Quantitative analysis of biomarker rutin in different species of genus *Ficus* by validated NP and RP-HPTLC methods

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Abstract: Biomarker rutin was analyzed in methanol extracts of leaves of five different species of genus *Ficus* (*Ficus carica*, *Ficus nitida*, *Ficus ingens*, *Ficus palmata* and *Ficus vasta*) by NP-HPTLC (Method I) and RP- HPTLC methods (Method II). The development and validation for method I was carried out with silica gel $60F_{254}$ plates using EA: GAA: FA: H_2O (10:1:1:2.5, v/v/v/v) as developing system. Method II was carried out on silica gel $60F_{254}$ RP-18 plates using mobile phase ACN: H_2O (4:6 v/v). Both analyses were scanned at 305 nm and were found to give well resolved peak of rutin at R_f 0.28±0.01 and 0.68±0.03 for Method I and Method II, respectively. The percentage of rutin was found to be 0.51% & 0.66% in *F. ingens*, 0.24% & 0.54% in *F. palmata* and 0.14% & 0.17% in *F. vasta* by Method I & Method II, respectively. Method II (RP-HPTLC) was found to be more accurate, precise and sensitive than Method I. Method II can be used as an important tool for standardization and quality control of bulk drugs and in-process formulations of rutin.

Keywords: Ficus species, rutin, NP& RP-HPTLC, validation.

INTRODUCTION

In recent years, because of its several benefits like low operation cost, high sample throughput and need for minimum sample clean up, the high performance thin layer chromatography (HPTLC) has become a conventional analytical approach for the quality control of herbal drugs (Alajmi *et al.*, 2013). It is widely used for the identification, assay, stability studies, uniform testing of raw materials and formulated products (Alam *et al.*, 2014).

The genus *Ficus* includes about 800 species of woody trees, shrubs and vines and their fruits generally known as figs (Harrison, 2005), distributed widely in tropical and subtropical regions (Hutchinson *et al.*, 1958). From ancient time figs were used as food and traditional medicine, contains laxative substances, flavonoids, sugars, vitamins A and C, acids and enzymes (Ronsted *et al.*, 2007). The various phytoconstituents reported in the genus *Ficus* are coumarins (Chunyan *et al.*, 2009), furocoumarin glycosides (Chang *et al.*, 2005), isoflavones (Kuo and Li, 1997), lignans (Li and Kuo, 2000), sterols and/or terpenes (Kuo and Chaiang, 1999). *Ficus* species have been used in folk medicine as anticancer and anti-inflammatory (Lansky *et al.*, 2008), antiepileptic (Noumi and Fozi, 2003) and as antioxidant (Caliskan and Polat, 2011).

Rutin (Fig. 1) is a naturally occurring flavonoid of the flavonol type widely distributed in many plants and serves as an important dietary constituent of several foods and plant-based beverages (Kuntic *et al.*, 2007). Rutin was reported to posses antioxidant (La Casa *et al.*, 2000), antitumor (Alonso-Castro *et al.*, 2013) and hepatoprotective (Khan *et al.*, 2012) activity. Rutin was isolated from several species of genus *Ficus* i.e. *Ficus carica* (Teixeira *et al.*, 2006), *Ficus ruficaulis* (Chang *et al.*, 2005), *Ficus indica* (Galati *et al.*, 2003). The quantitative estimation of rutin was done by HPLC (Carrillo-Lopez *et al.*, 2013), RP-HPLC (Fang *et al.*, 2013; Shen *et al.*, 2012), HPTLC (Soponar *et al.*, 2010; Pereira *et al.*, 2004) and RP-HPTLC (Bhandari *et al.*, 2007) in plant extracts, commercial formulations and biological fluids, but a validated HPTLC method has been not yet reported for the quantification of rutin in the different species of genus *Ficus*. Therefore, in this experiment we tried to develop and validate two sensitive and cost effective High performance thin layer chromatographic methods (Method I & II) for the analysis of rutin in methanol extracts of leaves of five species (*F. carica*, *F. nitida*, *F. ingens*, *F. palmata* and *F. vasta*) of genus *Ficus* grown in Kingdom of Saudi Arabia. The proposed method was validated as per ICH guideline, 1996.

MATERIALS AND METHODS

Material

About 500g leaves of *five* species of the genus *Ficus,* i.e., *F. carica* (sample 1) (voucher No. 15399; collected on 25/03/2008), *F. nitida* (sample 2) (voucher No. 14964; collected on 17/03/2006), *F. ingens* (sample 3) (voucher No. 15370; collected on 16/03/2009), *F. palmata* (sample 4) (voucher No. 15362; collected on 25/03/2008) and *F. vasta* (sample 5) (voucher No. 15046; collected on 15/03/2009), were collected from the southern region of Kingdom of Saudi Arabia and authenticated by Dr.

