Ravindra C. Sutar /J. Pharm. Sci. & Res. Vol. 14(8), 2022, 844-848

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Fingerprinting of Hydroalcoholic Extract for Flavonoids from *Ailanthus excelsa* (Roxb.) Leaves using High Performance Thin Layer Chromatography

Ravindra C. Sutar*

**Department of Pharmacology, Sanjivani College of Pharmaceutical Education and Research, Kopargaon. At-Sahajanandnagar, Post-Shinganapur (Pin code- 423603), Tal- Kopargaon, Dist- Ahmednagar, Maharashtra, India*

E-mail: ravisutarbpharm@sanjivani.org.in

Abstract

Objective: Natural remedies from medicinal plants are found to be safe and effective..Many plant species have been used in folklore medicine to treat various ailments. Standardisation of plant materials is the need of the day. To study flavonoid profile of the medicinal plant.

Ailanthus excelsa (Roxb.) using High Performance Thin Layer Chromatography (HPTLC) technique.

Methods: The extracts were tested to determine the presence of various phytochmeicals like alkaloids, phenolic compounds, flavonoids, carbohydrates, glycosides, saponins, terpenoids, tannins, fixed oils, fats and protein and aminoacids (Harborne and Harborne, 1998). HPTLC studies were carried out by Harborne and Wagner et al method. Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. CAMAG HPTLC system equipped with Linomat V applicator (Switzerland). Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungstant lamp. **Results:** HPTLC finger printing of flavonoids of hydroalcohol extract of leaves revealed seven polyvalent phytoconstituents (07 peaks) and corresponding ascending order of Rf values in the range of 0.063 to 0.927

Conclusion: With the results of HPTLC analysis and R_f values Flavonoids have been concluded in the extract. Hence it wa s concluded that the flavonoid compounds present in the Hydroalcohol extract could be responsible for antioxidant activities. Plant derived antioxidants, especially phenols and flavonoids, have been described to have various properties like anticancer, antiaging and prevention of cardiovascular diseases. Further, separation and characterization of the bioactive compound from the plant is to be evaluated and reported in near future.

Keywords: HPTLC, *Ailanthus excela* (Roxb.) leaves, Hydroalcohol extract, Phytochemicals, Flavonoids, Fingerprinting

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective.Many plant species have been used in folklore medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries[1]. Standardisation of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and
quantification of active constituents in the plant material may be useful for proper standardisation of herbals and it's formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards[2]. High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images[3]. *Ailanthus excels*(Roxb.) (Simaroubaceae) is commonly known as Mahanimba due to its resemblance with the neem tree (Azadirachita indica) and Maharukha due to its large size. Ailanthus is from ailanto which means tree of heaven and is the name for one of the species in the Moluccas, while in Latin excelsa means tall. The plant is known by different names like tree of heaven in English[4]. *Ailanthus excelsa (*Roxb.) a plant used in the Indian school/system of medicine for variety of purposes[5]. *Ailanthus excelsa (*Roxb.) belonging to family Simaroubaceae[6]. In Chinese system of medicine bark of *A. excelsa* is used to treat diarrhea and dysentery,especially when there is a blood in stool[7,8]. *Ailanthus excelsa* is a fast growing tree and is extensively cultivated in many parts of India in the vicinity of villages; it is cultivated as an avenue tree for its deep shade and can be used for ant-erosion purposes[9]. The bark has been used in Asian and Australian medicine to counteract worms, excessive vaginal discharge, malaria and asthma[10,11]. The plant shows Antifertility activity, Antifungal activity, Antimalarial activity, Hypoglycemic activity, Antipyretic activity, Antitumor and cytotoxicity, Hepatoprotective activity[12] . Research interest has focused on various herbs that possess hypolipidemic, antiplatlet, antitumour, immunestimulating properties, anti-inflammatory, anti-viral etc. that may be useful adjuncts in reducing the risk of cardiovascular disease, cancer and other diseases. A wide variety of active phytochemicals,including flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, phthalides, tannins, gallic acid, quercetin, phytosterols, alcohols, aldehydes have been identified from medicinal plants[13]. These phytochemicals are estimated by a variety of techniques such as spectroscopy and chromatography. High performance thin layer chromatography (HPTLC) chromatographic fingerprints can be applied for this kind of certification. Fingerprint analysis by HPTLC has developed into an effective and powerful tool for linking the chemical constituent's profile of the plants with botanical identity and for estimation of chemical and biochemical markers[14-

