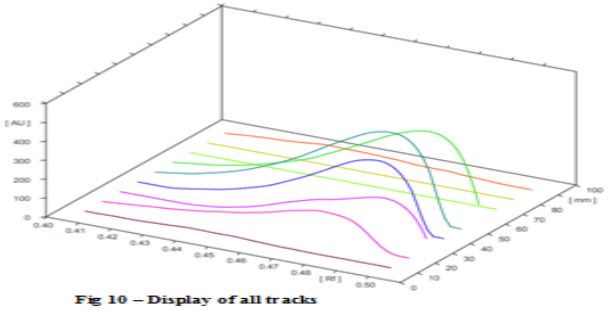


Fig 8- HPTLC fingerprints of different brands of *Rhus toxicodendron* mother tinctures (samples 1,2 &3) under UV 365 nm

Fig 9 - HPTLC fingerprints of different brands of *Rhus toxicodendron* mother tinctures (samples 1,2 &3) after derivatisation



Track	(Sample loading) µl	Color
1	2µl	
2	4µl	
3	6µl	
4	8µl	
5	10µ	
6	12µl	
7	LRT (2µl)	
8	NRT(2µl)	
9	IRT(2µl)	

Fig 10 – Display of all tracks
(First 6 tracks represents the concentrations of Quercetin while last 3 represents the LRT, NRT & IRT mother tincture)

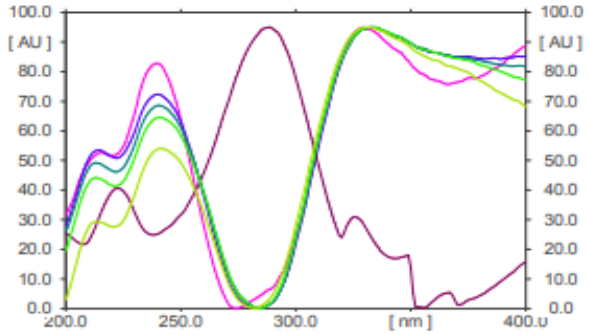


Fig 11 - Overlay of absorption spectra of standard & differentbrands of Rhus toxicodendron mother tincture.

Table 7: Content of Quercetin in different brands of *R. toxicodendron* tinctures

S.N	Name of Sample	% of Quercetin content
1.	LRTǾ	0.5381
2.	NRTǾ	0.00
3.	IRTǾ	0.0290

Comparison in the cost of different brands of mother tinctures:

The fig.13 represents the comparison in between the cost of the local, national and international brands of *Arnica montana* and *Rhus toxicodendron*mother tinctures. The cost of 30 ml of tincture of each sample has been compared and mentioned in the fig. 12. It was found that the LAMǾ (Local *Arnica montana*) is cheaper than NAMǾ (National *Arnica montana*) and IAMǾ (International *Arnica montana*) also LRTǾ (Local *Rhus toxicodendron*) is cheaper than NRTǾ (National *Rhus toxicodendron*) and IRTǾ (*Rhus toxicodendron*).

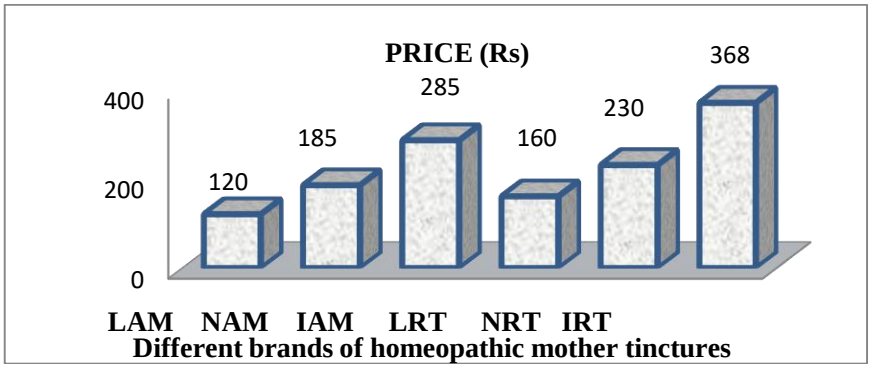


Fig 12: Cost comparison of different brands of mother tinctures

Conclusion:

