Original Article

HPTLC Densitometric Quantification of Kaempferol from Leaves of *Euphorbia neriifolia*

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ABSTRACT

Objectives: To study HPTLC densitometric quantification of kaempferol in aqueous extract fraction (EN6) of leaves of *Euphorbia neriifolia* Linn. **Methods:** Chromatographic method was employed on pre-washed and pre-activated 10.0 x 10.0 cm aluminum Lichrosphere HPTLC plates pre-coated by silica gel 60 F_{254} of 0.2 mm thickness layer as a stationary phase. Solvent system toluene: ethyl acetate: formic acid (6:4:1 v/v/v) was used. Densitometric analysis of kaempferol was carried out at 254 nm in absorption-reflectance mode. Percent content of kaempferol was quantified using proposed HPTLC densitometric method. The method was validated by ICH guidelines for its linear range, linearity, LOD and LOQ, specificity, precision, reproducibility and recovery studies. **Results:** HPTLC finger printing analysis showed that R_f value of peak of kaempferol (Rf 0.91) was closely compacted with aqueous extract fraction (EN6; R_f 0.90) that indicates identification of kaempferol. Percent content of kaempferol was quantified and found to be 0.024817% in aqueous extract fraction (EN6) for 10 μl volume applied. Linear range was found100-600 ng/spot with correlation coefficient 0.99834. LOD and LOQ were found 100 and 300 ng. Proposed method was specific. The % CV for intra-day was 2.422 and inter-day was 2.522 at concentration of 400 ng/spot. The % CV was found 2.1, hence the method was reproducible. Average percent recovery was 95.57% in aqueous extract fraction (EN6), therefore, the method was found to be accurate. **Conclusion:** Developed HPTLC method was found to be simple, specific, precise, reproducible and accurate. Here, the first time we have reported HPTLC method for kaempferol in aqueous extract fraction (EN6). However, there is a requirement to carry forward these studies by using some other standard markers in aqueous extract fraction (EN6) of *E. neriifolia* leaves for exploring its phyto-constituents that could have therapeutic potential in various diseases.

Key words: *Euphorbia neriifolia* Linn., Aqueous extract fraction, Leaves, HPTLC, Kaempferol, Identification, Quantification, Validation.

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INTRODUCTION

High performance thin layer chromatography (HPTLC) has become a regular investigative method due to its cost effectiveness and reliability in quantification of analyte at micro and nano gram levels. It is proved that HPTLC was very useful method for the reason of high sample throughput and requires minimum sample clean-up with less analysis or detection time.[1] The multi-coloured HPTLC images have provides an additional instinctive parameter of visible colour and fluorescence and unlike G.C and HPLC. Simultaneously HPTLC method can determine various samples on the same plate. This approach helps to sustain its instinctive advantage along with to find limitations of developing distance and plate efficiency.[2]

Euphorbia neriifolia Linn. Sp. Pl. (451.1753) belonging to family Euphorbiaceae. It is worldwide scattered in Baluchistan, Burma, India and Malaysian Islands. In India, it is found in rocky ground throughout Deccan Peninsula and Orissa. It is habitually cultivated for hedges in villages all over India.[3,4] *E. nerrifolia* leaves has several ethnomedicinal uses. As leaves are brittle, heating, carminative, improve the appetite,

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good for treatment of tumors, pain, inflammation, abdominal swelling and bronchial infection.[5] Various plant parts or whole *E. neriifolia* extract and its isolates have been reported scientifically using various *in-vivo* and *in-vitro* experimental methods for anti-carcinogenic / renal carcinogenesis/ hepatocarcinogenesis,[6-9] antidiabetic,[10] cytotoxicity,[11,12] immunomodulatory,[13,14] etc. properties. *E. neriifolia* is also reported to have neriifolin-S,[15] neriifolin,[16] neriifoliene,[17] euphol,[18] neriifolione and cycloartenol,[19] taraxerol,[20] quercetin and rutin,[21] antiquorin,[22] etc. phyto-constituents.