[18]. Alkaloids, tannins have been Identified with HPLTC Studies of this Plant[19,20]. but Hydroalcohol extract of this plant has not been explored for HPTLC Studies so in this present study the HPTLC fingerprinting of Flavonoids of Hydroalcoholic extract of leaves of *Ailanthus excelsa* (Roxb.) has been performed which may be used as markers for quality evaluation and standardization of the drug

MATERIALS AND METHODS

Plant material

Leaves of *Ailanthus excelsa (*Roxb.) were collected in the Month of August from the agricultural fields of Tirunelvel i district, Tamilnadu. The plant was identified and leaves of *Ailanthus excelsa* were authenticated and conf irmed from Dr.V.Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing morp hological features (leaf and stem arrangement, flower /in florescence arrangement, fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation and Extraction of Plant material

Preparation of Hydroalcohol extract by Soxhlet Extraction Method: The powder of *Ailanthus excelsa* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using Water and ethanol (1:1) proportion. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50ºC to get Hydroalcohol extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10º C. The Hydroalcohol extract of *Ailanthus excelsa* leaves was subjected to the following investigations

1.Preliminary phytochemical screening.

2. HPTLC Fingerprinting of Flavonoids

HPTLC Fingerprinting

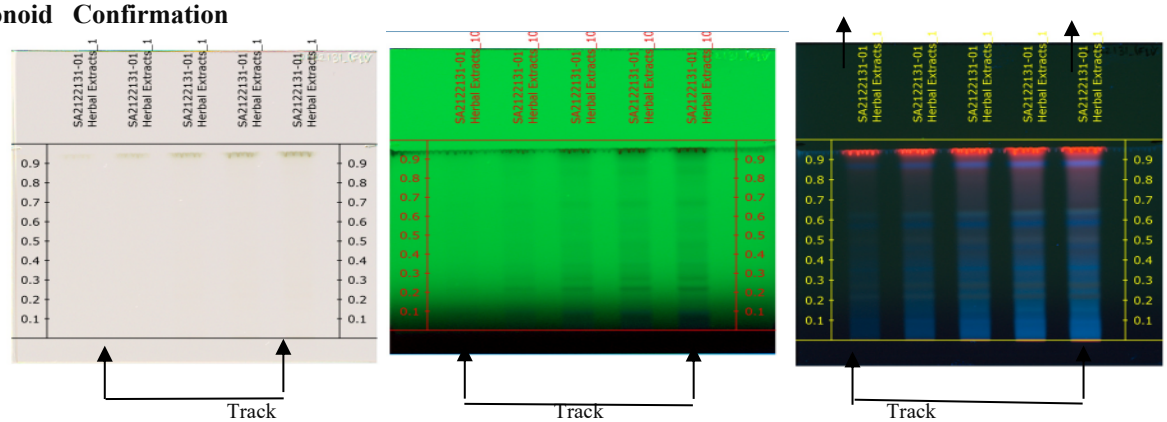
HPTLC studies were carried out following the method of Harborne[21] and Wagner *et al* [22].

HPTLC instrumentation and Chromatographic conditions

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microliter syringe on precoated silica gel aluminum plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The plates were activated at 120°C for 20 minutes prior to chromatography. A constant application rate of 1.0 µl/s was employed, and space between two bands was 5 mm. The slit dimension was kept at 6.0 mm × 0.45 mm and 10 mm/second scanning speed was employed. The slit bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase for fingerprinting of flavonoids consisted of ethyl acetate: formic acid: Glacial acetic acid: water in the volume ratio of 10: 0.5: 0.5:1.3 (v/v) and Anisaldehyde Sulphuric acid was used for derivatization of flavonoids. 20 ml of mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with filter paper whatman no: 1 in the mobile phase. The optimized chamber saturation time for mobile phase was 20 minutes at room temperature (25°C ± 2) at relative humidity of 60% ± 5. The length of the chromatogram run was 8.0 cm. Subsequent to the scanning; thin layer chromatography (TLC) plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungsten lamp. Subsequent to the development; TLC plate was dipped in anisaldehyde sulfuric acid reagent followed by drying in the oven at 110°C. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression[23-31].

