Rapid High Performance Thin Layer Chromatographic Method for Quantitation of Catechin from Extracts of Cashew Leaves – a Short Report

Yogini Jaiswal¹, Pratima Tatke²*, Satish Gabhe³, Ashok Vaidya²

¹C.U. Shah College of Pharmacy, S.N.D.T Women’s University, Mumbai – 400049, India
²ICMR Advanced Centre of Reverse Pharmacology in Traditional Medicine, Kasturba Health Society, Vile Parle-(W), Mumbai – 400 056, India

Key words: catechin, cashew leaves, HPTLC, quantitation

Cashew is a tropical tree with immense commercial value and the use of its various parts in the confectionary, food, herbal and tanning industry has gained tremendous importance. Thus, the development of quality control method for routine analysis of various products of this widely used plant would be a great help to analysts. The phytochemistry of this plant has not been explored in detail. Anacardium occidentale Linn. (cashew) is reported to contain many antioxidant and polyphenolic compounds. However, there have been no reports published for the HPTLC analysis of the extracts of cashew leaves. Hence, an attempt has been made to develop a HPTLC method for estimation of catechin from leaf extracts. The present paper reveals a rapid high performance thin layer chromatographic method developed for quantitation of catechin from extracts of cashew leaves. The chromatographic parameters such as solvent system, development time, saturation time, detection wavelength were optimized. The mobile phase toluene: ethyl acetate: methanol: formic acid (6:6:1:0.1v/v/v/v) gave the best resolution for various components. The separation of various components and quantitation of amount of catechin was successfully carried out from extracts of cashew leaves. The aqueous extract of leaves contained a higher amount of catechin as compared to ethanol extract. The presence of tannins and phenolics was visualised as bluish-black bands with 5% alcoholic FeCl₃ titration of amount of catechin was successfully carried out from extracts of cashew leaves. The aqueous extract of leaves contained a higher amount of catechin as compared to ethanol extract. The analysis of phytoconstituents is a difficult task because of their complex nature, usually low availability and variability even within the same plant species. Sensitivity is the major problem for the detection of phytoconstituents when various analytical techniques are used. Thus, most frequently chromatographic techniques in combination with different detections are the preferred techniques for this purpose. Due to extremely small sample volumes and the aspects mentioned above, HPLC and HPTLC are preferred for the analysis of herbal products [Liang et al., 2004].

The technique of HPTLC has the advantage that several samples can be analysed at the same time. The established importance of HPTLC fingerprints is attributed to the visual impression, which can be further explored by multiple detection (i.e. pre and post chromatographic visualisation). A wide spectrum of constituents can be detected at the same time in a single run in an experiment. Moreover performing HPTLC helps in identifying and quantifying various phytoconstituents present in the extracts.

The phytochemistry of the leaves of this plant has not been explored in detail. There have been no reports published for the HPTLC of the extracts of cashew leaves. Anacardium occidentale Linn. (cashew) is reported to contain many antioxidant and polyphenolic compounds [Kubo et al., 2006; Mathew & Parpia, 1970; Jaiswal et al., 2010]. Thus, an HPTLC method was developed and optimised for quantify...
ing catechin and obtaining a fingerprint of various extracts of leaves by this planar chromatographic method. This method can serve as a rapid, economic and specific alternative to other detection techniques for quantitation of catechin in cashew samples. Cashew is used in a number of beverages in various forms. The leaves are consumed as food in various forms in tropical countries like Malaysia and are reported to have a number of pharmacological effects [Abas et al., 2006; Kudi et al., 1999; Goncalves et al., 2005; Trevisan et al., 2006; Schmourol et al., 2005]. Thus development of an economic and rapid analysis method would help in quality control of various formulations and food products of cashew available commercially.

MATERIAL AND METHODS

Material

Cashew leaves were collected from Tungareshwar forests of Vasai Taluka, Dist. Thane in the state of Maharashtra, India. The plant specimen was authenticated and a herbarium of the plant specimen (voucher number no. YOGA1/No.BSI/WC/Tech/2008/69) was submitted at the Botany Department of Botanical Survey of India, Pune; (M.S), India.

Extraction of cashew leaves

Fully matured shade dried leaves of cashew were collected, cleansed and ground to coarse powder form. The samples were extracted by using Soxhlet extractor, with ethanol with a mass to volume ratio of 1:6. The ethanol extract was evaporated to dryness on the rotary evaporator. Aqueous extract was prepared by refluxing with water and mass to volume ratio of 1:6 (g/mL). The aqueous extract was freeze dried and used for analysis.

Preparation of solutions of extracts and catechin

Stock solutions (1 mg/mL) of reference catechin were prepared in methanol. Working solutions of catechin were prepared by appropriate dilutions of the stock solution with methanol. All solutions were prepared freshly prior to analysis. Working solutions of extracts (5 mg/mL) of cashew leaves were prepared with methanol.