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Mohammed Yusuf, Taxonomist, Medicinal Plant Collection and Survey Unit, Pharmacognosy Department, College of Pharmacy, KSU, Riyadh (KSA). Specimens of the plants were deposited in the herbarium of Pharmacognosy Department of College of Pharmacy, KSU, Riyadh (KSA).

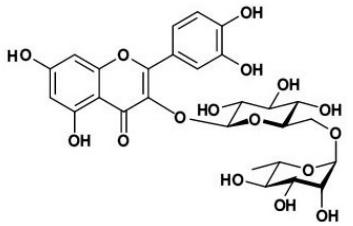

Fig. 1

Apparatus and reagents

Standard rutin was obtained from Sigma Aldrich. Analytical grade reagents and solvents (EA, GAA, FA and ACN) were purchased from WINLAB and BDH (U.K.). Glass-backed silica gel 60F_{254} (for method I) and Glass-backed RP-18 silica gel 60 F_{254} (for method II) HPTLC precoated plates (20×10cm) were procured from Merck (Darmstadt, Germany). The standard and the extracts were applied to HPTLC plates band wise with the help of CAMAG automatic TLC sampler-4 (CAMAG, Switzerland) and developed in ADC2 (automatic development chamber) (CAMAG, Muttenz, Switzerland). TLC Plates were then documented by CAMAG TLC Reprostar 3 and scanned by CATS 4 (CAMAG).

Preparation of standard stock solution

Stock solution of standard (Rutin) (1mg/mL) was made by dissolving 1mg of standard in 1mL methanol. Again 1mL of the stock solution was taken and 9mL methanol was added to it to make the final concentration of standard 100ng/µL. For calibration, 1-8µL of final standard solution was applied to both normal and reversed phase plate to provide concentration range of 100-800ng band^{-1}, respectively.

Preparation of samples

The leaves of Samples 1-5 were air-dried and pulverised. 500g of the powdered material of each sample was extracted with MeOH by the use of soxhlet extracter and filtered. The obtained extract was concentrated by using rotavapor and finally dried. The yield was found to be 4.2%, 4.9%, 5.8%, 4.7% and 6.8% w/w, respectively. Since marker compound was found to be soluble in methanol hence the same was used for the extraction of samples.

TLC instrumentation and chromatographic conditions

In Method I the quantitative analysis was carried out on 20 × 10cm glass-backed silica gel 60 F_{254} plates and in Method II the analysis was done on the 20×10cm glass-backed RP-18 silica gel 60 F_{254} plates. Automatic TLC Sampler 4 (ATS4) (CAMAG) fitted with a microlitre syringe was used to apply the samples and standard on the TLC plates and the application rate was 160nl/s. The plate was developed in previously saturated (For 20min at 22°C with mobile phase vapour) Automatic Developing Chamber 2 (ADC2) in linear ascending mode with EA: GAA: FA: H_2O (10:1:1:2.5, v/v/v/v) (for Method I) and ACN: H_2O (4:6 v/v) (for Method II) as mobile phases. Camag TLC scanner IV was used to scan the developed plates at 305 nm wavelength in absorbance mode by using the deuterium lamp. The slit dimensions were 4.00 × 0.45 mm and the scanning speed was 20mm/s.

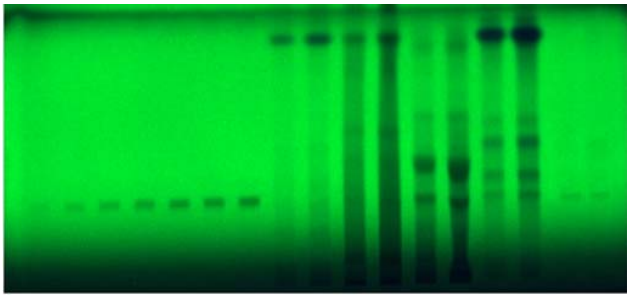

Fig. 2a: Picture of developed TLC plate (Method I) at 254nm, mobile phase: ethyl acetate: Glacial acetic acid: formic acid: water (10:1.1:1.1:2.5, v/v/v/v).

