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FINGERPRINT ANALYSIS FOR VALIDATION AND SIMULTANEOUS QUANTIFICATION OF QUERCETIN AND KAEMPFEROL IN *MORINGA OLEIFERA* BY RP-HPLC AND HPTLC

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Keywords:

Herbal formulations, Quercetin, *Moringa oleifera*, Kaempferol, RP-HPLC, Phytochemical analysis

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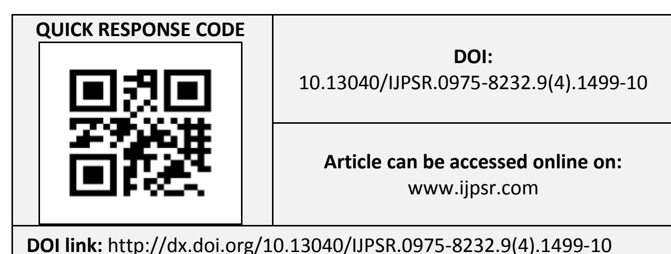
ABSTRACT: To establish authenticity and integrity for usage of herbal drugs quantitative analysis by sensitive analytical instrument is highly desirable. Quercetin and Kaempferol from whole plant of *Moringa oleifera* was chosen for simultaneous quantification by RP-HPLC and HPTLC method due to their pharmacological activities. HPTLC method of quercetin and kaempferol was developed on HPTLC plates using Toluene: Ethyl acetate: Chloroform: Formic acid (6:2:5:1.5) as mobile phase. Densitometric evaluation of flavonoids was performed at λ = 240 nm in absorbance mode. Furthermore, RP-HPLC method was developed using mobile phase containing acetonitrile and 0.1 M phosphate buffer (pH 2.5 adjusted by orthophosphoric acid) using C_{18} column in gradient mode. Chromatographic methods were validated as per ICH guidelines. Excellent linear relationships over the concentration range (1 - 40 µg/ml) were observed with the r^2 value higher than 0.999 for both the phyto-constituents by RP-HPLC. Limit of detection and quantification were found to be in range of 0.2 - 0.5 µg/ml with percent recovery of 98 - 101%; while r^2 value of quercetin and kaempferol were found to be 0.9764 and 0.9823 by HPTLC. The average recovery values for fruits of *Moringa oleifera* were within range of 98 - 101% by HPTLC. In conclusion, RP-HPLC method was found to be more precise and sensitive as compared to HPTLC for obtaining acceptable resolution between quercetin and kaempferol. Therefore, HPLC method can be used for the routine analysis of the quality of herbal extracts and formulations of *Moringa oleifera*.

INTRODUCTION: Phytochemical analysis is one of the tools to determine quality which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using advanced analytical techniques.



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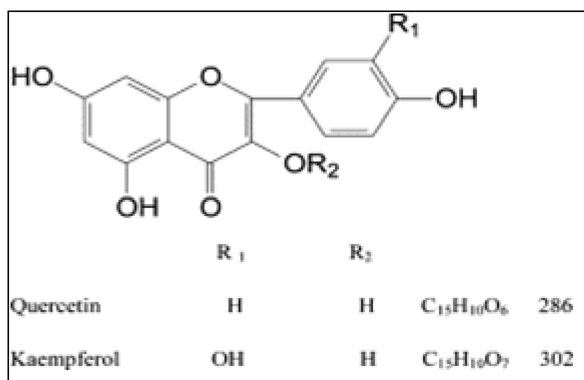
HPLC and HPTLC have been established as proficient tools for the phytochemical analysis and shown advanced sensitivity for analytical flexibility for phytoconstituents.

Such chromatographic analysis plays an important role in the pharmaceutical development, therefore, there is always a need to develop and validate the analysis method intended to be used for routine analysis [1, 2]. Routinely, plant extracts have been evaluated for their diverse and promising pharmacological properties, and therefore, development of methods for phytochemical analysis

is immensely important for various herbal formulations [3, 4, 5].

Moringa oleifera Lam. (syn. *M. pterygosperma* Gaerth) is one of the widely known, distributed and naturalized species of monogeneric family of Moringaceae **Fig. 1** [6, 7]. It is found in wild, can be cultivated throughout the plains, thrives best under the tropical weather, and is abundant near the sandy beds of rivers and streams [8]. All parts of the tree are believed to possess medicinal properties and its use has been well reported for the treatment of ascites, rheumatism, diabetes mellitus, venomous bites, and as cardiac and circulatory stimulant [9-11].

A



B

FIG. 1: (A) WHOLE PLANT OF DRUMSTICK (*MORINGA OLEIFERA*); (B) CHEMICAL STRUCTURE OF QUERCETIN AND KAEMPFEROL

The leaves contain more Vitamin A than carrots, greater calcium and iron than milk and spinach, and higher Vitamin C than citrus fruits like oranges, and more potassium than bananas, respectively, and that the protein quality of Moringa leaves rivals that of milk and eggs [12].

Leaves of Moringa plant have been reported to be a good source of natural antioxidants; and therefore, found to enhance the shelf-life of fat containing foods due to the presence of various types of antioxidant compounds such as flavonoids, ascorbic acid, phenolics and carotenoids [13, 14]. *Moringa oleifera* is also rich in compounds containing simple sugar, rhamnose and a unique group of compounds known as glucosinolates and isothiocyanates [15, 16]. The stem bark contains two alkaloids, known as moringine and moringinine [17]. Moreover, compounds like β-sitosterol, vanillin, β-sitostenone, 4-hydroxymellin and octaicosanoic acid have also been extracted from the stem of *M. oleifera* [18]. Flowers of *M. oleifera* is found to contain nine amino acids, D-glucose, sucrose, wax, traces of alkaloids, quercetin and kaempferol, while ash is rich in potassium and calcium [19]. They are also reported to contain flavonoid pigments such as alkaloids, kaempferol, isoquercitin, rhamnetin and kaempferitin. The flowers are known to be useful in treatment of inflammations and muscle diseases, hysteria, enlargement of spleen, reduce serum cholesterol, phospholipid, triglyceride, LDL, VLDL, cholesterol to phospholipid ratio, and increase the excretion of faecal cholesterol [20].

