Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 5, Issue 4, 2012 ISSN - 0974-2441

Research Article

HPTLC METHOD FOR QUANTITATIVE DETERMINATION OF GALLIC ACID IN ETHANOLIC ROOT EXTRACT OF *DIOSPYRUS FERREA (WILLD.) BAKH AND AERVA LANATA* (L.) JUSS. EX SCHULTES –A POTENT INDIAN MEDICINAL PLANTS.

R.VIJAYALAKSHMI [1*] AND R.RAVINDHRAN[2],

[1]Department of Plant biology &Plant Biotechnology Ethiraj College for Women, Chennai-8,[2]Department of Plant biology & Biotechnology Loyola College, Chennai-34.Email: rvijaya.lakshmi@yahoo.com

Received:1 June 2012, Revised and Accepted:21 June 2012

ABSTRACT

The present study was intended to establish the HPTLC gallic acid profile of the medicinally important plant *Aerva lanata* (L.) Juss. Ex Schultes and *Diospyrus ferrea (Willd.) Bakh*. HPTLC profiling was performed out by the method described by Harborne and Wagner *et al.* The Toluene-Ethyl acetate-Formic acid-Methanol (3: 3: 0.8: 0.2) was employed as mobile phase for gallic acid. Linear ascending development was carried out in 20 cm x 10cm twin trough glass chamber (CAMAG, Automatic TLC Sampler-4) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was sprayed with 5% Ferric chloride as spray reagent, dried at 100° C in hot air oven for 10 min. The ethanolic root extract of *A. lanata* and *D. ferrea* illustrated the presence of gallic acid with different range of Rf from 0.38 to 0.74. In general, higher degree of gallic acid diversity has been observed in vegetative parts especially in roots. The gallic acid with the Rf value 0.48, 0.47 was present in *Aerva lanata* and 0.46, 0.47 was reported in *Diospyrus ferrea (*synonym *Maba buxifolia)* corresponding as that of standard. The gallic acid with the Rf value 0.47 showed their joint presence in both the root extract. Maximum amount of gallic acid has been observed in root of *Diospyrus ferrea* compare to that of *A. lanata*. Detection and quantification were performed by densitometry at λ = 277 nm. The average recovery of gallic acid was found to be 47.50 μg/ml in *Diospyrus ferrea* and 2.61 μg/ml in *Aerva lanata* respectively. Validated method showed linear response over concentration range of 100 to 700 ng. The LOD and LOQ values were found to be 100, 300 ng/spot respectively. The results of the present study provided a valuable phytomarker for the identification and characterization of *Diospyrus ferrea* and *Aerva lanata.* The proposed HPTLC method provide a good resolution of gallic acid from other constituents present in ethanolic root extract of *Diospyrus ferrea* and *Aerva lanata*.

Keywords: HPTLC, Gallic acid, *Diospyrus ferrea*, *Aerva lanata,* Ethanolic root extract

INTRODUCTION

Diospyrus ferrea is a small bonsai tree, belongs to the family Ebenaceae, and grows in most areas of western and southern regions of Tamil Nadu as ornamental. They occur near the coastal region also in the Deccan plateau and dry evergreen forests of India. The uniqueness of the genus is the elaboration of a large number of pentacyclic triterpenes and juglone based 1, 4-naphthoquinone and phenol metabolites. These metabolites can be used as chemical markers for taxonomic studies. Its roots stem, bark and fruits constitute the drug, considered useful in treating the skin disorders [1]. Such secondary metabolites are highly varied in structure; many are aromatic substances, most of which are phenols or their oxygen-substituted derivatives [2]. In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., anti-inflammatory, anti-diabetic, anti-microbial, anti-parasitic and anti - helmentic activities.

Aerva lanata L. is an important medicinal plant, found throughout tropical India as a common weed in fields and wasteland. Because of its reputation in folk medicine, *A. lanata* has become the subject of intense pharmacological and chemical studies for the last 30 years. Traditionally, root of *Aerva lanata* are used as sap for eye-complaints; an infusion is given to cure diarrhea, kidney stone and in snake bite treatment. In addition to the traditional uses, the plant is reported for a number of pharmacological activities viz., anthelmintic, demulcent , anti-inflammatory [3], diuretic [4] expectorant, hepatoprotective [5] and nephroprotective [6], anti-diabetic [7], anti-hyperglycaemic , anti-microbial, cytotoxic [8] , urolithiatic , hypoglycemic, anti-hyperlipidemic , anti-parasitic and anti - helmentic activities . In order to identify the bioactive compounds responsible for the above pharmacological activities, phytochemical studies [9] have been carried out by several researchers with the report of phenolic compounds.