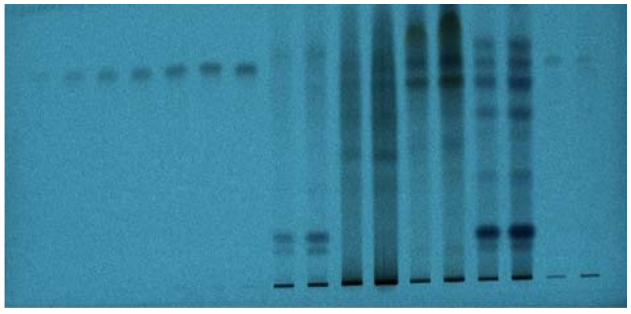

Fig. 2b: Picture of developed TLC plate (Method II) at 254nm, mobile phase: (acetonitrile: water, 4:6 v/v)

Preparation of calibration graphs

Calibration graph for standard rutin was prepared by applying a series of spots of standard with seven different volumes so as to get different amount of rutin per spot. They were prepared with respect to height and area vs amount per spot.

Method development

Chromatogram was developed for rutin by selecting the mobile phase after trying several combinations of solvents. The best resolution was observed in the selected mobile phase [EA: GAA: FA: H_2O (10:1.1:1.1:2.5, v/v/v/v)] for method I and [ACN: H_2O (4:6 v/v)] for method II. The same mobile phase has been employed for the separation of methanol extracts of samples 1, 2, 3, 4 and 5. The optimized saturation time was observed as 20min. The densitometric analysis was performed at absorption maxima of wave length 305 nm in absorbance mode.

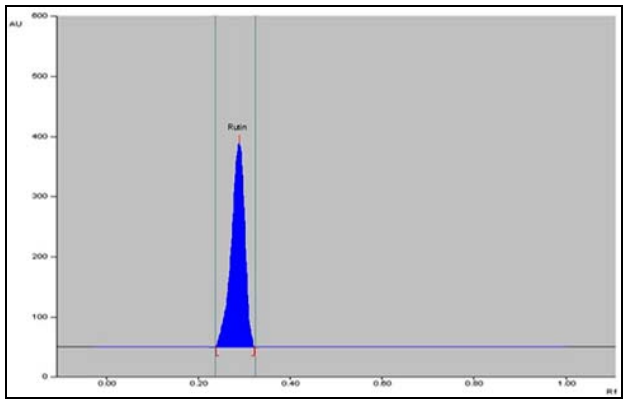

Fig. 3a: Chromatogram of standard rutin (700ng spot^{-1}), peak 1 (R_f = 0.28) scanned at 305nm; mobile phase: ethyl acetate: glacial acetic acid: formic acid: water (10:1.1:1.1:2.5, v/v/v/v) (Developed by Method I).

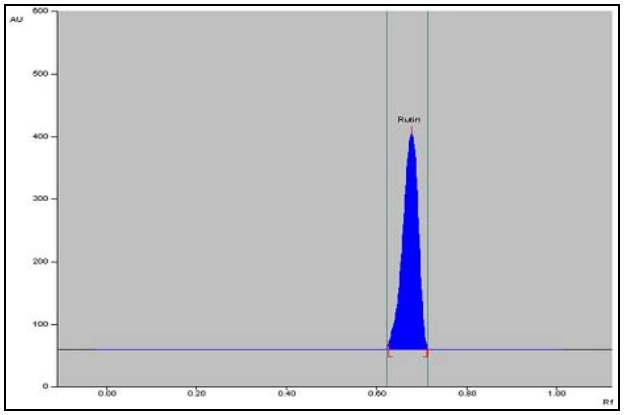

Fig. 3b: Chromatogram of standard rutin (700ng spot^{-1}), peak 1 (R_f = 0.68) scanned at 305nm; mobile phase: acetonitrile: Water (4:6, v/v) (Developed by Method II).

Method validation

The proposed methods I & II were validated according to ICH guidelines for linearity, precision, accuracy, LOD, LOQ and robustness.

Linearity range

For determining the linearity range of standard rutin, a series of spots of different volumes (1µl-8µl) were applied so as to get 100-800ng quantity of rutin per band for method I method II. Graph was plotted between concentration and peak area for linearity. Linearity data was statistically treated using least square linear regression analysis.

Accuracy

Accuracy was determined by standard addition method. The preanalyzed sample of rutin (300ng/spot) was spiked with the extra 0, 50, 100 and 150% of rutin and the solutions were reanalyzed in six replicates by the proposed method I and II. The % recovery and percent relative standard deviation (% RSD) were calculated.

Precision

Precision (inter and intraday) of the proposed methods (Method I and Method II) was evaluated by performing replicate analyses (n=6) at three different concentration levels 150, 300 and 600ng/spot of rutin. Inter-day precision was determined by repeating the intra-day assay on three different days.