Quercetin is plant derived flavonoid abundantly present in the plant kingdom as a secondary metabolite and is the neatly defined group of polyphenolic compounds [21]. Pharmacologically, its role is found as an antioxidant, anticancer and neuroprotective [22]. In phase I clinical trials, it has been reported to have inhibitory effect on tyrosine kinase which indicates its potential as an antitumor agent [23]. Kaempferol which is a member of flavonols is richly found in tea, broccoli, beans and berries. Pharmacologically, it is reported to exert different types of mechanisms in the regulation of cancer cells [24, 25]. It induces potent apoptosis and modifies a host of cellular signalling pathways [26]. Furthermore, it is reported to be less toxic to normal cells as compared to standard chemotherapeutic agents [27].

Recently, high performance liquid chromatography (HPLC) has been frequently used for fingerprint analysis for the quality control of herbal plants [28]. It is a highly versatile, simple, robust and widely used technique for the isolation of natural compounds which has been used in phytochemical and analytical chemistry to quantify, identify and purify the individual components from the mixture. Reversed-phase HPLC (RP-HPLC) is the one of the most commonly used separation techniques in

HPLC due to its wide range applications accounting for approximately 65% of all HPLC separations. Its popularity is due to its ability to handle compounds with diverse polarity and molecular mass, such as, secondary plant metabolites [29, 30].

High performance thin-layer chromatography (HPTLC) has also been emerged as a vital analytical tool for the qualitative and quantitative phytochemical analysis of the herbal drugs and formulations. HPTLC, also recognized as planar chromatography has a greater separation power and reproducibility compared to classical thin-layer chromatography (TLC) [31, 32]. Main difference between TLC and HPTLC is particle and pore size of the sorbents.

The present work carries out simultaneous fingerprinting estimation and validation of quercetin and kaempferol in different parts of *Moringa oleifera* using HPTLC and RP-HPLC method. The proposed methods were validated as per ICH guidelines [33].

MATERIALS AND METHODS:

Materials: Methanol (AR grade), toluene, chloroform, ethyl acetate, formic acid and potassium phosphate were purchased from Qualigens Fine chemicals, Mumbai, India. Quercetinand Kaempferol standards were obtained from Sigma Aldrich (USA) with purity found to be ≥ 98% and ≥ 90% by HPLC, respectively. Silica gel 60 F254 TLC plates with plate dimension 20 × 20 cm, 0.25 mm thickness were manufactured by Merck, Germany. Acetonitrile (HPLC grade) was purchased from HiMedia laboratories, Pvt. Ltd.

Plant Material: Leaves, Flowers and fruits of *Moringa oleifera* were collected from one local nursery at Surat, Gujarat, India. All the three part of the plant were authenticated by Dr. M. N. Reddy, Department of Biosciences, Veer Narmad South Gujarat University, Surat.

Method of Extraction: The fresh leaves, fruits and flowers of *Moringa oleifera* were dried under shade and were powdered using electrical grinder. Extraction was carried out by cold maceration method [34]. Approximately dried powder of leaves (300 gm), fruits (1 kg), and flowers (250 gm) were extracted by 5 times volume using methanol for 48 hours with intermittent shaking. The extract was collected, filtered and evaporated at less than 50 °C under reduced pressure in a rotary evaporator and was stored in a desiccator in a dry place. Extracts collected were weighed and the percent extractive values of different extracts are mentioned in **Table 1**.

TABLE 1: PERCENT EXTRACTIVE VALUES OF LEAVES, FRUITS AND FLOWERS OF *MORINGA OLEIFERA*

Moringa oleifera part	Methanolic extract (% w/w)
Leaves	8.9%
Flowers	15.4%
Fruits	5.9%

Preparation of Standard and Sample Solutions: Stock solutions (10 mg/ml) of a mixture of quercetin and kaempferol were prepared by dissolving 1 mg of standard compounds in 100 ml methanol and were sonicated for 10 minutes. 100mg of methanolic extract of dried leaves, fruits and flowers were dissolved individually in 1 ml methanol and filtered through 0.45 µ membrane filter to get sample solutions with concentration of 100 mg/ml. This solution was used for all the validation parameters for HPLC and HPTLC.

HPTLC Working Condition: Toluene-Ethylacetate - Chloroform-Formic acid (6:2:5:1.5) was used as a mobile phase for TLC separation of quercetin and kaempferol. A glass through chamber (20×10×4 cm) was saturated for 20 minutes with the stated mobile phase. The standard and sample solutions were spotted manually as bands by using capillary tubes. HPTLC was performed on 100×100 mm aluminium backed plates coated with 0.2 mm layers of silica gel 60 F254 (Merck, Mumbai, **Fig. 2**).

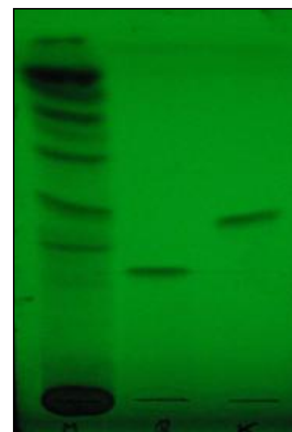


FIG. 2: PLATE DEVELOPED BY TLC OBSERVED AT 254 nm (BAND 1 TO 3 FROM LEFT TO RIGHT)
Band 1: *Moringa Oleifera* methanol extract of leaves;
Band 2: Quercetin standard; Band 3: Kaempferol Standard.

Standard and sample solutions of quercetin and kaempferol were applied on the plate size of bands 8 mm wide, 10 mm from the bottom edge of the chromatographic plate and 6 mm apart using a DESAGA applicator AS30 sample applicator quipped with a 100 µl Hamilton syringe (USA). Ascending development to a distance of 80 mm was performed at room temperature with Toluene-Ethyl acetate-Chloroform-Formic acid (6:2:5:1.5 v/v) as mobile phase **Fig. 3** in a DESAGA glass twin-trough chamber previously saturated with mobile phase vapor for 20 minutes. After development, the plates were dried in air and scanned at 240 nm with a HPTLC Densitometer CD 60 with ProQuant software using a deuterium lamp.