As per earlier report of percentage yields and primary phytochemical screening of extract fractions (EN1 to EN6) of *E. neriifolia* leaves.[23] The percentage yield of petroleum ether (EN1; 7.8 % yield), toluene (EN2; 1.19% yield), chloroform (EN3; 0.56% yield), ethyl acetate (EN4; 2.36% yield), n-butanol (EN5; 0.26% yield) and aqueous (EN6; 8.5% yield) was found. Among the findings of primary phytochemical screening of extract fractions (EN1 to EN6), the aqueous extract fraction (EN6) was comprised the presence of amino acids, alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponin, tannins, steroids and phenols class of phytocompounds. In reference with these studies, the aqueous extract fraction (EN6) was selected for primary HPTLC fingerprinting analysis. The HPTLC reports have also showed there is a presence of flavonoids, terpenes, steroidal saponins, alkaloids, glycosides, tannins and phenolic acids, etc. class of phyto-constituents. In present investigation authors have been reported identification, quantification and validation of kaempferol in EN6 extract fraction of leaves of *E. neriifolia* by HPTLC technique.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade and purchased from Merck, Germany and Sd Fine-Chem, Mumbai, India. Kaempferol (purity: 98.8% w/w) was purchased from Natural Remedies Pvt. Ltd., Bangalore, India.

Collection and authentication of *E. neriifolia* and its leaves

E. neriifolia plant and its leaves were collected from local region of Bhopal, Madhya Pradesh, India. Dr. Vijay V. Bhadane, Taxonomist, Department of Botany, Pratap College, Amalner-425401, Maharashtra, India was authenticated. Voucher specimen (No. PCA/Bot-P1637) was assigned and deposited in the department.

Extraction and fractionation of *E. neriifolia* leaves

The detailed procedure of extraction and fractionation of *E. neriifolia* leaves was mentioned in our earlier report.[23] Obtained extract fractions were concentrated, dried and designated as petroleum ether (EN1), toluene (EN2), chloroform (EN3), ethyl acetate (EN4), n-butanol (EN5) and aqueous (EN6) and used for HPTLC densitometric quantification of kaempferol.

Preparation of solution of aqueous extract fraction (EN6) of *E. neriifolia* leaves

Accurately weighted 500 mg aqueous extract fraction (EN6) was dissolved into 5 ml of methanol in volumetric flask separately. It was then sonicated for 20 min. The solution was kept a side for 30 min to settle down the aliquot. The concentration 100 µg/µl of aqueous extract fraction was used in the study.

Preparation of standard solution of kaempferol

Accurately weighted 5 mg of kaempferol was dissolved into 5 ml of methanol in volumetric flask. It was then sonicated for 15 min., 1 ml of this dilution was transferred into 10 ml volumetric flask and volume was adjusted with methanol to get the final concentration 0.1 µg/µl of kaempferol.

Solvent system

After several trails on different solvent systems, the better resolution was found in toluene: ethyl acetate: formic acid (6:4:1 v/v/v) and used for co-chromatography with kaempferol.

Chromatographic conditions and HPTLC fingerprinting for kaempferol

We have developed chromatographic conditions in this study, performed on pre-washed and pre-activated 10.0 x 10.0 cm aluminum Lichrosphere HPTLC plates pre-coated by silica gel 60 F_{254} of 0.2 mm thickness layer (Merck, Germany). Volumes of aqueous extract fraction (EN6) were applied at 5 and 10 µl and 2 and 5 µl of kaempferol at application position 8.00 mm with band length 8.00 mm by a Camag Linomat-V automatic HPTLC sample spotter (Camag) equipped with a 25 µl syringe (Hamilton) in continuous drying stream of nitrogen gas. Linear ascending development with solvent system, toluene: ethyl acetate: formic acid (6:4:1 v/v/v) ina 10.0x 10.0 cm twin trough glass chamber (Camag) formally saturated with solvent system for 15 min at relative humidity (40%) and room temp. (25 ± 2°C). The development distance was 70.00 mm (development time 20 min with filter paper) and 20 ml solvent system was used. After development, plate was dried with stream of hot air and densitometric scanning

was performed at 254 nm in absorption-reflectance mode by using a Camag HPTLC scanner 3 and Camag visualizer with automatic digital camera linked to win-CATS software (Version 1.4.6). The slit dimension of scanner at 6.00 x 0.45 mm (Micro) was set with 100 µm/step data resolution and 20 mm/s scanning speed. The fingerprinting of kaempferol was confirmed by superimposing the U.V spectra of aqueous extract fraction (EN6) and standard kaempferol within R_f ±0.1 values, scanned at 254 nm under D2 lamp with 190-400 nm start and end wavelengths. Colors of resolved bands were noted.