Development and optimisation of HPTLC parameters

To make a choice of suitable solvent system, initially the elutropic series of different solvents was tried by running on the HPTLC plates. Neat solvents of varying polarity and solvents in different combination ratios were used to optimise elution of various components and a combination of solvents that gave better resolution of the maximum number of components in extracts was selected. Formic acid was used as a modifier to affect better resolution of bands. Various time periods from 10–25 min (10, 15, 20, 25 mins) were attempted to select the optimum saturation time, suitable for maximum resolution and faster development of the HPTLC plate. The samples were applied at 1 cm distance from the bottom on the HPTLC plates and the solvent front was marked at 8 cm distance from the application position. The plates were allowed to dry and then placed in chambers saturated with the solvent system (mobile phase) for a period of 20 min prior to placement of plates. The qualitative evaluation of the plate was done by determining the migration behaviour of the separated substances by calculating Rf value.

HPTLC analysis

Precoated HPTLC plates used for analysis were preconditioned by overnight washing with methanol in a twin-trough chamber. Preconditioning in methanol has been shown to be effective for layer cleaning. The prewashed plates were then heated at 105°C for 5 min before use. HPTLC was performed on precoated silica gel GF254 aluminum backed HPTLC plates (20 cm×20 cm, 0.2 mm thickness, 5–6 µm particle size, E-Merck, Germany). Five microlitres of the sample solutions were spotted as bands of 6 mm width by using a 100 µL Hamilton syringe. The plates were developed using toluene: ethyl acetate: methanol: formic acid (6:6:1:0.1, v/v/v/v) as the solvent system with saturation time of 15 min in a CAMAG twin-trough plate development chamber. The developed plates were air dried and scanned. A spectro-densitometer (Scanner 3, CAMAG) equipped with ‘win CATS’ planar chromatography manager (version 1.3.0) software was used for the densitometry measurements, spectra recording and data processing. Absorption/remission were done in the measurement mode at a scan speed of 20 mm/s. Densitograms were recorded at the wavelength of 254 nm for catechin and various components of extracts. The analysis was performed in air-conditioned room maintained at 22°C and 55% humidity.

Spot visualisation on the HPTLC plate

A visualising agent was selected based upon the class of phytoconstituents found in the preliminary phytochemical screening tests. The visualising reagent helps in visualisation as well as confirmation of the identity of the phytoconstituents. A 5% alcoholic FeCl3 solution was used for visualisation of tannins and phenolic components in the extracts.

Quantitation of catechin in various extracts by HPTLC analysis

The extracts were dissolved in methanol and the solution of concentration 5 µg/µL was filtered through 0.45 µm PVDF filter and HPTLC was performed under the conditions optimised for the reference compound. The amount of catechin in the extracts was quantified by comparison with catechin bands from solutions of known concentration. The plates were then dipped in 5% alcoholic FeCl3 solution for a few seconds and then kept at 100°C for 5 min for visualisation of bands of polyphenols.

Calibration curve of catechin

In order to establish a calibration curve for estimation of catechin, the limit of detection (LOD) and limit of quantitation (LOQ) were determined.
RESULTS AND DISCUSSION

Ethanol and aqueous extract of cashew leaves were prepared and the extraction yields were calculated and listed in Table 1. In literature, reports indicate that Soxhlet extraction has been used as control for comparison with other extraction techniques [Ghosh et al., 2011; Ahmad et al., 2010; Chulet et al., 2010]. The extraction of cashew leaves with ethanol was carried out by Soxhlet extraction and with water by decoction process. Since cashew is reported to be a rich source of phenolic compounds, ethanol and water were selected as the solvents for extraction of leaves [Melo et al., 2006; Kamath et al., 2007; Rezali et al., 2008; Shobha et al., 1992; de Brito et al., 2007]. Such polar solvents would help in extraction of polar components like polyphenols. The extractive yield of ethanol extract was found to be higher than aqueous extract. This may be due to the ability of ethanol to extract polar as well as non-polar constituents and this is in agreement to the results of extraction yields obtained with ethanol in our previous work [Jaiswal et al., 2010, 2012].