Gallic acid (GA, 3, 4, 5-trihydroxy-benzoic acid) is a polyhydroxyphenolic compound widely distributed in various plants, fruits and foods, where it is present either in free form or, more commonly, as an ingredient of tannins [10]. GA and its derivates were found to be strong antioxidants which are able to scavenge reactive oxygen species (ROS), e.g., superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid[11, 12]. GA derivatives have also been found in many phytomedicines with a number of biological and pharmacological activities like inducing apoptosis of cancer cells[13, 14], inhibiting squalene epoxidase and interfering the signal pathways involving Ca (II) and oxygen free radicals[15, 16]. Autoxidation and oxidation of GA have been studied for many years due to its presence in various foods and its activity in living cells (mainly as antioxidant). Special attention was given to the autoxidation of GA in alkaline aqueous solutions because of its possible transformations during the food processing. Gallic acid can serve as a model for similar reactions in various foods and beverages[17, 18]. Gallic acid also has anti-viral and anti-fungal and anti oxidative properties. Gallic acid also has therapeutic applications for inflammatory allergic diseases, such as asthma, allergic rhinitis, sinusitis due to its ability to inhibit histamine release and the expression of pro-inflammatory cytokine[19]. Gallic acid as a remote astringent works to constrict tissue and help in the treatment of prolonged menstrual periods. When administered internally, is beneficial in the treatment of uterine, pulmonary, and nephritic hemorrhages, as well as all hemorrhages of a passive character.

The well developed quality standards can be achieved only through systematic evaluation of the plant material using modern analytical chromatographic techniques[20]. TLC and HPTLC are methods commonly applied for the identification, assay and the testing of purity, stability, dissolution or content uniformity of raw materials and formulated products. HPTLC is a valuable tool for the investigation of herbal products with respect to different aspects of their quality[21-25]. HPTLC analysis is comparatively short and many samples can conveniently be compared side by side on the same plate. This is particularly important for screening and inspection of selection of raw materials and for process control during manufacturing. HPTLC has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. HPTLC results are not only reported as peak data but can also be presented and communicated as images. Finger print analysis by HPTLC has become an effective and powerful tool for linking the chemical constituents' profile of the

plants with botanical identity and for the estimation of chemical and biochemical markers [22-24]. A lot of works have been done for the HPTLC profile of medicinal plants in pharmaceuticals industries [24-29]. Only a few reports are available for the HPTLC profile of *A. lanata* . But there is no report on the gallic acid profile of *A. lanata and Diospyros ferrea.*

Here, we used ethanol solvent for extraction because maximum chemical constituents soluble in it. A TLC method for detection of phenols has been reported in *A. lanata*[31- 33] but not in *Diospyros ferrea* However HPTLC method for quantitation of gallic acid from root extract *A. lanata and Diospyros ferrea* has not been reported. Densitometric HPTLC has been widely used for the phytochemical evaluation of the herbal drugs, due to its simplicity and minimum sample clean up requirement. Hence, a densitometric HPTLC method has been developed in the present work for quantitation of gallic acid from ethanolic root extract of plant *A. lanata and Diospyros ferrea* . The normal phase HPTLC method established in this research work uses aluminum backed silica gel 60 F254 plates which are less expensive than reversed-phase, preparative plates.

Hence, the present study was designed to determine the gallic acid HPTLC profile of the medicinally important plant *A. lanata* and *Diospyros ferrea* which will help in crude drug identification and in standardization of the quality and purity for various pharmaceutical industries.

MATERIAL AND METHODS

Methods of Preparation of plant extract

Fresh roots of *Diospyrus ferrea* and *A. lanata* was collected from Southern Western Ghats, South India. A voucher specimen (FLOR 24. 144) was deposited in the Herbarium Botanical Survey of India Coimbatore for authentication of plant. Roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, methanol, ethanol and aqueous for 48 hrs in a Soxhlet assembly. The extracts were concentrated, percentage yield calculated and then subjected to preliminary phytochemical analysis. The dried extract was properly stored in the desiccators for further experiment and analysis.