Preparation of calibration curve of kaempferol

Stock solution of kaempferol (0.1µg/µl) was prepared in methanol. Different volumes of stock solution 1, 2, 3, 4, 5 and 6 µl of kaempferol were applied on HPTLC plate to obtained concentration 100, 200, 300, 400, 500 and 600 ng/spot (Band width: 8 mm, distance between tracks 13.3 mm) using automatic sampler spotter. The developed plate was scanned at 254 nm under D2 lamp with 190-400 nm start and end wavelengths. Linear regression of standard curve was determined through R^2 ± SD = 0.99834 ± 3.53 % and linear regression equation was y = -86.03 + 10.56*X. The regression statistics was showed good linear relationship over the concentration range of 100-600 ng/spot. The calibration curve of kaempferol was obtained by plotting peak areas (AU) verses concentration (ng/spot) of kaempferol applied with the help of winCATS software (Version 1.4.6) as shown is (Figure 1).

Quantification of kaempferol in aqueous extract fraction (EN6)

Quantification of kaempferol in aqueous extract fraction (EN6) was performed by applying 10 µl of suitably diluted each extract fraction in triplicates on HPTLC plate. Developed plate was scanned at 254 nm under D2 lamp with 190-400 nm start and end wavelengths. The peak areas were recorded and amount of kaempferol was calculated. Therefore, based on the amount of kaempferol found, the percent content of kaempferol in aqueous extract fraction (EN6) was calculated.

Validation of developed HPTLC method for kaempferol

ICH guidelines were followed for the validation of developed HPTLC method for kaempferol (Topic Q2B - Step 4, Nov. 1996, CPMP/ICH/281/95 and Topic Q2 (R1) - Step 5, June 1995, CPMP/ICH/381/95) for its linear range, linearity (correlation coefficient), limit of detection (LOD) and limit of quantification (LOQ) (sensitivity), specificity (selectivity), precision (variation or variability), reproducibility and recovery (accuracy).[24-26]

Linear range

Linear range was determined by applying 1, 2, 3, 4, 5 and 6 µl of kaempferol on HPTLC plate to obtained concentration 100, 200, 300, 400, 500 and 600 ng/spot using automatic sampler spotter. Developed plate was scanned as mentioned in above chromatographic conditions and HPTLC fingerprinting for kaempferol section.

Linearity

Linearity of kaempferol was determined by plotting linearity curve of peak area (AU) verses concentration (ng/spot) of kaempferol as described in chromatographic conditions and HPTLC fingerprinting for kaempferol section. The correlation coefficient (r or r^2) and standard deviation (SD) of the calibration curves were estimated to determine the linearity of method.

LOD and LOQ

Sensitivity of method was determined by evaluating LOD and LOQ. Various concentrations of standard solutions of kaempferol were applied along with methanol as blank. LOD was determined on the basis of signal to noise ratio (S/N) of 3:1 and LOQ was (S/N) of 10:1.

Specificity

Specificity of developed method was studied by assay and impurity technique. The aqueous extract fraction (EN6), standard kaempferol, mobile phase used for kaempferol and methanol used as a diluent were applied simultaneously on the HPTLC plate and linear ascending development with the solvent system, toluene: ethyl acetate: formic acid (6:4:1 v/v/v) in a 10.0x 10.0 cm twin trough glass chamber (Camag) formerly saturated with solvent system for 15 min at relative humidity (40%) and room temp. (25 ± 2°C). The development distance was 70.00 mm (development time 20 min with filter paper) and 20 ml solvent system was used. After development, plate was dried with a stream of hot air and densitometric scanning was performed at 254 nm in absorption-reflectance mode by using a Camag HPTLC scanner 3 and Camag visualizer with automatic digital camera linked to win-CATS software (Version 1.4.6). The spot of kaempferol in aqueous extract fraction (EN6) was confirmed by comparing R_f value and superimposed spectra.

Precision

Variability of method was studied by evaluating nine aliquots of standard solution containing 400 ng/spot of kaempferol on same day (Intra-day) and on different days (Inter-day). Precision was expressed as coefficient of variation (CV%) of calculated concentrations of each calibration level.