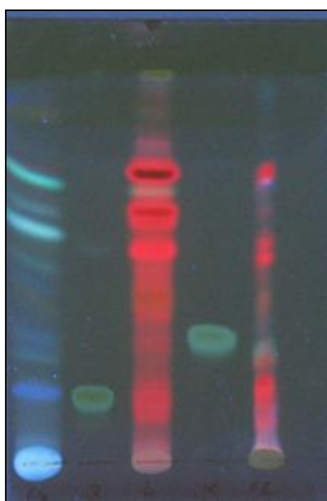


FIG. 3: PLATE DEVELOPED BY HPTLC OBSERVED AT 366 nm (BAND 1 TO 5 FROM LEFT TO RIGHT)
Band 1: *Moringa Oleifera* methanol extract of fruits;
Band 2: Quercetin standard;
Band 3: *Moringa Oleifera* methanol extract of leaves;
Band 4: Kaempferol Standard;
Band 5: *Moringa Oleifera* methanol extract of flowers.

HPLC Working Conditions: Quercetin and kaempferol was separated by using RP-HPLC chromatographic analysis. Analytical column SB-C18 with 4.6×150 mm was used for the separation. The mobile phase used was a mixture of acetonitrile and 0.01N potassium phosphate buffer pH 2.5 adjusted with orthophosphoric acid in the proportion of (time, % B): 0, 10; 8, 50; 15, 55; 18, 10; 19, 10 (gradient system). The flow rate of mobile phase was set at 1 ml/min and sample volume was adjusted to 20 µl. All the separations were carried out at a room temperature and were detected at wavelength of 240 nm.

Method Validation:

Specificity: The specificity of the method was determined by analyzing standard and sample solutions. The concentration of quercetin and kaempferol in sample were confirmed by comparing the UV spectra of the sample with standard. The peak purity of quercetin and kaempferol was analyzed by comparing the spectra at three different levels, *i.e.*, peak start, peak middle and peak end positions of the sample.

Linearity:

HPTLC Conditions: Calibration curves of quercetin and kaempferol (10 µg/ml) were obtained by applying different volumes of the standard mixture solution (10, 20, 40, 80 and 100 µl) to obtain concentrations of 100, 200, 400, 800 and 1000 ng/band of quercetin and kaempferol respectively. A sample solution of methanolic extract (100 mg/ml) of fruit, leaves and flowers of *Moringa oleifera* were prepared in methanol and 10µl of this solution was spotted as a band (n = 3). The densitograms were recorded and mean peak areas (Y-axis) of quercetin and kaempferol were plotted against the corresponding concentrations (X-axis).

HPLC:

HPLC Conditions: 20µl of different concentrations of the standard mixture solution were injected to obtain the calibration curves of quercetin and kaempferol (1, 2.5, 5, 10, 20 and 40 µg/ml). A sample solution of methanolic extract of leaves, fruits and flowers (100 mg/ml) of *Moringa oleifera* were prepared in methanol and 20µl of this solution was injected for analysis (n = 3). The chromatograms were obtained and mean quercetin and kaempferol peak areas (Y-axis) were plotted against the corresponding concentrations (X-axis).

Limit of Detection and Quantification: The detection limit of an individual analytical procedure is the lowest amount of analyte which can be detected in a sample but not necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is particularly

used for the determination of impurities and/or degradation products. The limit of detection (LOD) and limit of quantification (LOQ) were determined as per the ICH guidelines using following formulae for calculating LOD and LOQ:

$$LOD = 3.3\ \sigma/S$$
$$LOQ = 10\ \sigma/S$$

Where, σ = standard deviation of the response and S = slope of calibration curve.

Precision:

HPTLC Conditions: Intraday precision was evaluated by analysis of six replicate applications of three concentrations at 100, 120 and 140 ng/band obtained by applying three different volumes *i.e*., 10, 12 and 14 µl of freshly prepared solutions of both the standards *i.e*., quercetin and kaempferol (10 µg/ml) on the same day. Interday precision was analyzed with the same concentrations as mentioned above on two different days.

HPLC Conditions: Intraday precision was analyzed by six replicated injections of both the standards, *i.e*., quercetin and kaempferol at three different concentrations mixtures, *i.e*., 5, 10 and 20µg/ml on the same day. However, for interday precision, six replicated injections of standard solution mixtures were injected on two different days.

Sample Analysis and Recovery:

HPTLC and HPLC Conditions: The accuracy of analytical method for HPTLC was determined by performing recovery experiments at three different levels using the standard addition method. Known volume of quercetin and kaempferol standards (100, 200 and 400 ng/spot) were spiked by 10 µl (100 mg/ml) of methanolic extract of *Moringa oleifera*. The plate was developed under similar conditions mentioned earlier. The average values of percent recovery for quercetin and kaempferol were calculated respectively and the procedure was repeated in triplicates. For HPLC conditions, the concentrations of quercetin and kaempferol standards were kept at 0.01, 0.005 and 0.0025 mg and rest of the method was followed as mentioned earlier.

RESULTS AND DISCUSSION:

Optimization of the HPTLC and HPLC Conditions: Initial optimization experiments were performed using previously known mobile phase systems for flavonoids separation. Combination of toluene: ethyl acetate with ration of 9:1 (v/v) [16] was employed which had led to poor resolution of the standards and phytoconstituents present in the extract. Therefore, mobile phase was adapted by including formic acid and chloroform in various proportions. Out of the various combinations evaluated, A:B:C:D; where A is toluene, B is ethyl acetate, C is chloroform and D is formic acid, ration of 6:2:5:1.5 (v/v) gave the best resolution for quercetin (R_f = 0.35) and kaempferol (R_f = 0.48). TLC analysis was performed by spotting two standards, *i.e.* quercetin and kaempferol as well as sample extracts of all the three parts of *Moringa oleifera* on the same TLC plate. From the extracts of different parts of the herb, only leaves and flowers showed some presence of both compounds, *i.e.,* quercetin and kaempferol **Fig. 4** and **6**. *Moringa oleifera* fruits showed presence of quercetin only **Fig. 5**.