RESULTS AND DISCUSSION

Flavonoid Confirmation



Track 1-6: Hydroalcohol extract of *Ailanthus excelsa* leaves

Fig. 1 : HPTLC fingerprint profile of Flavonoids of leaf extract of *Ailanthus excelsa* Detection of Flavonoids in Hydroalcohol extract

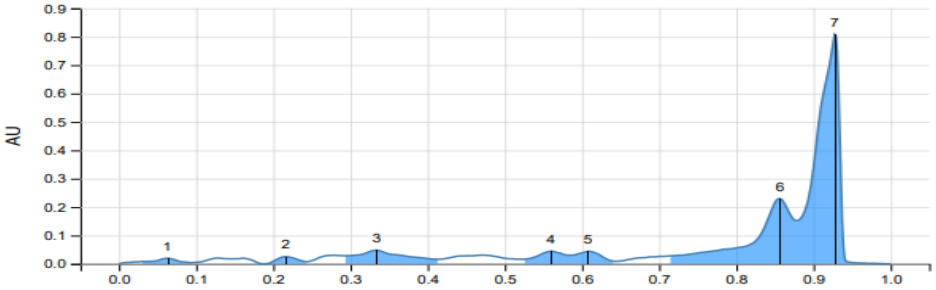


Fig. 2: Flavonoids confirmation at visible derivatisation with Anisaldehyde Sulphuric acid reagent

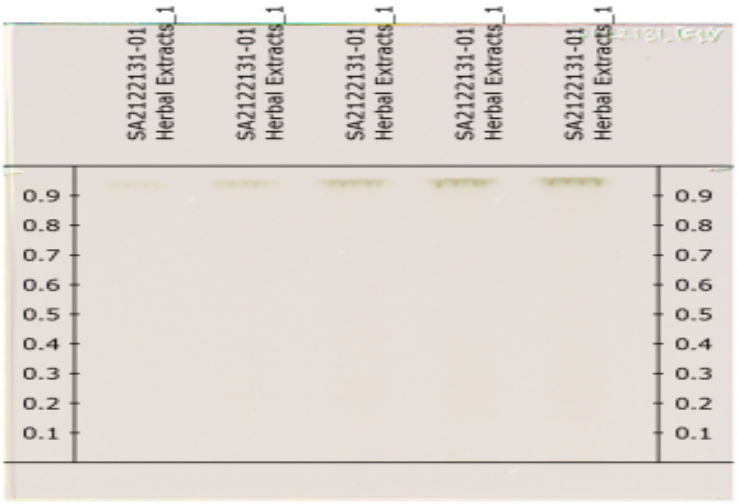


Fig 3: Chromatogram for flavonoids in Hydroalcohol extract of *Ailanthus excelsa* leaves

Table 1: Rf Values for flavonoids in Hydroalcohol extract of Ailanthus excelsa leaves

Peak #	Start		Max			End		Area		Manual peak
	R_F	H	R_F	H	%	R_F	H	A	%	
1	0.026	0.0073	0.063	0.0199	1.63	0.094	0.0041	0.00076	1.52	No
2	0.189	0.0000	0.216	0.0254	2.08	0.244	0.0077	0.00080	1.60	No
3	0.292	0.0286	0.334	0.0480	3.94	0.413	0.0162	0.00371	7.40	No
4	0.518	0.0163	0.560	0.0447	3.67	0.582	0.0303	0.00198	3.94	No
5	0.582	0.0303	0.608	0.0442	3.62	0.644	0.0088	0.00186	3.70	No
6	0.708	0.0265	0.856	0.2296	18.84	0.877	0.1521	0.01421	28.31	No
7	0.877	0.1521	0.927	0.8072	66.22	0.973	0.0015	0.02688	53.55	No

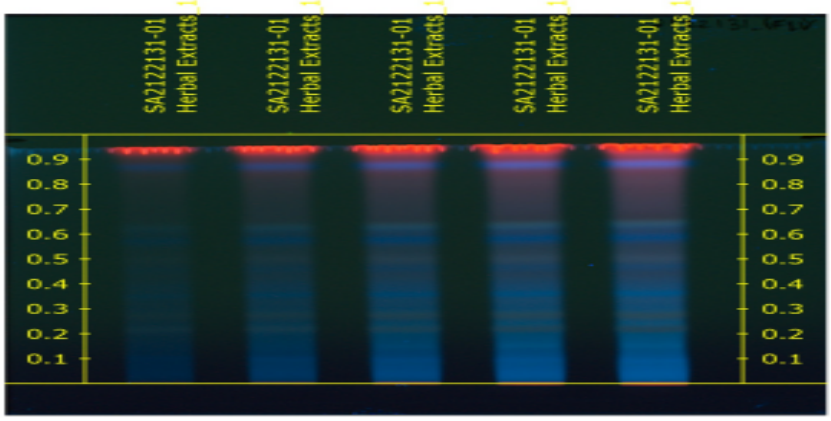


Fig 4: R_f Values for Flavonoids in Hydroalcohol extract of *Ailanthus excelsa* leaves

It was observed that track 1-5 of Figure 1 shows Hyderoalcohol extract. Figure 3 shows separation of constituents. The Fluorescence shows the presence of Flavonoids in the extract. It was observed that there is a separation of different phytoconstituents, in Hydroalcohol extract .