Reproducibility

Reproducibility of method was performed by evaluating nine aliquots of standard solution containing 400 ng/spot of kaempferol. Reproducibility was expressed as coefficient of variation (CV %) of measured concentrations of each calibration level.

Recovery

Recovery studies were performed by spiking known amounts of marker corresponding to 80%, 100% and 120% of kaempferol on the aqueous extract fraction (EN6). Each level was analyzed in triplicates. The recovery of kaempferol at different levels in aqueous extract fraction (EN6) fraction was calculated.

RESULTS

HPTLC fingerprinting and co-chromatography

The chromatograph of aqueous extract fraction (EN6) and kaempferol with their R_f values and colour of band resolved where taken into consideration for identification as shown in (Table 1 and Figure 2 and Figure 3). R_f value of peak of kaempferol (R_f 0.91) was closely compacted with R_f value of peak of aqueous extract fraction (EN6; R_f 0.90).

Quantification of kaempferol in aqueous extract fraction (EN6)

There was no report of quantification of kaempferol in aqueous extract fraction (EN6) of *E. neriifolia* leaves by using HPTLC technique. Therefore, we have developed a simple and precise method for quantification of kaempferol. HPTLC procedure was optimized by a view to quantify herbal extracts. The solvent system toluene: ethyl acetate: formic acid (6:4:1 v/v/v) gave better, sharp and well-defined peak resolution. Developed HPTLC plate of kaempferol and aqueous extract fraction (EN6) for quantification was scan at 254 nm before and 366 nm after derivatization. The percent content of kaempferol was quantified using proposed HPTLC densitometric method. It was found to be 0.024817% in aqueous extract fraction (EN6) for the 10 µl volume applied.

Validation of developed HPTLC method for kaempferol

Linear range

Linear range for kaempferol was 100-600 ng/spot with correlation coefficient 0.99834 (Table 2).

LOD and LOQ

LOD for kaempferol was100 ng and LOQ was 300 ng which indicated adequate sensitivity of the method. LOD and LOQ were determined from slope of the lowest part of calibration plot. This also indicated that proposed method exhibits a good sensitivity for quantification of kaempferol (Table 2).

Specificity

Proposed method was found to be specific for kaempferol quantification (Table 2).

Precision

The peak area was measured at nine different concentration levels which showed % CV for intra-day was 2.422 and inter-day was 2.522 at concentration of 400 ng/spot (Table 3). As per ICH guidelines, the acceptance criteria for precision were % CV ≤ 2% when

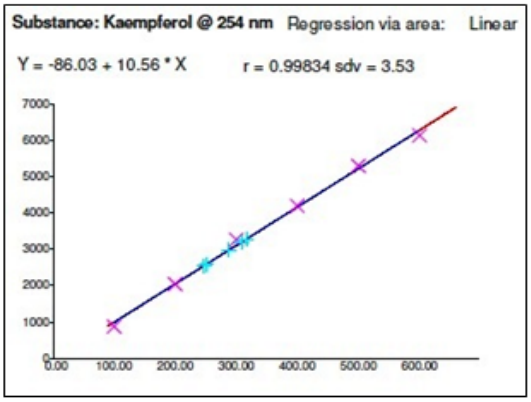

Figure 1: Calibration curve for standard kaempferol (*n*=6).

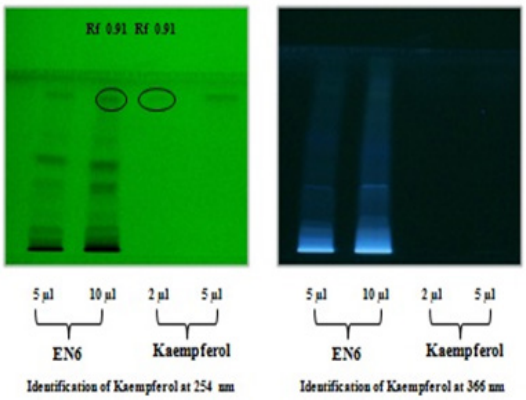

Figure 2: Scanned HPTLC plate using EN6 extract fraction and standard kaempferol at 254 nm and 366 nm

Table 1: R_f value and colour of band for identification of kaempferol in aqueous extract fraction (EN6) at 254 nm.

Samples	R_f value	Colour of band
Aqueous extract fraction (EN6)	0.90	Pink
Kaempferol	0.91	Dark blue

Table 2: Method validation parameters for quantification of kaempferol by proposed HPTLC densitometric method.