Preparation of gallic acid standard solution

A stock solution of standard gallic acid (50 μg/mL) was prepared by transferring 5 mg of gallic acid, accurately weighed, into a 100 mL volumetric flask, dissolving in 50 mL methanol. It was then sonicated for 10 minutes and the final volume of the solutions was made up to 100 mL with methanol to get stock solutions containing 50μ g/mL.

Preparation of sample solution

Accurately weighed 10 mg of *Aerva lanata* root extract was transferred to a 100 mL volumetric flask dissolving in 80 mL of methanol. It was then sonicated for 10 minutes and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 100 mL with methanol to get stock solution containing 1.00 mg/ mL. Accurately weighed 5 mg of *Diospyrus ferrea* root extract was transferred to a 100 mL volumetric flask dissolving in 1 mL of methanol. It was then sonicated for 10 minutes and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 100 mL with methanol to get stock solution containing 1.00 mg/ mL. A stock solution of standard gallic acid (50μg/mL) was prepared in methanol. Different volume of stock solution 1,2, 4, 6, 8, 10,12,14,16,18 μL, were spotted on TLC plate to obtained concentration 40, 80, 160, 240, 320, 400,560,640,720 ng/spot of gallic acid, respectively. The data of peak areas plotted against the corresponding concentration (Fig- 1).

HPTLC studies were carried out following Wagner et al method. For the present study, CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used for HPTLC analysis was obtained from MERCK. The samples (4,8,16μL for *Aerva* and 1,2,4 μL for *Diospyrus ferreae*) were spotted in the form of bands of width 8 mm with a Camag 25 μL microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 × 10 cm with 50 μm thickness (E. Merck,) using a Camag Linomat IV (Switzerland). The plates were pre-washed by methanol and activated at 60°C for 5 min prior to chromatography. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (gallic acid) and the plate was developed in the respective mobile phase up to 90 mm. The Toluene-Ethyl acetate-Formic acid-Methanol (3: 3: 0.8: 0.2) was employed as mobile phase for gallic acid. Linear ascending development was carried out in 20 cm x 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25 ± 2°C). The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with 5% Ferric chloride as spray reagent and dried at 100°C in hot air oven for 10 min. The plate was photo-documented at UV 366 nm and UV 254 using Photo-documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 200-700 nm. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR3) and captured the images under, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag). Blue-brown coloured zones at Day light mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of gallic acid in the given standard and may be in the samples.

Linearity

10 μL in different concentrations (1-50 μg mL−1) of gallic acid was applied to a plate, developed, spots were derivatized, and the detector responses at different concentrations were measured at λ = 277 nm. A graph of peak area against amount of gallic acid was plotted. The experiment was performed in triplicate. The data were analyzed by linear regression least-squares fitting.

Precision

Instrumental precision

On plate, 10 μL gallic acid solution (50 μg mL−1) was applied in six bands. The plate was developed, the spots were derivatized, and the detector response for these spots was measured by use of scanning densitometry at λ = 277 nm. The mean, standard deviation, and % RSD were calculated. Intraday and Interday precision was studied using gallic acid 100, 300 ng/spot aliquots. Experiment was performed in triplicate.

Specificity

Specificity of method was determined by overlaying gallic acid in extract to standard spectra.

Gallic acid acid quantification in root extract

10 μL and 1 μL root extract and gallic acid solutions in linear range was applied and developed according to method described in manuscript. From line equation amount of gallic acid in extract of roots were calculated.