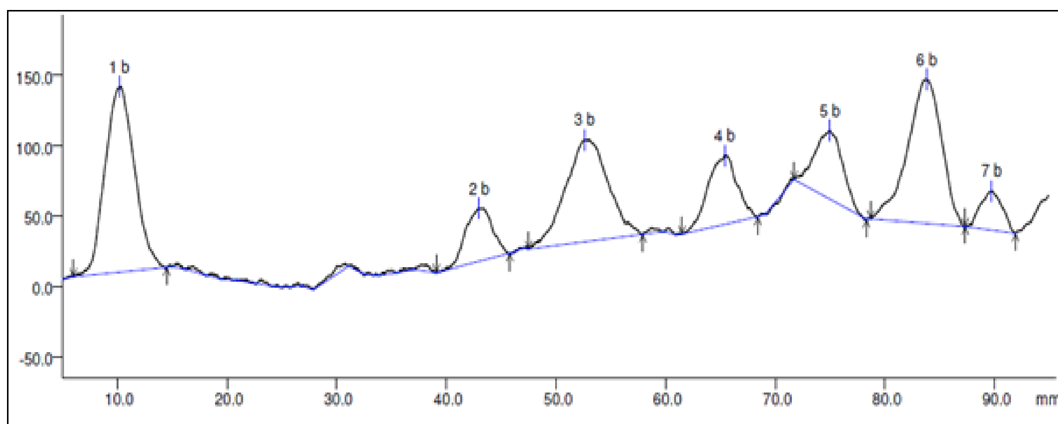


FIG. 4: CHROMATOGRAM REPRESENTING PEAKS FOR QUERCETIN AND KAEMPFEROL IN METHANOLIC EXTRACT OF LEAVES OF *MORINGA OLEIFERA*

2b-Quercetin peak, 3b-Kaempferol peak

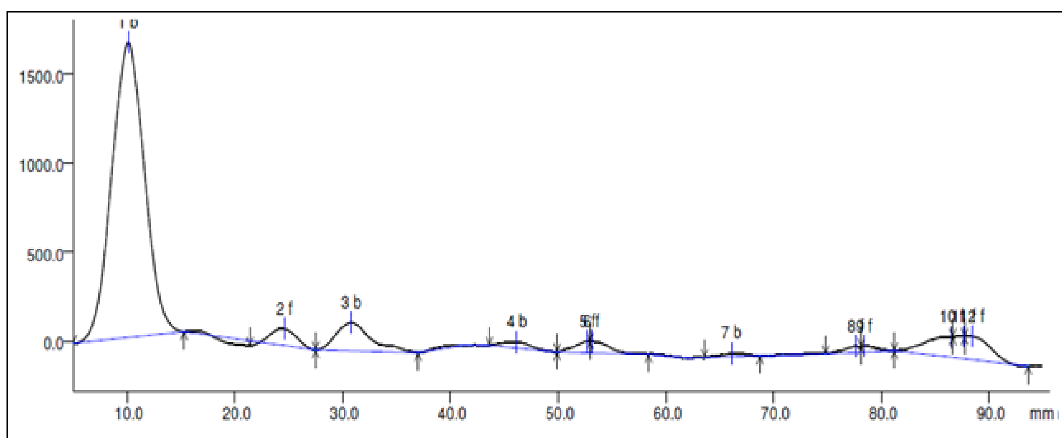


FIG. 5: CHROMATOGRAM REPRESENTING PEAK FOR QUERCETIN IN METHANOLIC EXTRACT OF FRUITS OF *MORINGA OLEIFERA*
4b-Quercetin peak

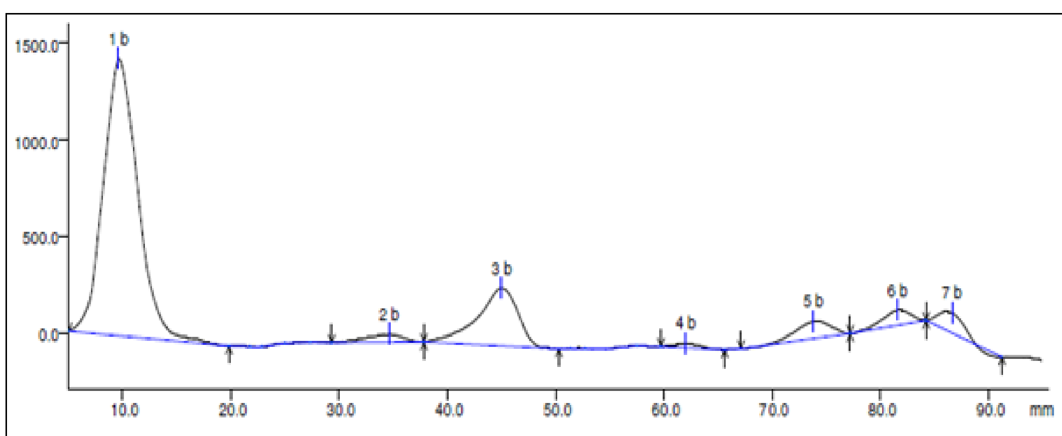


FIG. 6: CHROMATOGRAM REPRESENTING PEAKS FOR QUERCETIN AND KAEMPFEROL IN METHANOLIC EXTRACT OF FLOWERS OF *MORINGA OLEIFERA*
2b-Quercetin peak; 3b-Kaempferol peak