Parameters	Kaempferol
Scanned wavelength	254 nm
Linear range (ng/spot)	100-600
LOD (ng)	100
LOQ (ng)	300
Linearity (r or r^2)	0.99834
Specificity	Specific
Reproducibility (% CV, n=6)	2.1

Table 3: Intra-day and inter-day precision of kaempferol.

Marker	Concentration (ng/spot)	Intra-day precision*	Inter-day precision*
Kaempferol	400	2.422	2.522

*Coefficient of variation (% CV, *n*=9)

Table 4: Recovery studies of kaempferol at 80%, 100% and 120% addition by proposed HPTLC densitometric method.

Marker	Amount of marker spiked (%)	Amount of marker added (µl)	Recovery* (%)	Average recovery (%)
			Aqueous extract fraction (EN6)	Aqueous extract fraction (EN6)
Kaempferol	80	1.6	94.06	95.57
	100	2	96.21	
	120	2.4	96.44	

*average of three determinations

minimum of 6 determinations. But we have used nine different concentrations. Therefore, proposed method was found to be precise.

Reproducibility

The % CV was found to be 2.1(Table 2). Hence, proposed method was reproducible.

Recovery studies

The average percent recovery was 95.57% for aqueous extract fraction (EN6), which was within the acceptable limits (Table 4). Hence, proposed method was found to be accurate.

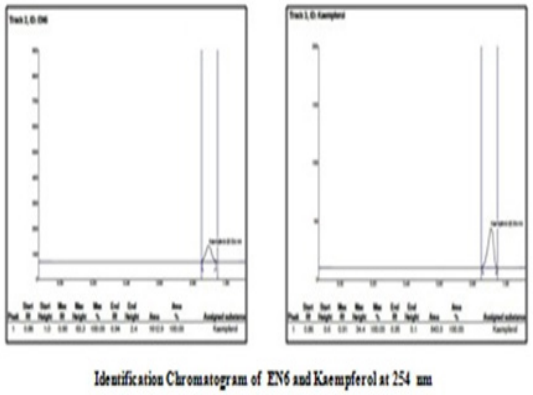

Figure 3: HPTLC chromatogram of EN6 extract fraction and standard kaempferol at 254 nm.

DISCUSSION AND CONCLUSION

HPTLC has potential to identify more compounds than HPLC, even though its poor resolution. In this view, the compounds that cannot be eluted still can be detected. Furthermore, the compounds having no U.V absorption e.g. sugar still can be detected by reagent spraying. HPTLC chromatogram pattern assessment seems to be promising for identification of active compounds in plant extracts. Hence, HPTLC can be used as a tool in quality control in order to assurance that the active compounds are extracted. Through data analysis system and optimized experimental conditions, HPTLC is also possible for the expansion of chromatographic fingerprint techniques to identify and determine composite of plant extracts as like G.C and HPLC.[27] In reference with the above statement, we have studied chromatographic fingerprinting, quantification and validation of HPTLC method for standard marker kaempferol in aqueous extract fraction (EN6) of *E. neriifolia* leaves. The results of HPTLC finger printing analysis showed that R_f value of peak of kaempferol (R_f 0.91) was closely compacted with aqueous extract fraction (EN6; R_f 0.90) which indicates the identification of kaempferol. The percent content of kaempferol was quantified and found to be 0.024817% in aqueous extract fraction (EN6) for the 10 µl volume applied. Linear range was found100-600 ng/spot with correlation coefficient 0.99834. LOD and LOQ were found 100 ng and 300 ng. The proposed method was specific. The % CV for intra-day was 2.422 and inter-day was 2.522 at concentration of 400 ng/spot. The % CV was found 2.1, hence the method was reproducible.

The average percent recovery was 95.57% in aqueous extract fraction (EN6), therefore, the method was found to be accurate. Therefore, the developed HPTLC method was found to be simple, specific, precise, reproducible and accurate. Here, the first time we have reported this HPTLC method for kaempferol in the aqueous extract fraction (EN6). This HPTLC method has provided sufficient information and parameters for complete identification and separation of kaempferol. Hereby, it is also confirmed that the presence of kaempferol in the aqueous extract fraction (EN6) was justified its recognition in primary phytochemical studies, fluorescence and colour reactions examination. However, there is a requirement to carry forward these studies by using some other standard markers in the aqueous extract fraction (EN6) of *E. neriifolia* leaves for exploring its phyto-constituents that could have therapeutic potential in various diseases.