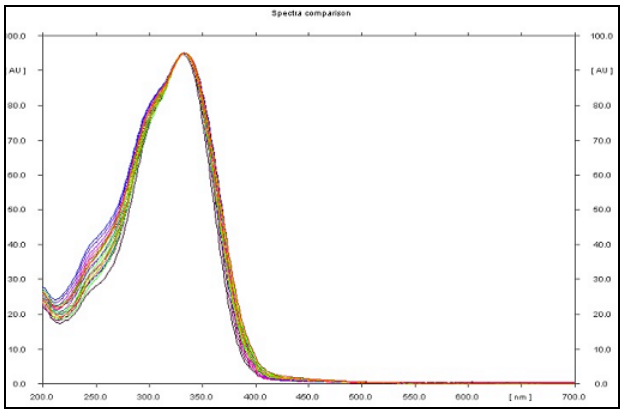

Fig. 4: Spectral comparison of all tracks with standard rutin scanned at 305nm.

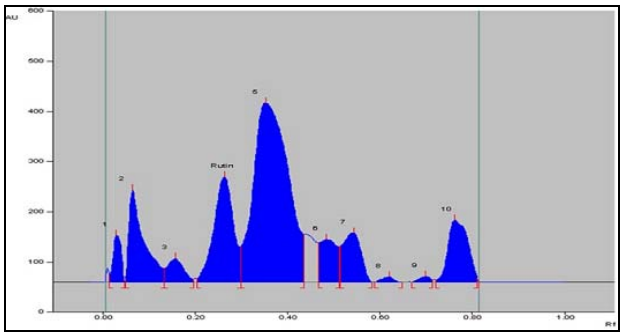

Fig. 5a: Chromatogram of sample 3 scanned at 305nm (rutin; R_f = 0.28); mobile phase: ethyl acetate: glacial acetic acid: formic acid: water (10:1.1:1.1:2.5, v/v/v/v). (Developed by Method I).

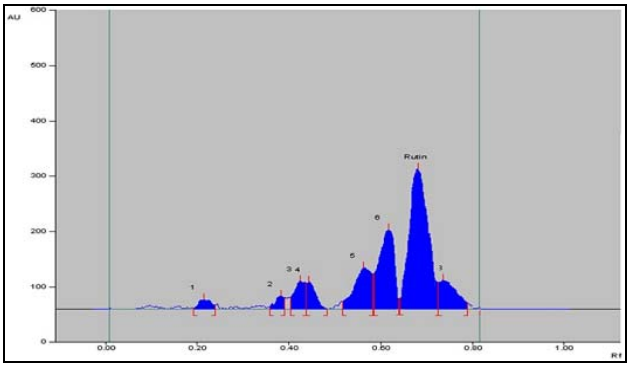

Fig. 5b: Chromatogram of sample 3 scanned at 305nm (rutin; R_f = 0.68); mobile phase: acetonitrile: water (4:6, v/v) (Developed by Method II).

Robustness

Robustness was studied in triplicate at 500ng band^{-1} by making small changes to mobile phase composition,

mobile phase volume and duration of saturation. The results were studied in terms of SD and % RSD of peak areas. Mobile phases prepared from EA: GAA: FA: H_2O (10:1.1:1.1:2.5, v/v/v/v; 10:1:1:1.5v/v/v/v; 9.5:1:1:2, v/v/v/v; 10:2:2:1.5, v/v/v/v) were used for chromatography in method I and mobile phases prepared from ACN: H_2O in different proportions (4:6,v/v; 3.9:6.1,v/v; 4.1:5.9,v/v; 4.2:5.8,v/v) were used for chromatography in method II. Mobile phase volume and duration of saturation investigated were 20±2mL (18, 20, and 22mL) and 20±10min (10, 20 and 30min), respectively. The plates were activated at 110°C for 30 minutes before chromatography.

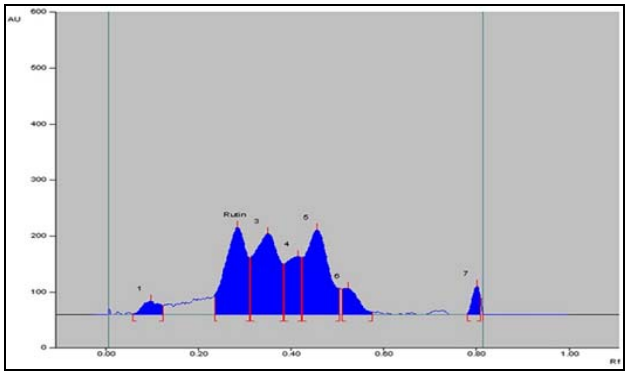

Fig. 6a: Chromatogram of sample 4 scanned at 305nm (rutin; R_f = 0.28); mobile phase: ethyl acetate: glacial acetic acid: formic acid: water (10:1.1:1. 1:2.5, v/v/v/v) (Developed by Method I).