Fingerprinting study of Hydroalcohol extract at 366 nm shows seven Rf between the range of 0.063-0.927. Rf 0.927 has maximum 66.22 % concentration in Table 1, Figure 4

The evaluation of crude extract is an integral part of correct identity. HPTLC is useful as a phytochemical marker[32, 33]. and more effective in the field of plant taxonomy also for the identification of plants through secondary metabolites[34]. HPTLC fingerprinting is proved to be a linear, precise, and accurate method for herbal identification[35]. Such finger printing is useful in quality control of herbal products and checking for the adulterants[36]. Therefore, it can be useful for the evaluation of different marketed pharmaceutical preparations [37–39].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine[40]. These natural products were known for their beneficial effects on health long before flavonoids were isolated as the effective compounds. More than 4000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves[41]. The antiviral activity of flavonoids was shown in a study by Wang et al[42]. Some of the viruses reported to be affected by flavonoids are herpes simplex virus, respiratory syncytial virus, parainfluenza virus, and adenovirus.Plant derived antioxidants, especially polyphenols and flavonoids have been ascribed to various properties like anticancer, antidiabetic, antiaging and prevention of cardiovascular diseases[43,44]. Polyphenolic compounds like flavonoids have been labelled as “high level” natural antioxidants based on their abilities to scavenge free radicals and active oxygen species[45]. They contain conjugated ring structures and hydroxyl groups that have the potential to function as antioxidants in vitro or cell free system by scavenging superoxide anion, singlet oxygen, lipid peroxyradicals and stabilizing free radicals involved in oxidative processes through hydrogenation or complexing with oxidizing species[46]. There is now a strong consensus that flavonoids and related polyphenols are responsible for much of anti oxidant activity of fruits and vegetables[47,48,49]. Many fruits and vegetables are rich in flavonoid content, consuming flavonoid regularly increases longevity by reducing inflammation and contributing to the amelioration of atherosclerosis from CHD[48]. Green tea is the commonly used beverage in Asian countries is a significant source of polyphenols. These polyphenols have recently attracted the medicinal attention as bioactive agents with anticancer, antidiabetic, antiviral, antimalarial, hepatoprotective, neuroprotective and cardioprotective effects.

CONCLUSION

It is observed in the above HPTLC studies that, Hydroalcohol extract of *Ailanthus excelsa* (Roxb.) contain a lot of polyvalent chemical constituents with different R_f values. The developed fingerprint analysis of leaf extract of *Ailanthus excelsa* will help to isolate and identify new Flavonoids which will offer a possibility to discover lead a molecule for drug development.

ACKNOWLEDGMENT

The author wish to thank Anchrom Test Lab Pvt. Ltd. Mulund (E), Mumbai - 400081 for their excellent and generous help for the HPTLC analysis.

AUTHOR CONTRIBUTIONS

Dr. Ravindra C. Sutar conceptualized and designed the study, curated the data and prepared the original draft, discussed the methodology and analysed the data, prepared results and contributed to the final manuscript.

FUNDING

I acknowledge the resource support for the study was provided by the Hon’ble Management of Sanjivani Group of Institutes, Sanjivani College of Pharmaceutical Education and Research, Kopargaon. The excellent support for carrying out the HPTLC study at Anchrom Test Lab Pvt. Ltd. Mulund (E), Mumbai – 400081, is also acknowledged.

REFRENECES:

1. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aegle marmelos* (L.) Correa extracts. J Pharm Res 2011; 4(1):252-255.
2. Sharma P, Kaushik S, Jain A, Sikarwar S M. Preliminary phytochemical screening and HPTLC fingerprinting of Nicotiana tobacum leaf. J Pharm Res 2010; 3(5):1144-1145.
3. Johnson M, Mariswamy Y, Ganaraj WE. Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique. Asian pac j Trop Biomed 2011; 1:428-433.
4. Database, 2000. Medicinal Plants Used in Ayurveda. Central Council for Research in Ayurveda and Siddha. New Dhli, India, pp: 50-59.
5. Kirtikar, K.R. and B.D. Basu, 1995. Indian Medicinal Plants. Vol. 1, International Book Distributors,Dehradun, India, 1995; 371-372.
6. Anonymous.The Wealth of India, Raw Materials. Publication and information Directorate, New Delhi,1985:116-118.
7. Chopra, R.N., I.C. Chopra, K.L. Handa and L.D. Kapur, 1958. Chopra's Indigenous Drugs of India. 2nd Edn.,UN. Dhar and Sons Private Ltd., Calcutta, 1958: 408.
8. Dash, S.K. and S. Padhy. Review on ethnomedicines for diarrhoea diseases from *Orissa*: Prevalence versus culture. J. Hum. Ecol., 2006,20: 59-64.
9. Anonymous, 1956. The Wealth of India: Raw Materials. Council of Industrial and Scientific Research, New Delhi.
10. Kirtikar, K.R. and B.D. Basu, 2003. Indian Medicinal Plant. 2nd Edn., Mohan Basu Publisher, Allahabad, India.
11. Chevallier, A., 1996. The Encyclopedia of Medicinal Plants. 1st Edn., DK Publishing Inc., New York, USA.:259.
12. Lavhale, M.S. and S.H. Mishra, 2007. Nutritional and therapeutic potential of *Ailanthus excelsa*: A review. Pharmacognosy Rev., 1: 105-113.
13. Craig. W, Beck.L Phytochemicals:Health protective effects. Can J Diet Pract Res. 1999; 60(2): 78-84
14. Patil AG, Koli SP, Patil DA, Chandra N, Pharmacogonostical standardization and HPTLC fingerprint of Crataeva tapia Linn.SSP. Odora(Jacob.) Almeida leaves. Int.j.pharm.Biosci.2010; 1(2): 1-14
15. Ramya V, Dheena Dhayalan V, Umamaheswari S. In vitro studies on antibacterial activity and separation of active compounds of selected flower extracts by HPTLC. J.Chem.Pharm.Res., 2010; 2(6): 86-91.
16. Manikandan A,Victor Arokia Doss A.Evaluation of biochemical bontents, nutritional value,trace elements,SDS-PAGE and HPTLC