As suggested by the experimental findings, while bearing in mind the limitations of the study with respect to the numbers of homeopathic tinctures tested, the selected locally manufactured homeopathic mother tinctures (LAMǾ and LRTǾ) demonstrated a superior quality as compared to the selected nationally and

internationally manufactured homeopathic mother tinctures (NAMǾ, NRTǾ,IAMǾ and IRTǾ). Also, the fig. 12 shows the comparison in price of all the mother tincture which reveals that although the local brand is cheaper than the national and international, but it is of superior quality and thus, its use can be promoted. This also indicates the need for increased regulation and quality assurance of all complementary and alternative medicines manufactured at national and international level. This piece of work can be helpful to all the stakeholders dealing with the standardization, quality control and quality assurance of the homeopathic mother tinctures.

REFERENCES

1. PradhanPK, Homoeopathy for All, Vol.7 No 9 (81) 15, 2006; p.51-52.
2. BanerjeeDD,Augmented Textbook of Homoeopathic Pharmacy. B. Jain Publishers, New Delhi, 2006; p.309-449.
3. DeweyWA, Essentials of Homoeopathic *materiamedica* and Homoeopathic Pharmacy.B. Jain Publishers, New Delhi, 1998; p.29-30.
4. PathakS, The Homoeopathic Heritage. Vol 34 (9), B.Jain publisher (P) Ltd., 2009; p.26-27, 51-52.
5. Sahani, MK, Principles and Practice of Homeopathic Pharmacy for Students,1st ed; B.Jain Publishers (P) Ltd, 2007; p.120-30.
6. MandalPP and MandalB, Text Book of Homeopathic Pharmacy. New Central Book Agency, 1994; p.140-148.
7. Goel S, Art and Science of Homoeopathic Pharmacy, Ahmedabad, India: Leo enterprises, 2007; p.390-392.
8. Homoeopathic Pharmacopoeia of India, (H.P.I.). Ministry of Health, Government of India; 1st edn.; Vol. 1, 1971; p.257-59.
9. VarmaPNandVaidI,Encyclopedia of Homoeopathic Pharmacopoeia & Drug Index, New Delhi. B. Jain Publishers, 2017; p.9-15.
10. ThakurT, Ethnobotanical, Phytochemical, Pharmacological &Homoeopathic Review of*Arnica Montana* Linn. World Journal of Pharmaceutical and Medical Research, 3(6), 2017;p.152-157.
11. Jadhav HP,Chaudhari GG, PatilDD, Jadhav RB, Reddy NM, Shirkhedkar AA, *et al*., Standardization of homeopathic mother tincture of *Toxicodendronpubescens*and correlation of its flavonoid markers with the biological activity. Homeopathy, 2015; 105(01), p.48-54.
12. Skoog DA, Holler FJ and Crouch, SR, Principles of instrumental analysis, Cengage learning. 6th edition, 2004; p.495-496.
13. KhandelwalKR, Preliminary phytochemical screening, Practical Pharmacognosy Techniques and Experiments, 8, 2008; p.149-156.
14. Khadabadi SS, Deore SL and BaviskarBA, Experimental Phyto Pharmacognosy A Comprehensive Guide. Pune: NiraliPrakashan, 2011; p.25-30.
15. SadasivamSand Manickam A, Biochemical methods, new age international publishers,1996;p.50-63.
16. ParejoI, Viladomat F, BastidaJ, Rosas-Romero A, FlerlageN, Burillo J, *et al.,* Comparison between the radical scavenging activity and antioxidant activity of six distilled andnon distilled Mediterranean herbs and aromatic plants. Journal of Agricultural and Food Chemistry, 2002; 50(23), p.6882-6890.
17. Stahl E and Chromatography, TL, A laboratory handbook. Thin-layer chromatography, 1969; p.669- 712.
18. WagnerHand Bladt S, Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media, 1996;p.275-278.
19. DwivediBK, Kumar M, Arya, BS, Sundaram EN and Manchanda RK, Comparative standardization study for determination of reserpine in *Rauwolfiaserpentina* homoeopathic mother tinctures manufactured by different pharmaceutical industries using HPTLC as a check for quality control. Indian Journal of Research in Homoeopathy, 2017; 11 (2), p.109-112.