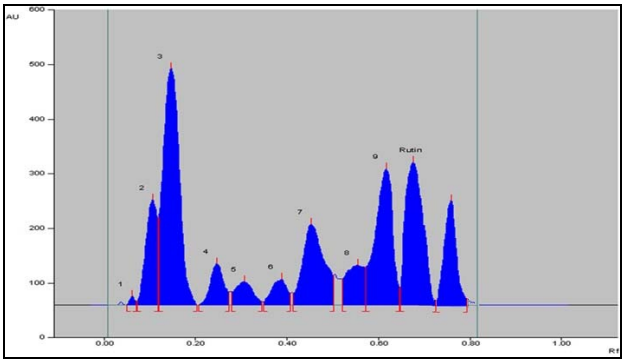

Fig. 6b: Chromatogram of sample 4 scanned at 305nm (rutin; R_f= 0.68); mobile phase: acetonitrile: water (4:6, v/v) (Developed by Method II).

LOD and LOQ

The LOD is the lowest amount of an analyte that may be differentiated from the assay background at a distinct level of confidence and the LOQ is the minimum amount that can be quantified at a distinct level of precision or accuracy.

Assay of rutin

Standard rutin and test samples were spotted on HPTLC plates. The percentage of rutin present in test samples (sample 1 to 5) was determined by measuring area for the standard and test samples. Thereby the percentage of rutin was calculated for all the five samples of *Ficus* species and reported in table 5.

RESULTS

Method development and validation

The two developed methods (method I and method II) were found to be effective in separation of constituents present in the samples (1, 2, 3, 4 and 5) (Figs. 2a & 2b) and exhibiting sharp peaks of standard (Rutin) as well, with the selected mobile phase under chamber saturation conditions at a wave length of 305 nm in absorbance mode. The mobile phases EA: GAA: FA: H_2O (10:1.1:1.1:2.5, v/v/v/v) and ACN: H_2O (4:6 v/v) were found to furnish a sharp peak of rutin at R_f values 0.28 (Fig. 3a) and 0.68 (Fig. 3b) for Method I and Method II, respectively. The developed methods were found to be quite selective with good baseline resolution. The bands of rutin in the samples (1-5) were authenticated by overlaying it with the bands of standard (rutin) (Fig. 4).

The calibration curve of rutin was found to be linear in the range 100-800ng/spot by Method I and Method II. Linear regression data for the plot confirmed the good linear relationship (table 1). The correlation coefficient (R^2) for rutin were 0.998 and 0.998 for Method I and Method II, respectively and found to be highly significant ($P<0.0001$). The linear regression equation was $Y= 3.758x + 38.945$ and $Y=5.367x + 61.381$ for rutin for Method I and Method II, respectively where Y is response and X is amount of reference standards.

The accuracy was calculated by recovery analysis which afforded recovery of 98.40-99.45% and 98.84-99.50% for Method I and II, respectively and the different values are listed in table 2. Low values of % RSD 0.92-1.55 (for method I) and 1.13-1.61 (for method II) indicated good accuracy of the proposed method.

Intra-day and inter-day precision of the assay of rutin at three different concentration levels (150, 300 and 600ng $band^{-1}$) were expressed as RSD (%) in table 3. % RSD was in the range 1.51-1.73 & 1.37-1.54 for intra-day and 1.47-1.81 & 1.35-1.73 for inter-day precision of Method I & Method II, respectively. These low values indicated that the method was precise.

Results of robustness are shown in table 4. Low values of % RSD (0.420-0.436) and (0.422-0.454) for method I and method II, respectively proved the robustness of the proposed HPTLC method.

LOD & LOQ of the proposed method were found to be 35 & 103ng/spot for method I and 38 & 111ng/spot for method II, which suggested that the proposed method might be used in wide range for detection and quantification of rutin.

Table 1: R_f, Linear regression data for the calibration curve of Method I and Method II (n=6)

Parameters	Method I	Method II
R_f Linearity range (ng/spot)	0.28±0.01 100-800	0.68±0.03 100-800
Regression equation	Y= 3.758X+38.945	Y=5.367X + 61.381
Correlation coefficient	(r^2) 0.998	0.998
Slope ± SD	3.758±0.013	6.178±0.042
Intercept ± SD	38.945±0.30	71.594±0.91
Standard error of slope	0.023	0.031
Standard error of intercept	2.18	2.79
LOD	35ng band^{-1}	38ng band^{-1}
LOQ	103ng band^{-1}	111ng band^{-1}

Table 2: Accuracy of the proposed method (n=6)

Excess drug added to analyte (%)	Theoretical content (ng)	Conc. Found (ng) ± SD	% Recovery	% RSD
Method I				
0	300	295.40 ± 4.59	98.40	1.55
50	450	444.90 ± 5.85	98.86	1.31
100	600	594.98 ± 6.59	99.16	1.10
150	750	745.88 ± 6.91	99.45	0.92
Method II				
0	300	296.54 ± 4.78	98.84	1.61
50	450	445.29 ± 6.59	98.95	1.48
100	600	596.35 ± 7.84	99.39	1.32
150	750	746.21 ±8.42	99.50	1.13

Table 3: Precision of the proposed method of Method I and Method II.