RESULTS

Various solvent compositions of the mobile phase for HPTLC analysis were examined in order to achieve high resolution and reproducible peaks. The mobile phase with the composition of Toluene-Ethyl acetate-Formic acid-Methanol (3: 3: 0.8: 0.2) showed high resolution and repeated results confirmed their efficiency and accuracy (Table -1, 2, 3. Fig -1). The ethanolic root extract of *A. lanata* illustrated the presence of gallic acid with different Rf values with range 0.47 to 0.79 (Table-2). In general, higher degree of gallic acid diversity has been observed in *Diospyrus ferrea* (Table-3) when compared to that of *A. lanata*. Maximum number of Rf value 0.47,0.48,0.53,0.57,0.65,0.78,0.62,0.66,0.69,0.78 has been observed in root extract of *Aerva lanata* (Table-2). Among the five different

gallic acid with Rf values0.46,0.47,0.59,0.68,0.76, are unique to *Diospyrus ferrea* root extract (Table 3). The gallic acid with the Rf value 0.47 is present commonly in both *Diospyrus ferrea* and *Aerva lanata*. The gallic acid with Rf values 0.46 showed its unique presence only in the *Diospyrus ferrea* root. The gallic acid with the Rf values 0.47 show their joint presence in root of *A. lanata* and *Diospyrus ferrea.* A photograph of a TLC plate after chromatography of gallic acid standard and a ethanolic extract of *Diospyrus ferrea* and *Aerva lanata* are shown in Figure 1. The identity of the gallic acid bands in sample chromatograms was confirmed by comparison of the chromatogram obtained from the sample with that obtained from the reference standard solution (Figure-1) and by comparing retention factors of gallic acid from sample and standard solutions. The peak corresponding to gallic acid from the sample solution had same retention factor as that of the retention factor from the gallic acid standard (Rf 0.47). A preparative TLC method reported in the literature was developed for isolation of gallic acid.

Table 1: HPTLC – standard gallic acid profile.

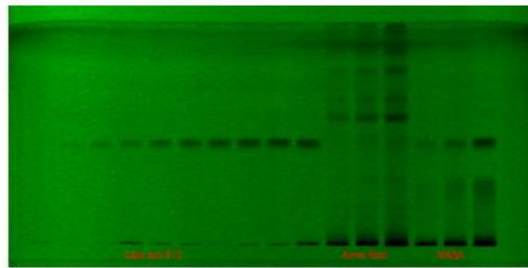
PEAK	RF	HEIGHT	AREA	ASSIGNED SUSBTANCE
1	0.47	21.5	516.5	GA
2	0.45	57.1	1405.9	GA
3	0.46	107.9	2663.6	Unknown
4	0.46	132.5	3408.6	GA
5	0.46	178.3	4632.1	GA
6	0.46	217.9	5888.9	GA
7	0.47	250.9	6790.7	GA
8	0.47	277.8	7733.8	Unknown
9	0.470	281.1	8111.9	Unknown
10	0.58	21.1	117.5	Unknown
11	0.47	297.8	8397.9	Unknown

Table 2:HPTLC – gallic acid profile of the ethanolic root extracts of *aerva lanata.*

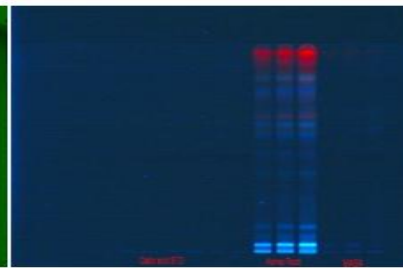
Peak	Rf	Height	Area	Assigned substances
1	0.48	35.0	1370.5	GA
2	0.53	40.5	866.5	Unknown
3	0.57	171.1	4068.9	Unknown
4	0.65	44.0	842.2	Unknown
5	0.78	67.0	1582.8	Unknown
6	0.47	56.7	2415.3	GA
7	0.53	62.4	1391.1	Unknown
8	0.57	249.6	6168.0	Unknown
9	0.65	71.6	1403.6	Unknown
10	0.78	104.7	2573.7	Unknown
11	0.47	89.7	4016.8	GA
12	0.53	93.1	2094.6	Unknown
13	0.57	340.9	8869.9	Unknown
14	0.62	64.4	1078.5	Unknown
15	0.66	103.0	2006.2	Unknown
16	0.69	18.5	368.8	Unknown
17	0.78	152.3	4047.9	Unknown

Table 3: HPTLC – gallic acid profile of the ethanolic root extracts of *diospyrus ferrea.*