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CONFLICT OF INTEREST

No conflicts of interest are declared.

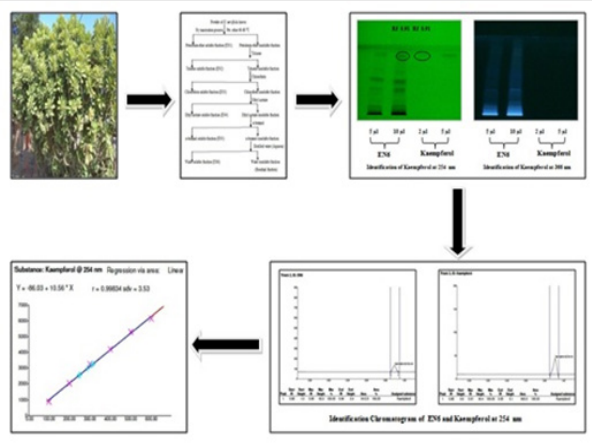
ABBREVIATIONS

HPTLC: High performance thin layer chromatography; **EN1:** Petroleum ether extract fraction of *E. neriifolia* leaves; **EN2:** Toluene extract fraction of *E. neriifolia* leaves; **EN3:** Chloroform extract fraction of *E. neriifolia* leaves; **EN4:** Ethyl acetate extract fraction of *E. neriifolia* leaves; **EN5:** n-butanol extract fraction of *E. neriifolia* leaves; **EN6:** Aqueous extract fraction of *E. neriifolia* leaves; **ICH:** International Conference on Harmonization; **LOD:** Limit of detection; **LOQ:** Limit of quantification; **%:** Percent / Percentage; **CV:** Coefficient of variation.