Method I						
	Repeatability (Intraday precision)			Intermediate precision (Interday)		
Conc. (ng/spot)	Avg Conc. ± SD (n=6)	Standard error	% RSD	Avg Conc. ± SD (n=6)	Standard error	% RSD
150	218.16±3.77	5.81	1.73	211.21±3.82	6.11	1.81
300	354.66±5.74	11.44	1.62	336.79±5.65	10.29	1.68
600	637.43±9.62	14.51	1.51	632.63±9.29	14.18	1.47
Method II						
150	224.39±3.45	4.83	1.54	201.78±3.49	4.90	1.73
300	366.45±5.16	11.18	1.41	325.71±5.14	9.19	1.58
600	659.71±8.22	14.49	1.37	614.29±8.29	12.28	1.35

Table 4: Robustness of the proposed HPTLC method of Method I and Method II.

Method I		
Mobile phase composition (Glacial acetic acid: formic acid: water, 10:1.1:1.1:2.5, v/v/v/v)		
Optimization condition	Rutin	
	SD	%RSD
Mobile phase from ethyl acetate		
Glacial acetic acid: formic acid: water		
(10:1.1:1.1:2.5, v/v/v/v; 10:1:1:1.5v/v/v/v;		
9.5:1:1:2, v/v/v/v; 10:2:2:1.5, v/v/v/v)	2.18	0.436
Mobile phase volume (18, 20 and 22mL)	1.69	0.338
Duration of saturation (10, 20 and 30min)	2.10	0.420
Method II		
Mobile phase composition (Acetonitrile: water, 4:6 v/v)		
Optimization condition	Rutin	
	SD	SD
Mobile phase from acetonitrile: water (4:6, v/v; 3.9:6.1, v/v; 4.1:5.9, v/v; 4.2: 5.8, v/v)	2.27	0.454
Mobile phase volume (18, 20 and 22mL)	1.89	0.386
Duration of saturation (10, 20 and 30min)	2.09	0.422

Table 5: Estimation of rutin in different *Ficus* species (samples1-5) by Method I and Method II.

Sample No.	Name of Ficus species	Method I	Method II
		Rutin content (%)	Rutin content (%)
Sample 1	*F. carica* Linn	Not found	Not found
Sample 2	*F. nitida* Vahl	Not found	Not found
Sample 3	*F. ingens* Miq	0.51%	0.66%
Sample 4	*F. palmata* Forssk.	0.24%	0.54%
Sample 5	*F. vasta* Forssk.	0.14%	0.17%

HPTLC analysis of prepared samples

The utility of the proposed methods were evaluated by applying these methods for the quantification of rutin in samples 1, 2, 3, 4 and 5. Out of these five samples evaluated for the quantification of rutin, only three samples i.e. sample 3 (*F. ingens*), sample 4 (*F. palmata*) and sample 5 (*F. vasta*) were found to contain rutin (Figs. 5a & 5b, 6a & 6b and 7a & 7b). Rutin was not found in the remaining two samples i.e. sample 1(*F. carica*) and 2 (*F. nitida*). The content of rutin evaluated in the different samples by method I and method II are given in table 5. Both the methods were found to be suitable for the analysis of rutin in the different *Ficus* species, but method II was found to be more suitable for the analysis of rutin because its gives more precise and accurate result in comparison to the method I.

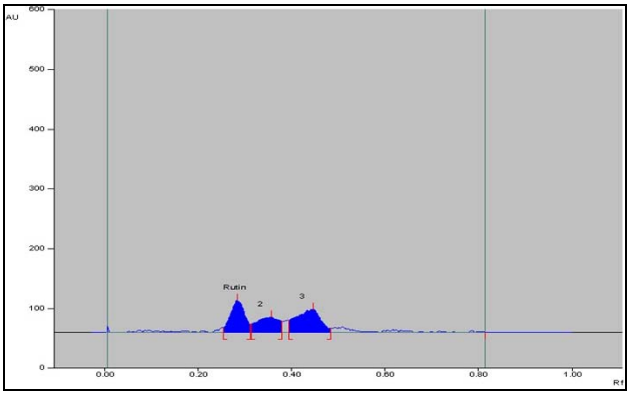

Fig. 7a: Chromatogram of sample 5 scanned at 305nm (rutin; R_f = 0.28); mobile phase: ethyl acetate: glacial acetic acid: formic acid: water (10:1.1:1.1:2.5, v/v/v/v) (Developed by Method I).