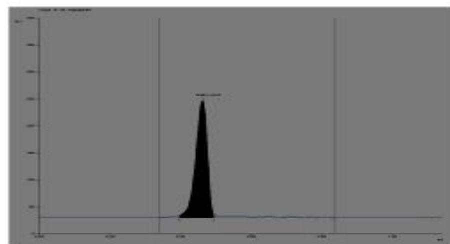
Peak	Rf	Height	Area	Assigned substances
1	0.46	104.1	2702.3	GA
2	0.47	184.4	4950.9	GA
3	0.59	22.6	641.6	Unknown
4	0.47	360.5	10996.3	GA
5	0.59	30.1	907.9	Unknown
6	0.68	10.4	197.2	Unknown
7	0.76	11.4	255.6	Unknown



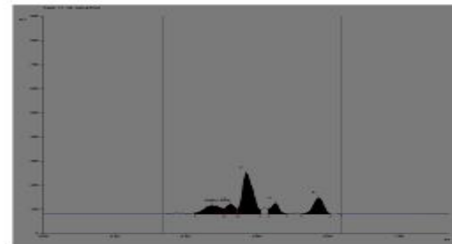
A-254nm



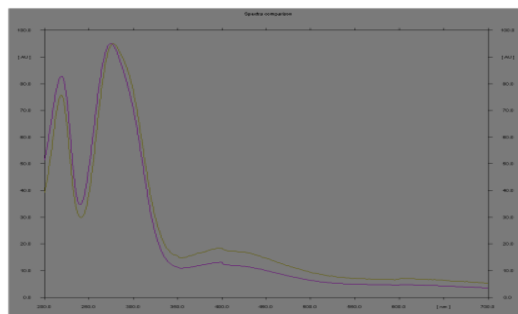
B-365 nm



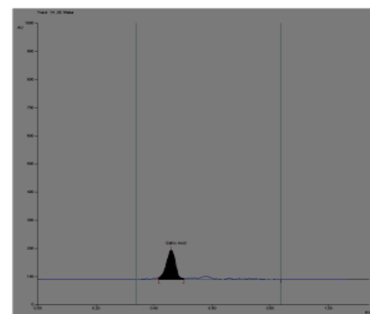
C



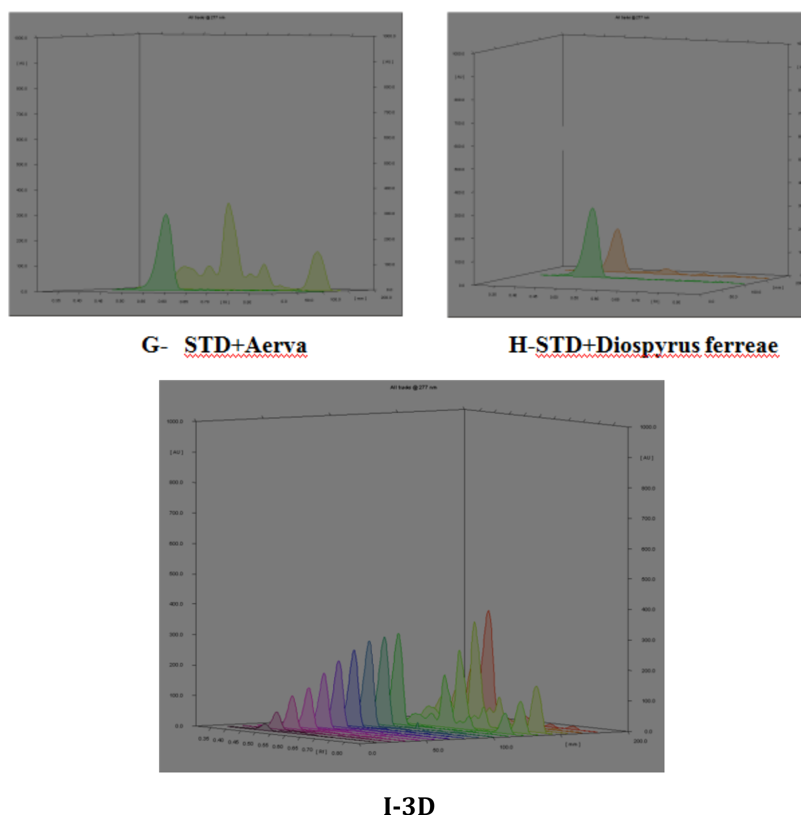
D



E



F



G- STD+Aerva

H-STD+Diospyrus ferreae

I-3D

Fig 1: HPTLC studies on the gallic acid of the medicinally important plant *aerva lanata* and *diospyrus ferreae* ethanolic root extract