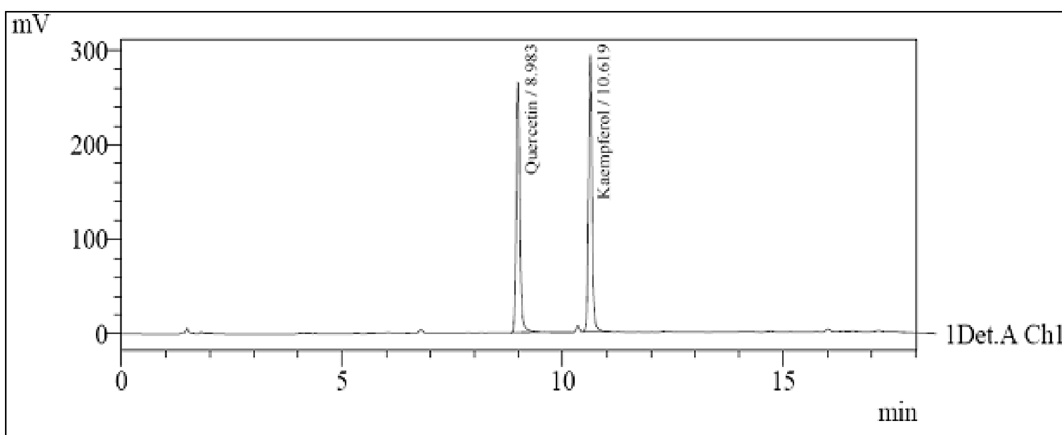


FIG. 7: CHROMATOGRAM OF MIXTURE OF STANDARDS QUERCETIN AND KAEMPFEROL

Separation on RP-HPLC was carried out using a gradient program with mobile phase of acetonitrile and 0.01 N potassium phosphate buffer pH 2.5. Good separation of flavonoids (quercetin and kaempferol) was observed **Fig. 7** with retention time of about 8.98 minutes and 10.61 minutes respectively. All the peaks were found to be sharp and symmetrical with good resolution. Also, methanolic extract of leaves and flowers showed presence of quercetin and kaempferol with retention time of about 9 minutes and 10 minutes respectively at 240 nm **Fig. 8** and **9**, while, methanolic extract of fruits showed presence of quercetin with retention time of 9 minutes **Fig. 10**.

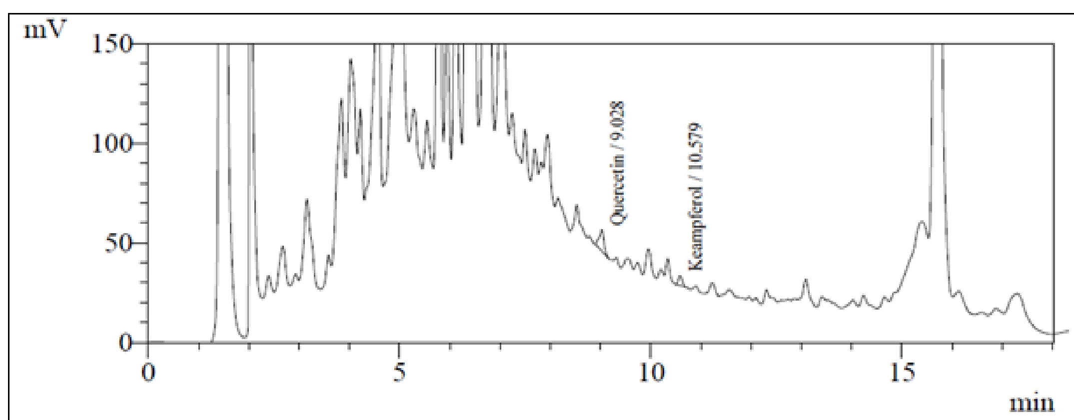


FIG. 8: CHROMATOGRAM OF *MORINGA OLEIFERA* LEAVES METHANOLIC EXTRACT

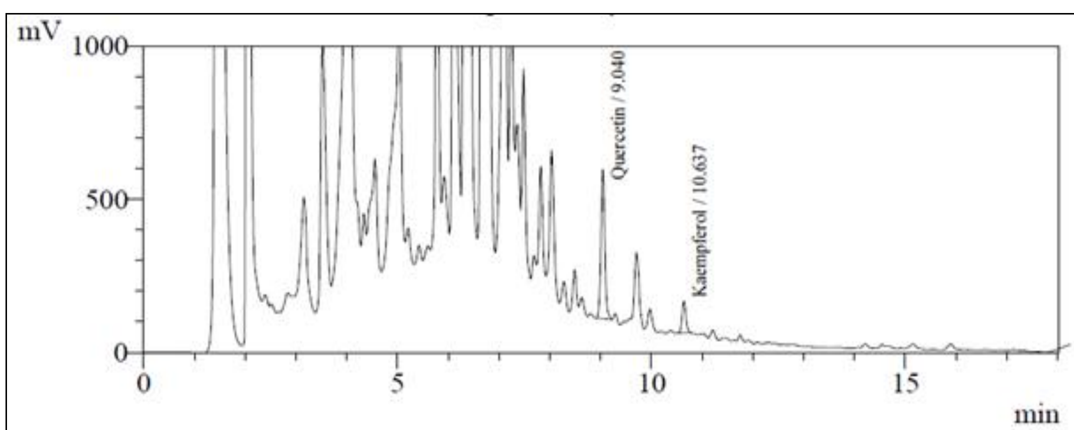


FIG. 9: CHROMATOGRAM OF *MORINGA OLEIFERA* FLOWERS METHANOLIC EXTRACT

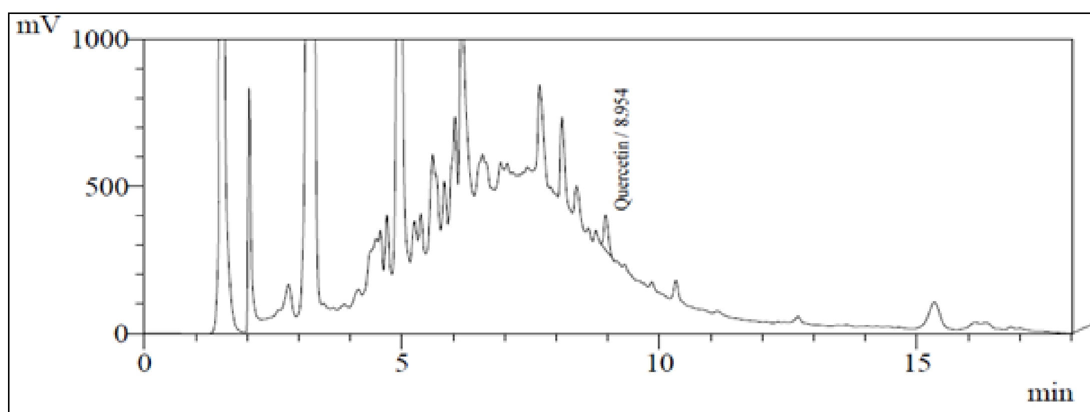
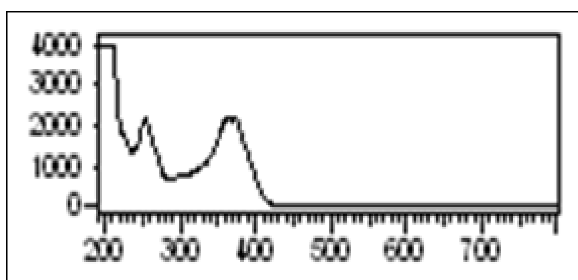