DISCUSSION

In this research we performed this comparative estimation of rutin for the first time in above-mentioned five species of genus *Ficus* collected from Kingdom of Saudi Arabia by two validated HPTLC methods (method 1 and method 2). The quantity of rutin found in *F. ingens*, *F. palmate* and *F. vasta* by using methods I & II were 0.66% & 0.51%, 0.54% & 0.24% and 0.17% & 0.14%, respectively. The obtained results clearly indicated that out of all five species of *Ficus* quantified for rutin content *F. ingens* was found to contain highest amount of rutin. The outcomes of this experiment may be utilized to select the species having high content of rutin (*F. ingens*) for herbal formulations. The formation of secondary metabolites in the plants is greatly affected by extrinsic factors (eg. climate, altitude, soil pH etc.) as well as intrinsic factors (eg. age, gender, genotype etc.), which are most often beyond our control. To compensate the effect of these external and internal factors on the production of secondary plant metabolites, the developed HPTLC method II was found to be an important analytical technique for separation, detection, identification and quantification of rutin in this experiment.

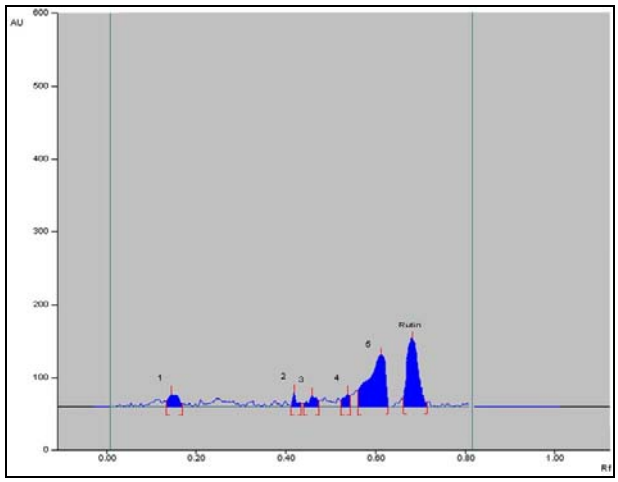

Fig. 7b: Chromatogram of sample 5 scanned at 305nm (rutin; R_f = 0.68); mobile phase: acetonitrile: Water (4:6, v/v) (Developed by Method II).

CONCLUSION

Cancer is the most dreaded disease in the world as well as in kingdom of Saudi Arabia and causes large number of deaths every year. It is reported that the *Ficus* species were used traditionally as an anticancer drug. Since, rutin has good anti-oxidant and antitumor activity, so the detection of rutin in the extracts of three species of *Ficus* in this experiment justifies the use of *Ficus* as anti-cancer drug in folk medicines. Further studies can be designed to explore the bioavailability (concentration in blood plasma) and degradation kinetics of rutin. The above proposed High Performance Thin Layer Chromatographic method (Method II) can also be employed for exploration of rutin in chemo taxonomically related genera of the plant kingdom.

ACKNOWLEDGEMENT

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REFERENCES

Alajmi MF, Alam P and Shakeel F (2013). Quantification of bioactive marker β-amyrin by validated high-performance thin-layer chromatographic-densitometric method in different species of *Maytenus* grown in Saudi Arabia. *J. Planar Chromat.*, **26**(6): 475-479.

Alam P, Siddiqui NA, Al-Rehaily AJ, Alajmi MF, Basudan OA and Khan TH (2014). Stability-Indicating Densitometric High-Performance Thin-Layer Chromatographic Method for the Quantitative Analysis of Biomarker Naringin in the Leaves and Stems of *Rumex vesicarius* L. *J. Planar Chromat.*, **27**(3): 204-209.

Alonso-Castro AJ, Dominguez F and Garcia-Carranca A (2013). Rutin exerts antitumor effects on nude mice bearing SW480 tumor. *Arch. Med. Res.*, **44**(5): 346-351.

Bhandari P, Kumar N, Gupta AP, Singh B and Kaul VK (2007). A rapid RP-HPTLC densitometry method for simultaneous determination of major flavonoids in important medicinal plants**.** *J. Sep. Sci.*, **30**(13): 2092-2096.

Caliskan O and Polat AA (2011). Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern mediterranean region of Turkey. *Sci. Hort.*, **128**: 4730-4738.

Carrillo-Lopez A and Yahia E (2013). HPLC-DAD-ESI-MS analysis of phenolic compounds during ripening in exocarp and mesocarp of tomato fruit. *J.* Food *Sci.,* **78**(12): C1839-1844.