REFERENCES

1. Rathee D, *et al*. Simultaneous quantification of bergenin, (+)-catechin, gallicin and gallic acid and quantification of β-sitosterol using HPTLC from *Bergeniaciliata*(Haw.) Sternb. *Forma ligulata* Yeo (Pasanbheda). Pharm Anal Acta. 2010;1:104.
2. Kpoviessia DSS, *et al*. Validation of a method for the determination of sterols and triterpenes in the aerial part of *Justicia anselliana* (Nees) T. Anders by capillary gas chromatography. J Pharm Biomed Anal. 2008;48(4):1127-35.
3. Anonymous. The wealth of India. A dictionary of Indian raw materials and industrial products (raw materials), (D–E).New Delhi: Central Institute of Medicinal and Aromatic Plants. 2003;3.
4. Ved DK, Sureshchandra ST, Barve V, Srinivas V, Sangeetha S, Ravikumar K, *et al.* Plant details. Bangalore: FRLHT's ENVISCentre on Medicinal Plants. 2016. Available from:http://envis.frlht.org/plant_details.php?disp_id=936&parname=0
5. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd ed., Allahabad: Lal it Mohan Basu. 2006;3.
6. Bigoniya P, Rana AC. Protective effect of *Euphorbia neriifolia* saponin fraction on CCl4-induced acute hepatotoxicity. Afri J Biotech. 2010;9(42):7148-56.
7. Sharma V, Janmeda P, Paliwal R, Singh L, Sharma V, Sharma S. Anticarcinogenic potential of *E. neriifolia* leaves against n-nitrosodiethylamine-induced nephrotoxicity in mice. Biochem Cell Arch. 2011;11(2):393-8.
8. Sharma V, Janmeda P. Chemopreventive role of *Euphorbia neriifolia* (Linn) and its isolated flavonoid against n-nitrosodiethylamine-induced renal histopathological damage in male mice. Toxicol Int. 2013;20(1):101-7.
9. Janmeda P, Sharma V, Singh L, Paliwal R, Sharma S, *et al.* Chemopreventive effect of hydroethanolic extract of *Euphorbia neriifolia* leaves against DENA induced renal carcinogenesis in mice. Asian Pac J Cancer Prev. 2011;12(3):677-83.
10. Mansuri MI, Patel VM. Anti-diabetic potential of *Euphorbia neriifolia* Linn. alloxan induced diabetic rats. Journal of Pharmacy Research. 2012;5(5):2571-3.
11. Babar RS, Kataware UP, Mali NN, Patil SB, Naikwade NS. *In-vitro* cytotoxicity activity of *Euphorbia hirta*, *Euphorbia tirucalli* and *Euphorbia neriifolia* extract against B16F10 melanoma cell line. Inven Impact Ethnopharmacol. 2012;3(3). Available from: http://inventi.in/journal/article/impact/3/3174/ethnopharmacology/pi
12. Patil SB, Magdum CS. Determination of LC50 values of extracts of*Euphorbiahirta* Linn and *Euphorbia neriifolia* Linn. Using brine shrimp lethality assay. Asian J Res Pharm Sci. 2011;1(2):42-3.
13. Bigoniya P, Rana AC. Immunomodulatory activity of *Euphorbia neriifolia* leaf hydro-alcoholic extract in rats. Indian Drugs. 2008;45(2):90-7.
14. Gaur K, Rana AC, Chauhan LS, Sharma CS, Nema RK, Kori ML, *et al*. Investigation of immunomodulatory potential of *Euphorbia neriifolia* Linn. against betamethasone induced immunosuppression. Int J Pharmacog Phytochem Res. 2009;1(1):8-11.
15. Yadav RP, Patel AK, Jagannadham MV. Neriifolin S, a dimeric serine protease from *Euphorbia neriifolia* Linn.: Purification and biochemical characterization. Food Chemistry. 2012;132(3):1296-304.
16. Yadav RP, Patel AK, Jagannadham MV. Purification and biochemical characterization of a chymotrypsin-like serine protease from *Euphorbia neriifolia* Linn. Process Chem. 2011;46(8):1654-62.
17. Mallavadhani UV, Satyanarayana KV, Mahapatra A, Sudhakar AV. A new tetracyclic triterpene from the latex of *Euphorbia nerifolia*. Natural Product Research 2004;18(1):33-7.
18. Mallavadhani UV, Satyanarayana KVS, Mahapatra A, Sudhakar AVS, Narasimhan K, *et al*. Development of diagnostic microscopic and chemical markers of some *Euphorbia* Latexes. J Integrative Plant Biology. 2006;48(9):1115-21.
19. Ilyas M, Parveen M, Amin KM. Neriifolione, a triterpene from *Euphorbia neriifolia*. Phytochemistry. 1998;48(3):561-3.
20. Anjaneyulu V, Row LR. The crystalline principles of *Euphorbiaceae*. Part IV. Curr Sci. 1965;21:608-9.
21. Sharma V, Janmeda P. Extraction, isolation and identification offlavonoid from *Euphorbia neriifolia* leaves. Arab J Chem. 2014. http://dx.doi.org/10.1016/j.arabjc.2014.08.019.
22. Sharma V, Janmeda P. Chromatography fingerprinting profile studies on the flavonoids of *Euphorbia neriifolia* (Linn.) leaves. Int J Drug Dev Res. 2013;5(1):286-96.
23. Mali PY, *et al* .Pharmacognostical and physico-chemical standardization of *Euphorbia neriifolia* Leaves. Pharmacog J. 2017;9(5):696-705.
24. International conference on harmonization. Harmonized tripartite guideline. Validation of analytical procedures: text and methodology, Q2 (R1) Geneva. 2005.
25. International conference on harmonization. European medicines agency, ICH Topic Q2(R1) - Validation of analytical procedures: text and methodology (Step 5, June 1995, CPMP/ICH/381/95), UK. 1995.

PICTORIAL ABSTRACT



SUMMARY

The present study was a first report of HPTLC densitometric quantification of kaempferol in aqueous extract fraction (EN6) of leaves of *E. neriifolia*. The method has been validated by ICH guidelines for its linear range, linearity, LOD and LOQ, specificity, precision, reproducibility and recovery studies. The HPTLC finger printing analysis showed that R_f value of peak of kaempferol (R_f 0.91) was closely compacted with aqueous extract fraction (EN6; R_f 0.90) which indicates the identification of kaempferol. The percent content of kaempferol was quantified and found to be 0.024817% in aqueous extract fraction (EN6) for the 10 μl volume applied. The developed HPTLC method was found to be simple, specific, precise, reproducible and accurate.

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