A. HPTLC profile of the ethanolic extract of *Aerva lanata and Diospyrus ferreae* under UV 254 nm
B. HPTLC profile of the ethanolic extract of *Aerva lanata and Diospyrus ferreae* under UV 366
C. HPTLC Chromatogram of *Aerva lanata and Diospyrus ferreae* root - Peak densitogram display [Scanned at 277nm]
D. HPTLC Chromatogram of Standard Gallic acid [Scanned at 277nm]
E. HPTLC Chromatogram of *Aerva lanata and Diospyrus ferreae* - Baseline display [Scanned at 277nm)
F. HPTLC Chromatogram of standard Gallic acid Peak densitogram display [Scanned at 277 nm]
G. HPTLC Chromatogram of *Aerva lanata and standard* Root - Baseline display [Scanned at 277 nm]
H. HPTLC Chromatogram of *Diopyrus ferrea* and standard Root - Peak densitogram display [Scanned at 277 nm]
I.3D display of HPTLC Chromatogram of *Aerva lanata* and *Diospyrus fereae*.

HPTLC profiles obtained from the ethanol root extracts of *Diospyrus ferrea* and *Aerva lanata* revealed the strong presence of gallic acid. In Fig.1 gallic acid was linear in range100-700 ng per band with a correlation coefficient of 0.99903 (Y = 24.8(X) + 0.3822) for *Aerva lanata* and (Y=408.8(X) + 10.74) in *Diospyrus ferreae*. Amount of gallic acid in ethanolic extract of *Aerva lanata* was found to be 2.6 µg/ml and 47.50 µg/ml in *Diospyrus ferreae*. The method was validated by determining linearity, peak purity, limit of detection, repeatability, and percentage recovery of gallic acid from samples (Table2 &3).

DISCUSSION

The gallic acid compounds are widely distributed in many species of plants, where they play a role in protection from predation, perhaps also as pesticides and in plant growth regulation. In the present study we observed the gallic acid presence in root of *A. lanata* and *Diospyrus ferreae*. Previous biological and pharmagological studies showed that gallic acid possess anti-inflammatory, anti-viral, anti-bacterial, anti-parasitic, anti-oxidant, anthelmintic, anti-cancer, anti-septic, anti-diuretic properties. The results of the present study confirmed the presence of gallic acid in *A. lanata* and *Diospyrus ferreae root extract.* The presence of gallic acid confirmed the pharmacological applications of *A. lanata* and *Diospyrus ferreae*. Chromatographic fingerprint has been suggested to be practical and comprehensive approach for identifying authenticity and evaluating the quality, consistency and the stability of raw herbal materials and herbal extracts [34-36]. HPTLC is a valuable tool for reliable identification of the medicinally important plants. In the present study also we established the HPTLC profile for the medicinally important plant *A. lanata* and *Diospyrus ferreae*. The HPTLC profile will be used to distinguish the medicinally important plant from its adulterant.

Rakesh *et al., (*2009) proved the presence of gallic acid in hydroalcoholic extract of *N. stellata.* The dried flower extract was chromatographed on silica gel 60 F254 plates with chloroform: ethyl acetate: formic acid, 7.5: 6: 0.5 (v/v/v), as mobile phase. Detection and quantitation were performed by densitrometric scanning at 292 nm; by using deuterium lamp. The average recovery of gallic acid was found to be 98.33% [37]. The proposed HPTLC method provide a good resolution of gallic acid from other constituents present in ethanolic root extract of *Diospyrus ferrea* and *Aerva lanata.*

CONCLUSION

The results of the present study provided a valuable phytomarker for the identification and characterization of *A. lanata* and *Diospyrus ferreae* root extract. A rapid, simple, accurate and specific HPTLC method for quantitative estimation of gallic acid present in the *A. lanata* and *Diospyrus ferreae* has been developed and validated. The method used in this work resulted in good peak shape and enabled good resolution of gallic acid from other constituents of the plant material. Because recovery (98.33%) was close to 100%, there was no interference with the gallic acid peak from other constituents present in the plant. The plants studied here are shown as a potential source of useful drugs. Further pharmacological studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

ACKNOWLEDGEMENTS

The author is thankful to System in charge, Center for Advanced Research Facility, Ramachandra Medical College, for providing facilities to carry out the research work.