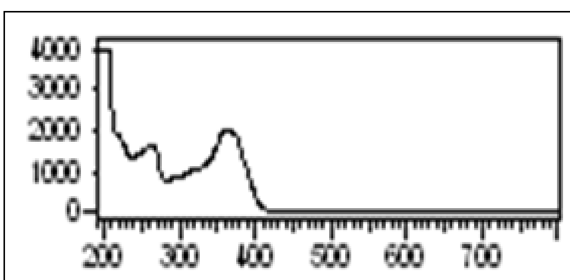
FIG. 10: CHROMATOGRAM OF *MORINGA OLEIFERA* FRUITS METHANOLIC EXTRACT

Method Validation:

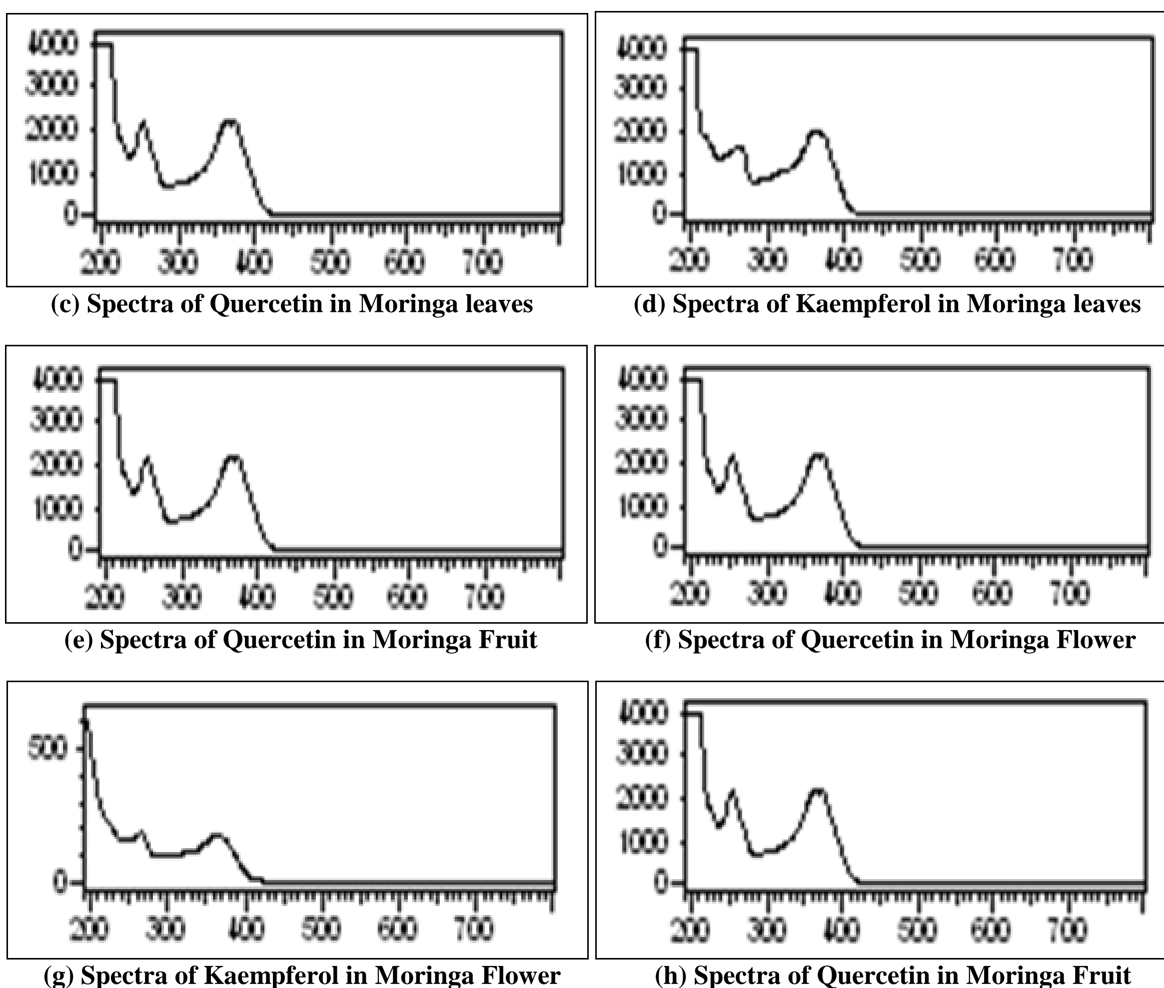
Specificity: Presence of quercetin and kaempferol in the methanolic extract of fruits, leaves and flowers of *Moringa oleifera* was confirmed by comparing their UV absorption spectra with those of the standards **Fig. 11**.



(a) Spectra of Quercetin standard



(b) Spectra of Kaempferol standard



(c) Spectra of Quercetin in Moringa leaves

(d) Spectra of Kaempferol in Moringa leaves

(e) Spectra of Quercetin in Moringa Fruit

(f) Spectra of Quercetin in Moringa Flower

(g) Spectra of Kaempferol in Moringa Flower

(h) Spectra of Quercetin in Moringa Fruit

FIG. 11: UV SPECTRA OF THE STANDARDS (A) AND (B) AND SAMPLES (C) TO (H)

Linearity and Detection Limits:

HPTLC and HPLC: The linear regression analysis of HPTLC for the calibration plots showed a linear relation within concentration range of 100 - 1000 ng/band with a correlation coefficient (R^2) of 0.9764 and 0.9823 for quercetin and kaempferol, respectively **Fig. 12 - 14**. LOD for quercetin and kaempferol was found to be 54.29 and 68.45 ng, respectively; and LOQ was found to be 164.51 and 207.41 ng, respectively.