profiling in the leaves of Ruellia tuberose L.and Dipteracanthus patulus (Jacq.).J.Chem.Pharm.Res.2010; 2(3): 295-303
17. Yamunadevi M, Wesely EG, Johnson M. Chromotographic fingerprint analysis of steroids in Aerva lanata L.by HPTLC technique.Asian Pac.J.Trop.Biomed.2011; 1: 428-433.
18. Yamunadevi M, Wesely EG, Johnson M.Chemical profile studies on the alkaloids of medicinally important plant Aerva lanata L using HPTLC.J.Nat.Conscientia.2011; 2(2): 341-349.
19. Ranjana Sharma, Sudhir Singh Gangwar, Amita Tilak and Ravindra C. Sutar. High performance thin layer chromatography fingerprinting of the alkaloids from *Ailanthus excelsa* (Roxb.) Leaves**.** WJPR.2020; 9(1):1596-1601.
20. Sudhirsingh Gangwar, AmitaTilak,RanjanaSharma,Ravindra C.Sutar. HPTLC finger printing analysis of the tannins from *Ailanthus excelsa* (Roxb.) Leaves.WJPR.2018;8(2):1023-1029.
21. Harborne J B. *Phytochemical methods;* 3rd edition, London: Chapman and Hall; 1998.
22. Wagner H, Baldt S. *Plant drug analysis;* Berlin: Springer; 1996. R.P.W. Scott, Encyclopedia of Chromatography, 10th edn, Marcel Dekker, USA, 2001; 252–254.
23. ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures–Methodology, 1996.
24. J. Cazes and R.P.W. Scott, Chromatography Theory, Marcel Decker, NY, 2002; 443-454.
25. Reviewer Guidance, Validation of Chromatographic Methods,1994.
26. P.D. Sethi, HPTLC: Quantitative Analysis of Pharmaceutical Formulations, CBSPublications, New Delhi, 1996;162–165.
27. E. Heftman, Chromatography Fundamentals and Applications of Chromatography and Related Differential Migration Methods.Vol. 69A, 6th edn, Elsevier, Amsterdam. 2004;253–291.
28. British Pharmacopoeia, International edn, Vol. II, HMSO, Cambridge, 2002; Appendix 112 (IB).
29. J. Sherma, Encyclopedia of Pharmaceutical Technology, 2nd edn, Marcel Dekker, USA, 2001; 252–254.
30. ICH/CPMP guidelines Q2A, Text on Validation of Analytical Procedures, 1994.
31. USP 23, NF 19, Asian edn, United States Pharmacopeial Convention, Rockville, M.D., 982, 1225.
32. M. Attimarad, K. Mueen Ahmed, B. E. Aldhubaib, and S. Harsha, "High-performance thin layer chromatography: a powerful analytical technique in pharmaceutical drug discovery," *Pharmaceutical Methods*, vol. 2, no. 2, pp. 71–75, 2011.
33. H. Misra, D. Mehta, B. K. Mehta, and D. C. Jain, "Extraction of artemisinin, an active antimalarial phytopharmaceutical from dried leaves of *Artemisia annua* L., using microwaves and a validated HPTLC-visible method for its quantitative determination," *Chromatography Research International*, vol. 2014, Article ID 361405, 11 pages, 2014.
34. K. Salim, K. S. Rajeev, and Z. A. Malik, "Assessment of phytochemical diversity in *Phyllanthus amarus* using HPTLC fingerprints," *Indo-Global Journal of Pharmaceutical Sciences*, vol. 1, pp. 1–12, 2011.
35. N. Cortés, C. Mora, K. Muñoz et al., "Microscopical descriptions and chemical analysis by HPTLC of *Taraxacum officinale* in comparison to *Hypochaeris radicata*: a solution for mis-identification," *Brazilian Journal of Pharmacognosy*, vol. 24, no. 4, pp. 381–388, 2014.
36. P. Teo, F. Ma, and D. Liu, "Evaluation of Taurine by HPTLC reveals the mask of adulterated edible Bird's nest," *Journal of Chemistry*, vol. 2013, Article ID 325372, 5 pages, 2013.
37. S. P. Gandhi, M. G. Dewani, T. C. Borole, and M. C. Damle, "Development and validation of stability indicating HPTLC method for determination of diacerein and aceclofenac as bulk drug and in tablet dosage form," *E-Journal of Chemistry*, vol. 9, no. 4, pp. 2023–2028, 2012.
38. S. Meena and S. M. Sandhya, "Validated HPTLC method for simultaneous analysis of pyrimethamine and sulphadoxine in pharmaceutical dosage forms," *Journal of Chemistry*, vol. 2013, Article ID 698490, 6 pages, 2013.
39. K. G. Patel, N. R. Jain, and P. A. Shah, "Stability indicating HPTLC method for analysis of rifaximin in pharmaceutical formulations and an application to acidic degradation kinetic study," *ISRN Analytical Chemistry*, vol. 2013, Article ID 613218, 9 pages, 2013.
40. Middleton EJ. Effect of plant flavonoids on immune and inflammatory cell function. Adv Exp Med Biol 1998;439:175–82. Medline
41. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol 1998; 12:249–255.
42. Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH. Recent advances in the discovery and development of flavonoids and their analogues as antitumor and antiHIV agents. Adv Exp Med Biol 1998;439: 191–225.
43. Muthukumaran, A, Singh.N Atteneuting effect of Acorus calamus extract in chronic constriction induced neuropathic pain in rats;an evidence of anti-oxidative, anti-inflammatory,neuroprotective and calcium inhibitory effects, BMC complement.Altern.Med. 2011; 11(24):1-14. [13]
44. Gupta, R.C, Sharma,V Sharma,N Kumar ,N Singh. B In vitro antioxidant activity from leaves of Oroxylum indicum- A North Indian highly threatened and vulnerable medicinal plant. J. Pharma. Res. 2008; 1(1): 65-72.
45. Birt, D. S. Hendrich , W. Wang. Dietary agents in cancer prevention:flavonoids and isoflavonoids. Pharmacology and Therapeutics, 2001 ; 90: 157-177.
46. Klahorst, S. Exploring antioxidants.Wd.Food Ingred.2002:54-59
47. Frankel, E.N. J. Kanner, J.B. German, E. Parks, J.E. Kinsella. Inhibition of oxidation of human low density lipoprotein by phenolic substances in red wine. The Lancet, 1993; 341: 454 - 457.
48. Pietta, P.G. Flavonoids as antioxidants, Journal of Natural Products, 2000; 63:1035-1042.
49. Vinson J, Jang J, Yang J, Dabbagh Y, Liang X, Serry M, et al. Vitamins and especially flavonoids in common beverages are powerful invitro antioxidants which enrich low density lipoproteins and increase their oxidative resistance after exvivo spiking in human plasma. Journal of Agricultural and Food Chemistry 1999; 47: 2502-2504.