REFERENCES

1. Pankaj Oudhia's et al., 2001 *Diospyros ferrea* root extract based Traditional Herbal Formulations used for Diabetes Complications.
2. Acharya, Deepak and Shrivastava Anshu (2008): Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices, Aavishkar Publishers Distributor, Jaipur- India. ISBN 978-81-7910-252-7. pp 440. 1
3. T. Vertichelvan, M. Jegadeesan, S. Senthil Palaniappan, N.P. Murali, and K. Sasikumar, Diuretic and anti-inflammatory activities of *Aerva lanata* in rats, *Indian J. Pharm. Sci., 62, 2000*, 300-302.
4. M. Udupihille, and M.T.M. Jiffry, Diuretic effect of *Aerva lanata* with water, normal saline and coriander as controls, *Indian J Physiol and Pharmacol., 30, 1986,* 91-97 .
5. S. Manokaran, A. Jaswanth, S. Sengottuvelu, J. Nandhakumar, R. Duraisamy, D. Karthikeyan, and R. Mallegaswari, Hepatoprotective Activity of *Aerva lanata* Linn. Against Paracetamol Induced Hepatotoxicity in Rats, *Research J. Pharm. and Tech., 1(4), 2008,* 398-400.
6. A. Shirwaikar, D. Issac, and S. Malini, Effect of *Aerva lanata* on cisplatin and gentamicin models of acute renal failure, *J. Ethnopharmacol., 90, 2004*, 81–86.
7. T. Vetrichelvan, and M. Jegadeesan, Anti-diabetic activity of alcoholic extract of *Aerva lanata* [L.] Juss. Ex Schultes in rats, *J. Ethnopharmacol., 80, 2002,* 103–107.
8. S. Manokaran, A. Jaswanth, S. Sengottuvelu, J. Nandhakumar, R. Duraisamy, D. Karthikeyan, and R. Mallegaswari, Hepatoprotective Activity of *Aerva lanata* Linn. Against Paracetamol Induced Hepatotoxicity in Rats, *Research J. Pharm. and Tech., 1(4), 2008,* 398-400.
9. J.B. Harbone, Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, *1998*, pp. 182-190.
10. Niemetz R, Gross GG. Enzymology of gallotannin and ellagitannin biosynthesis. Phytochemistry 2005; 66: 2001.
11. Polewski K, Kniat S, Slawinska D. Gallic acid, a natural antioxidant, in aqueous and micellar environment:
12. Kim YJ. Antimelanogenic and antioxidant properties of gallic acid. Biol Pharm Bull 2007; 30: 1052-5.
13. Sakagami H, Satoh K, Hatano T, Yoshida T, Okuda T. Possible role of radical intensity and oxidation potential for gallic acid-induced apoptosis. Anticancer Res1997; 17:377-80.
14. Serrano A, Papacios C, Roy G, Cespon C, Villar ML, Nocito M, Gonzalez-Porque P. Derivatives of gallic acid induce apoptosis in tumoral cell lines and inhibit lymphocyte proliferation. Arch Biochem Biophys 1998; 350: 49-54.
15. Inoue M, Sakaguchi N, Isuzugawa K, Tani H, Ogihara Y. Role of reactive oxygen species in gallic acid-induced apoptosis. Biol Pharm Bull 2000; 23: 1153-7.
16. Sakaguchi N, Inoue M, Ogihara Y. Reactive oxygen species and intracellular Ca2+, common signals for apoptosis induced by gallic acid. Biochem Pharm 1998; 55: 1973-81.
17. Tulyathan V, Boulton RB and Singleton VJ. Oxygen uptake by gallic acid as a model for similar reactions in wines. J Agric Food Chem 1989; 37: 844-9.
18. Friedman M and Jürgens HS. Effect of pH on the stability of plant phenolic compounds. J Agric Food Chem 2000; 48: 2101-10.
19. Krores, B. H., Van den Berg, A. J. J. Ufford, H. C. Q., Van Dijk, H., & Labadie, R. P. (1992). Antiinflammatory activity of gallic acid. *Planta Medica. 58,* 499-504.
20. Rakesh SU, Patil PR, Salunkhe VR, Dhabale PN, Burade KB. HPTLC method for quantitative determination of quercetin in hydroalcoholic extract of dried flower of *Nymphaea stellata* willd. International Journal of Chem Tech Research 2009; 1(4): 931-936.