Chang MS, Yang YC, Kuo YC, Kuo YH, Chang C, Chen CM and Lee TH (2005). Furocoumarin glycosides from the leaves of *Ficus ruficaulis* Merr. var. antaoensi. *J. Nat. Prod.*, **68**(1): 11-13.

Chunyan C, BO S, Ping L, Jingmei L and Ito Y (2009). Isolation and purification of rutin and bergapten from *Ficus carica* L. leaves by high-speed countercurrent chromatography. *J. Liq. Chromatogr. RT.*, **32**: 136-143.

Fang X, Wang Y, Wang J, Zhang J and Wang X (2013). Microwave-assisted extraction followed by RP-HPLC for the simultaneous extraction and determination of forsythia side A, rutin and phillyrin in the fruits of *Forsythia suspensa*. *J. Sep. Sci.*, **36**(16): 2672-2679.

Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S and Taviano MF (2003). Chemical characterization and biological effects of Sicilian Opuntia *ficus indica* (L.) mill. Fruit juice: Antioxidant and antiulcerogenic activity. *J. Agr. Food Chem*., **51**(17): 4903-4908.

Harrison RD (2005). Figs and the diversity of tropical rainforests. *Bioscience*, **55**: 1053-1064.

Hutchinson J, Dalziel JM and Keay RWJ (1958). Flora of West Tropical Africa. second ed: Crown Agents, London, 1958.

International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Harmonized Triplicate Guideline on Validation of Analytical Procedures: Text and Methodology Q2 (R1), Complementary Guideline on Methodology incorporated in November 2005 by the ICH Steering Committee, IFPMA, Geneva, Pp 6-13.

Khan RA, Khan MR and Sahreen S (2012). CCl4-induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat**.** *BMC Complement Altern. Med.*, **12**: 178.

Kuntic V, Pejic N, Ivkovic B, Vujic Z, Ilic K, Micic S and Vukojevic V (2007). Isocratic RP-HPLC method for rutin determination in solid oral dosage forms. *J. Pharm. Biomed. Anal.*, **22**: 718-721.

Kuo YH and Chaiang YM (1999). "Five new taraxastane-type triterpenes from the aerial roots of *Ficus microcarpa. Chem. Pharm. Bull*, **47**: 498-500.

Kuo YH and Li YC (1997). Constituent of the bark of *Ficus Microcarpa* (L. f). *J. Chin. Chem. Soc.*, **44**: 321-325.

La Casa C, Villegas I, Alarcon de la Lastra C, Motilva V and Martin Calero MJ (2000). Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *J. Ethnopharmacol.*, **22**: 45-53.

Lansky EP, Paavilainen HM, Pawlus AD and Newman RA (2008). *Ficus* spp. (fig): Ethno botany and potential as anticancer and anti-inflammatory agents. *J. Ethnopharmacol.*, **119**: 195-213.

Li YC and Kuo YH (2000). Four new compounds, ficusal, ficusesquilignan a, b and ficusolide diacetate from the heartwood of *Fiucs microcarpa*. *Chem. Pharm. Bull*, **48**: 1862-1865.

Noumi E and Fozi FL (2003). Ethno medical botany of epilepsy treatment in fongo-tongo village, western province Cameroon. *Pharm. Biol.*, **41**: 330-339.

Pereira CA, Yariwake JH, Lanças FM, Wauters JN, Tits M and Angenot L (2004). A HPTLC densitometric determination of flavonoids from *Passiflora alata, P. edulis, P. incarnata* and *P. caerulea* and comparison with HPLC method. *Phytochem. Anal*, **15**(4): 241-248.

Ronsted N, Salvo G and Savolainen V (2007). Biogeographical and phylogenetic origins of African fig species (*Ficus* section Galoglychia). *Mol. Phylogenet. Evol.*, **43**(1): 190-201.

Shen Y, Yin H, Chen B, Xia G, Yang H and Jia X (2012). Validated reversed phase-high performance liquid chromatography-diode array detector method for the quantitation of Rutin, a natural immunostimulant for

improving survival in aquaculture practice, in *toonea sinensis* folium. *Pharmacogn. Mag.*, **8**(29): 49-53.

Soponar F, Mot AC and Sarbu C (2010). High-performance thin-layer chromatography and three-dimensional image analysis for the determination of rutin in pharmaceutical preparations**.** *J. AOAC Int.,* **93**(3): 804-810.

Teixeira DM, Patao RF, Coelho AV and DaCosta CT (2006). Comparison between sample disruption methods and solid-liquid extraction (SLE) to extract phenolic compounds from *Ficus carica* leaves. *J. Chromatogr. A.*, **1103**(1): 22-28.