An HPTLC method was optimised and developed for estimation of catechin from extracts of leaves of cashew. A 5% alcoholic FeCl₃ solution was used as a visualising agent to visualise the presence of polyphenols and bluish black coloured bands indicated their presence. Polyphenols are considered as one of the important secondary metabolites produced by plants, and hence our HPTLC studies focused on visualisation of polyphenols in the leaves of cashew [Mamyrbekova-Bekro et al., 2008]. Catechin is known to be a bioactive and potent polyphenolic compound, and hence we estimated and quantified its presence in the extracts prepared from cashew leaves. The peak areas obtained for standard catechin were used as a standard to quantify the amount of catechin present in ethanol and aqueous extracts of cashew leaves. The Rₛ value of catechin was found to be 0.4 by comparison with the reference catechin that showed same Rₛ value. Other 7 spots in addition to catechin were found to have Rₛ values of 0.3, 0.45, 0.52, 0.6, 0.74, 0.76 and 0.8 respectively. There were some spots which on spraying with ferric chloride showed clear patches and were not visualised. Spraying with the FeCl₃ solution on an HPTLC plate without any sample displays very faint brown or yellowish in colour after drying, due to deposition of ferric hydroxide on the plate. But in the HPTLC fingerprint of extracts of cashew leaves, number of clear patches (between Rₛ value 0.6 to 0.75) were observed where the plate retained its original white colour. The clear patches may thus be attributed to the presence of free acids which apparently stop the precipitation of ferric hydroxide [Roberts & Wood, 1951]. Identification of all the bands in those patches was not successful, but one of them corresponded to citric acid which is in agreement to our previous research work that explains the qualitative phytochemical tests of extracts of cashew leaves [Jaiswal et al., 2012].

With a decrease in the intensities of the individual spots up to Rₛ value 0.3, there was a marked increase in the intensity observed in the diffused area at and near the origin, after spraying with FeCl₃ solution. Such diffused area is possibly to be identified as the various condensation products that are formed from the preliminary oxidation products of the polyphenols [Harrison & Roberts, 1939; Roberts & Wood, 1951]. The presence of gallic acid was observed at Rₛ value 0.45. However the other bands at Rₛ value 0.52 and 0.6 were polyphenols which gave bluish colouration with ferric chloride solution and were present in trace amounts only. Only cat-

### TABLE 1. Extractive yield of various extracts of cashew leaves.

| Cashew leaf extracts | Extractive yields (%)±SEM |
|----------------------|---------------------------|
| Ethanol extract      | 25.94±0.70                |
| Aqueous extract      | 8.64±0.50                 |

* n=3 determinations for each of the values mentioned above.

### TABLE 2. Catechin content in various extracts of cashew leaves.

| Cashew leaf extracts | Content of catechin (%)±SEM |
|----------------------|-----------------------------|
| Ethanol extract      | 4.75±0.7                    |
| Aqueous extract      | 5.70±0.9                    |

* n=3 determinations for each of the values mentioned above.
Echin and gallic acid were found to be present in appreciable amounts, and since gallic acid is a very common constituent, catechin was selected as a marker compound for quantitation. Catechin is a well known bioactive compound with significant antioxidant activity with a great commercial value. The bands with R<sub>f</sub> value 0.74, 0.76 and 0.8 were chlorophyll pigments that did not respond to the ferric chloride solution after spraying.

The values obtained for LOD and LOQ were 0.1 and 0.3 µg/µL, respectively. The linear response was obtained between 0.4 and 2.0 µg/µL. The chromatograms for both the extracts of cashew leaves and for catechin are depicted in Figures 1, 2 and 3, respectively. The calibration curve is depicted in Figure 4 indicating excellent linear relationship with the correlation coefficient as 0.9990.

The amount of catechin in various extracts was estimated and listed in Table 2. It was found that an aqueous extract of leaves contained the maximum amount of catechin as compared to the ethanol extract. Similar results were obtained in our previous work, where HPLC method was used for quantitation of catechin in various extracts of cashew leaves and aqueous extracts were found to contain a higher content of catechin as compared to the ethanol extract [Jaiswal et al., 2010]. This may be due to the higher solubility of catechin in water as compared to ethanol. The video images of HPTLC fingerprints of the extracts and fractions at different wavelengths and after spraying with 5% ferric chloride are shown in Figure 5. The various components of the extracts were well resolved. A distinct band of catechin can be seen in both the extracts at R<sub>f</sub> 0.4.

The developed method can be recommended for quality assurance of extracts and herbal formulations of cashew leaves using catechin as a marker. The method finds its merit in being an economic method of analysis with lower sample preparation and analysis time as compared to other chromatographic techniques like LC and GC [De Brito et al., 2007; Jaiswal et al., 2010].

**CONCLUSION**

The high performance thin layer chromatography with toluene: ethyl acetate: methanol: formic acid (6:6:1:0.1, v/v/v/v) as the mobile phase is a reliable method for fast and easy separation of components and quantitation of catechin from crude extracts of cashew leaves. The method can be used as a cost effective method with reduced analysis time as compared to other alternative methods of analysis.
ACKNOWLEDGEMENTS

This research project was supported by the Indian Council of Medical Research (ICMR), New Delhi, India within Project No. 2005–05180. The authors are also thankful to Dr. Vinayak Naik, Nicholas Piramal India Ltd, Mumbai; for his kind support in procuring the plant specimen.