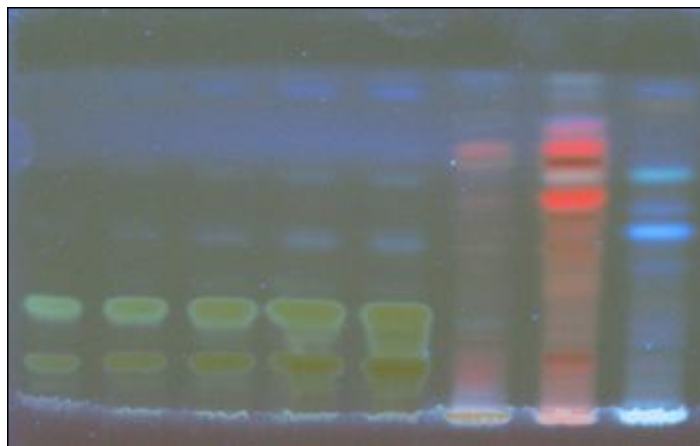


FIG. 12: LINEARITY PLATE DEVELOPED BY HPTLC OBSERVED AT 366 nm (BAND 1 TO 8 FROM LEFT TO RIGHT)
Band 1: Quercetin and Kaempferol Standard 100ng; Band 2: Quercetin and Kaempferol Standard 200ng;
Band 3: Quercetin and Kaempferol Standard 400ng; Band 4: Quercetin and Kaempferol Standard 800ng;
Band 5: Quercetin and Kaempferol Standard 1000ng; Band 6: *Moringa Oleifera* flowers methanolic extract;
Band 7: *Moringa Oleifera* leaves methanolic extract, Band 8: *Moringa Oleifera* fruits methanolic extract.

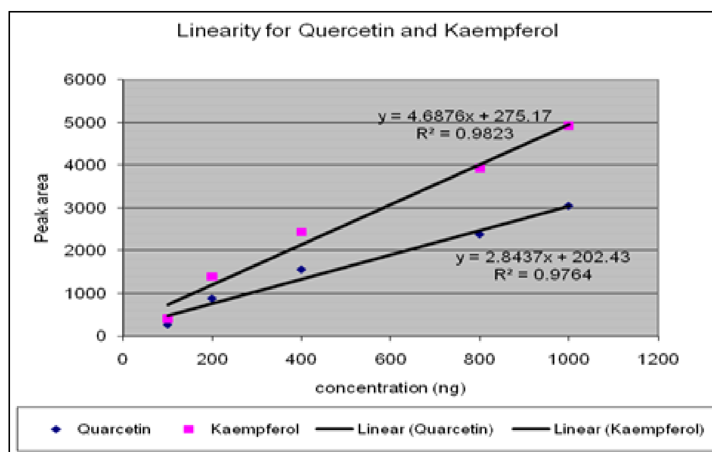


FIG. 13: RESULTS FROM LINEAR REGRESSION ANALYSIS (CALIBRATION STANDARDS) – QUERCETIN AND KAEMPFEROL BY HPTLC

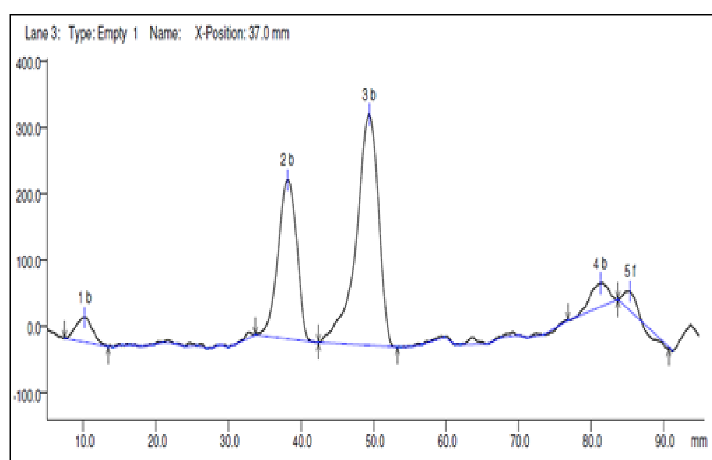


FIG. 14: CHROMATOGRAM REPRESENTING QUERCETIN PEAK - 2B, KAEMPFEROL PEAK - 3B, CONCENTRATION - 140 ng

In case of HPLC, calibration plots showed a linear relationship in the concentration range of 0.001 - 0.05 mg/ml with R^2 of 0.9994 for both quercetin and kaempferol **Fig. 15** and **16**. LOD for quercetin and kaempferol was found to be 0.164 and 0.197 µg/ml, while; LOW was found to be 0.289 and 0.816 µg/ml, respectively. The percentage amount of quercetin and kaempferol based on HPTLC and HPLC present in methanolic extract of fruits, flowers and leaves of *Moringa oleifera* is shown in **Table 2** and **3**, respectively. The method showed lower limit of detection which was found to be 5-10 times lower than the HPLC method reported by Dubber MJ *et al.*, [35] for quercetin and kaempferol, thus, the method was found to be highly sensitive.

TABLE 2: HPTLC VALIDATION DATA FROM CALIBRATION CURVES OF QUERCETIN AND KAEMPFEROL IN METHANOLIC EXTRACT OF *MORINGA OLEIFERA* (n = 3)

Plant part	Quercetin				Kaempferol			
	R^2	LOD (ng)	LOQ (ng)	Content % w/w	R^2	LOD (ng)	LOQ (ng)	Content % w/w
Drumstick leaves	0.9764	54.29	164.51	0.52	0.9823	68.45	207.41	0.74
Drumstick flowers				0.64				0.222
Drumstick fruits				0.13				-

TABLE 3: HPLC VALIDATION DATA FROM CALIBRATION CURVES OF QUERCETIN AND KAEMPFEROL IN METHANOLIC EXTRACT OF *MORINGA OLEIFERA* (n = 3)

Plant part	Quercetin				Kaempferol			
	R^2	LOD (µg/ml)	LOQ (µg/ ml)	Content % w/w	R^2	LOD (µg/ml)	LOQ (µg/ml)	Content % w/w
Drumstick leaves	0.9994	0.164	0.497	0.567	0.9994	0.289	0.816	0.402
Drumstick flowers				0.44				0.61
Drumstick fruits				0.15				-