21. Rajkumar T, Sinha BN. Chromatographic finger print analysis of budmunchiamines in *Albizia amara* by HPTLC technique. Int. J. Res. Pharm. Sci. 2010; 1(3): 313-316.
22. Manikandan A, Victor Arokia Doss A. Evaluation of biochemical contents, nutritional value, trace Elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* [Jacq.]. J. Chem. Pharm. Res. 2010; 29(3): 295-303.
23. Tripathi AK, Verma RK, Gupta AK, Gupta MM, Khanuja SPS. Quantitative Determination of Phyllanthin and Hypophyllanthin in *Phyllanthus* Species by High-performance Thin Layer Chromatography. Phytochem. Anal. 2006; 17: 394–397.
24. 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.
25. Patil AG, Koli SP, Patil DA, Chnadra N. Phamacognostical standardization and HPTLC finger print of *Crataeva tapia* Linn. SSP. Odora [Jacob.] Almeida leaves. International Journal of Pharma and Biosciences 2010; 1(2): 1-14.
26. Sharma V, Sharma N, Singh B, Gupta RC. Cytomorphological studies and HPTLC fingerprinting in different plant parts of three wild morphotypes of *Datura metel* L. "Thorn Apple" from North India. Int J Green Pharm 2009; 3: 40-6
27. Sasikumar JM, Jinu U, Shamna R. Antioxidant Activity and HPTLC Analysis of *Pandanus odoratissimus* L. Root. European Journal of Biological Sciences 2009; 1 (2): 17-22.
28. Priyabrata Pattanaya, Ranjan Kumar Jena, Sangram Keshri Panda. HPTLC fingerprinting in the standardization of Sulaharan Yoga: An Ayurvedic tablet formulation. International Journal of Pharmaceutical Sciences Review and Research 2010; 3(2): 33-36.
29. Khan S, Singla RK, Abdin MZ. Assessment of phytochemical diversity in *Phyllanthus amarus* using HPTLC Fingerprints Indo-Global Journal of Pharmaceutical Sciences 2011; 1(1): 1-12.
30. Sasikumar, Meenaa, Kavitha Srilakshmi, Sriram HPTLC analysis of various market samples of a traditional drug source – Kodiveli (*Plumbago zeylanica* L.) Int J Pharm Pharm Sci. 2010; 2 (Suppl 4): 130132
31. M. Yamunadevi, E.G. Wesely, and M. Johnson, Phytochemical studies on the terpenoids of medicinally important plant *Aerva lanata* L. using HPTLC, *Asian Pacific Journal of Tropical Biomedicine, 2011,* S220-S225.
32. M. Yamunadevi, E.G. Wesely, and M. Johnson, Chromatographic fingerprint analysis on flavonoids constituents of the medicinally important plant *Aerva lanata* L. by HPTLC technique, *Asian Pacific Journal of Tropical Biomedicine, 2012*, S8-S12.
33. M. Yamunadevi, E.G. Wesely, and M. Johnson, Chemical Profile Studies on the Alkaloids of medicinally important plant *Aerva lanata* L. using HPTLC, *Journal of Natura Conscientia, 2(2), 2011,* 341-349.
34. M. Yamunadevi, E.G. Wesely, and M. Johnson, Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique, *Asian Pacific Journal of Tropical Biomedicine, 2011,* 428-433.
35. T. Rajkumar, and B.N. Sinha, Chromatographic finger print analysis of budmunchiamines in *Albizia amara* by HPTLC technique, *Int. J. Res. Pharm. Sci., 1(3), 2010,* 313-316.
36. A. Manikandan, and A. Victor Arokia Doss, Evaluation of biochemical contents, nutritional value, trace Elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* [Jacq.]., *J. Chem. Pharm. Res., 29(3), 2010,* 295-303.
37. Rakesh SU, Salunkhe VR, Dhabale PN, Burade KB. HPTLC Method for Quantitative Determination of Gallic Acid in Hydroalcoholic Extract of Dried Flowers of *Nymphaea stellata* Willd. Asian J. Research Chem. 2009; 2(2): 131-134.