REFERENCES

1. Abas F., Lajis N.H., Israf D.A., et al., Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. Food Chem., 2006, 95, 566–573.
2. Ahmad A., Alkarkhi A.F., Hena S., Siddique B.M., Dur K.W., Optimization of Soxhlet extraction of Herba Leonuri using factorial design of experiment. Int. J. Chem. 2010, 2, 198–205.
3. Chulet R., Jospeh L., George M., Pradhan P., Pharmacognostic standardization and phytochemical screening of Albizia lebbeck. I. Chem. Pharm. Res., 2010, 2, 432–443.
4. De Brito E.S., De Araujo M.C.P., Lin L.-Z., Harnly J., Analytical, nutritional and clinical methods: determination of the flavonoid components of cashew apple (Anacardium occidentale) by LC-DAD-ESI/MS. Food Chem., 2007, 105, 1112–1118.
5. Ghosh U., Haq M.A.B., Chakrabarty S., Application of systematic technologies for the extraction of novel phytoconstituents from pharmacologically important plants. Int. J. Chem. Anal. Sci., 2011, 2, 1153–1158.
6. Goncalves J.L.S., Lopes R.C., Oliveira D.B., et al., In vitro anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea. J. Ethnopharmacol., 2005, 99, 403–407.
7. Harrison C., Roberts E., Tea tannins and its fermentation products. Biochem. J., 1939, 33, 1408.
8. Jaiswal Y., Naik V., Tatke P., Gabhe S., Vaidya A., Pharmacognostic and preliminary phytochemical investigations of Anacardium occidentale (Linn.) leaves. Int. J. Pharm. Pharm. Sci., 2012, 4, 625–631.
9. Jaiswal Y., Tatke P., Gabhe S., Vaidya A., Isolation and quantitative analysis of a bioactive polyphenol – Catechin in Anacardium occidentale Linn. (leaves and testa) by HPLC analysis. Res. J. Pharmacog. Phytochem., 2010, 5, 372–376.
10. Kamath V., Rajm P.S., The efficacy of cashew nut (Anacardium occidentale L.) skin extract as a free radical scavenger. Food Chem., 2007, 103, 428–433.
11. Kubo I., Masuoka N., Ha T.J., Tsujimoto K., Antioxidant activity of anacardic acids. Food Chem., 2006, 99, 555–562.
12. Kudi A.C., Umoh J.U., Edauvie L.O., Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol., 1999, 67, 225–228.
13. Liang Y.Z., Xie P.S., Chan K., Quality control of herbal medicines. J. Chromatogr. B., 2004, 812, 53–70.
14. Mahady G.B., Fong H.H., Farnsworth N.R., Botanical Dietary Supplements: Quality, Safety and Efficacy. 2001, Swets and Zeitlinger Publishers, Lisse, The Netherlands, pp. 283–289.
15. Mamyrbekova-Bekro J.A., Konan K.M., Bekro Y.A., Gobin M., Bi D., Bi T.J.Z., Mambo V., Boua Boua B., Phytochemicals of the extracts of four medicinal plants of Côte d’Ivoire and assessment of their potential antioxidant by thin layer chromatography. Eur. J. Sci. Res., 2008, 24, 219–228.
16. Mathew A.G., Parpia H.A.B., Polyphenols of cashew kernel testa. J. Food Sci., 1970, 35, 140–143.
17. Melo E. de A., De Lima V.L.A.G., Maciel M.I.S, Caetano A.C. de Silva, Leal F.L.L., Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. Braz. J. Food Technol., 2006, 9, 89–94.
18. Rezali N., Razab R., Junit S.M., Aziz A.A., Radical scavenging and reducing properties of extracts of cashew shoots (Anacardium occidentale). Food Chem., 2008, 111, 38–44.
19. Roberts E., Wood D., A study of the polyphenols in tea leaf by paper chromatography. Biochem. J., 1951, 414–422.
20. Schmourlo G., Mendonca-Filho R.P., Alviano R.C.S., et al., Screening of anti-fungal agents using ethanol precipitation and bioautography of medicinal and food plants. J. Ethnopharmacol., 2005, 96, 563–568.
21. Shohba S., Krishnaswamy P., Ravindranath B., Phenolic lipid composition during development of cashew. Phytochemistry, 1992, 31, 2295–2297.
22. Trevisan M.T.S., Pfundstein B., Haubner R., Württele G., Spiegelhalter B., Bartsch H., Owen R., Characterization of alkyl phenois in cashew (Anacardium occidentale) products and assay of their antioxidant capacity. Food Chem. Toxicol., 2006, 44, 188–197.
23. WHO, WHO Traditional Medicine Strategy. 2002–2005. World Health Organization. 2005, Geneva, pp. 43–48.

Submitted: 16 March 2012. Revised: July 2012. Accepted: 7 August 2012. Published on-line 15 February 2013.