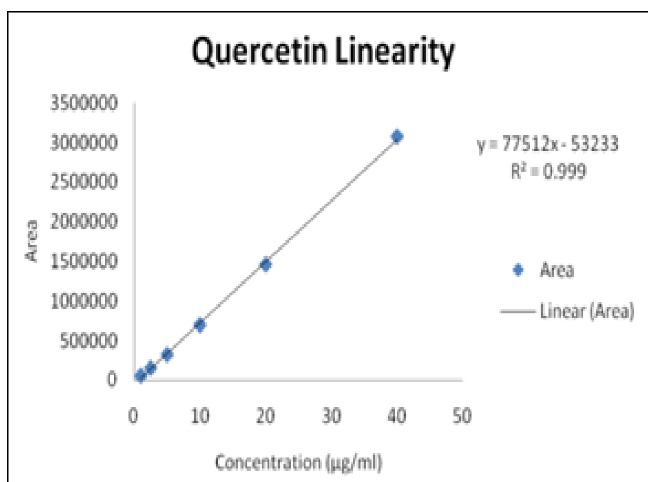


FIG. 15: RESULTS FROM LINEAR REGRESSION ANALYSIS (CALIBRATION STANDARDS) – QUERCETIN BY HPLC

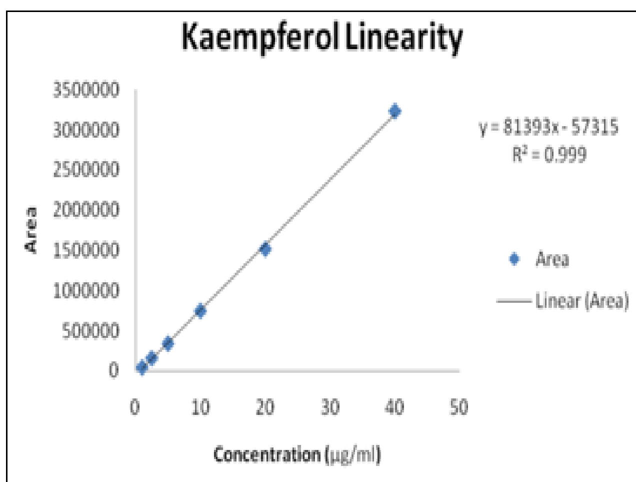


FIG. 16: RESULTS FROM LINEAR REGRESSION ANALYSIS (CALIBRATION STANDARDS) – KAEMPFEROL BY HPLC

Precision:

HPTLC and HPLC: For HPTLC, intraday and interday precisions expressed as relative standard deviation (RSD) for peak area was determined by repeated analysis (n = 6) for both standards of quercetin and kaempferol. Intraday relative standard deviation for quercetin was found to be between 5.34 and 6.53% and for kaempferol between 5.05 - 11.36%. Interday RSD of quercetin was between 2.10 - 6.64% and for kaempferol; it was found to be between 2.60 - 5.79%. The results showed that intraday and interday RSDs for peak area are within acceptable limits **Table 4**.

TABLE 4: INTRADAY AND INTERDAY PRECISION USING HPTLC METHOD (n = 6)

Parameters	Concentration (ng)					
	100		120		140	
	Quercetin	Kaempferol	Quercetin	Kaempferol	Quercetin	Kaempferol
	Intraday precision					
% RSD	5.37	11.36	6.53	8.13	5.74	5.04
	Interday precision					
% RSD	2.09	3.46	6.94	2.60	4.92	5.80

In case of HPLC **Table 5** as well, the results showed that relative standard deviations for retention time and for peak area are quite low and were found to be well within the acceptable criteria.

TABLE 5: INTRADAY AND INTERDAY PRECISION USING HPLC METHOD (n = 6)

Parameters	Concentration (µg/ml)					
	5		10		20	
	Quercetin	Kaempferol	Quercetin	Kaempferol	Quercetin	Kaempferol
	Intraday precision					
% RSD	0.519426	0.78576	1.786636	1.289036	2.796165	2.040243
	Interday precision					
% RSD	2.8	4.7	2.1	1.5	2.8	2.2

Sample Analysis and Recovery:

HPTLC and HPLC: The recovery experiments of HPTLC for quercetin and kaempferol were performed by spiking standards at known concentration in the methanolic extract of *Moringa oleifera* in triplicate as per the procedure described earlier. The % recoveries of the flavonoids are presented in **Table 6**. Similarly, for HPLC, the procedure for recovery experiments was repeated in triplicates as mentioned earlier. The recoveries for both the flavonoids found within acceptable limits are mentioned in **Table 7**.

TABLE 6: PERCENT RECOVERY OF QUERCETIN AND KAEMPFEROL FROM THE METHANOLIC EXTRACT OF LEAVES, FRUITS AND FLOWERS OF *MORINGA OLEIFERA* USING HPTLC METHOD (n = 3)

Plant part	Quercetin	Kaempferol
Leaves	98.59	90.08
Flowers	95.10	97.08
Fruits	99.12	-

TABLE 7: PERCENT RECOVERY OF QUERCETIN AND KAEMPFEROL FROM THE METHANOLIC EXTRACT OF LEAVES, FRUITS AND FLOWERS OF *MORINGA OLEIFERA* USING HPLC METHOD (n = 3)

Plant part	Quercetin	Kaempferol
Leaves	98.03	98.80
Flowers	95.90	100.44
Fruits	99.64	-

CONCLUSION: An accurate and sensitive HPTLC and RP-HPLC method was developed for simultaneous qualitative and quantitative analysis of combination of quercetin and kaempferol in the extract of *Moringa oleifera*. Moreover, RP-HPLC method can also be used as quality control of quercetin and kaempferol in the extract of *Moringa oleifera* with greater accuracy and precision. The average recoveries were found to be higher with the current method. This method was found to be more precise and has potential to successfully analyze commercially available solid oral dosage forms of *Moringa oleifera* and to assay other pharmaceutical herbal dosage forms of quercetin and kaempferol.

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