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Quantitative Determination and Source Variation of Rutin in *Fagopyrum Tataricum*

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Abstract

Buckwheat (Fagopyrum sp.) is a traditional crop of Uttarakhand (India) known for its high Rutin content, a bioflavonoid with significant medicinal properties. This study explores the quantitative measurement of Rutin in various buckwheat varieties grown in different regions of Uttarakhand using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC). Twelve seed sources of Fagopyrum tataricum from the Garhwal and Kumaon regions, along with Fagopyrum esculentum seeds for comparison, were collected. Preliminary phytochemical screening of the extracts revealed the presence of flavonoids, alkaloids, tannins, and carbohydrates, with methanolic extracts showing higher concentrations. TLC analysis was employed to determine the most effective solvent system for Rutin separation, leading to the identification of three samples with substantial Rutin content. HPTLC was then used for quantitative Rutin assessment, with a calibration curve established using standard Rutin solutions. The three selected samples, along with the standard, were applied to HPTLC plates, and the Rutin content was measured based on peak areas and peak heights. The sample collected from Joshimath, Uttarakhand, known as "JOG," exhibited the highest Rutin content. This study provides valuable insights into the phytochemical composition of buckwheat varieties in Uttarakhand, which can inform the development of high-quality functional foods. Rutin, a potent antioxidant with various health benefits, underscores the significance of buckwheat as a naturally occurring functional food source. The results contribute to understanding the Rutin-rich potential of buckwheat grown in Uttarakhand and its applications in functional foods and nutraceuticals. Further research in this field can promote the cultivation and utilization of buckwheat as a source of this valuable bioactive compound with numerous health benefits.

Keyword: *Bioactive, High-Performance*

1. Introduction

Buckwheat (*Fagopyrum* sp.) is a traditional crop of Uttarakhand (India) that is grown at high altitudes among other plants, both medicinal and culinary. Buckwheat is still regarded as a significant dietary source of Rutin today. The dicotyledonous plant buckwheat (also known as pseudo cereal) is a member of the *Polygonaceae* family. Out of the several buckwheat species known to exist, only two are known to be farmed for human consumption: common buckwheat (*Fagopyrum esculentum* Moench.) and tartary buckwheat (*Fagopyrum tataricum* Gaertn.). Because it benefits people (low glycemic index, decreased capillary fragility, etc.) and doesn't require additional ingredients, buckwheat is a naturally occurring functional food. In addition to being very high in proteins (12–15%) and important amino acids like lysine (5-7%), which are lacking in other main cereal crops, buckwheat also contains, but it is also rich in minerals (zinc, manganese, selenium, iron, phosphorus, and copper), fats, fibres, and vitamins B1 and B2 (Stibilj *et al*., 2004; Luthar *et al*., 2021). One of the primary causes of the variety of buckwheat foods produced is the presence of rutin in buckwheat plants (Kalinova *et al*, 2006).

As one of the many phenolic secondary metabolites of plants known as bioflavanoids, which contain over 2000 distinct compounds, rutin is one of them (Xu *et al*., 2007). Rutin is a significant medicinal ingredient that has a positive impact on the treatment of antherosclerosis, circulatory diseases, and the augmentation of blood vessel flexibility. It lowers blood pressure, increases the body's use of vitamin C, and has antioxidant activity. It has been shown that rutin, a phenolic antioxidant, can scavenge superoxide radicals involved in the Fenton process, which produces reactive oxygen species (Nešović *et al*., 2021). Additionally, rutin may help maintain levels of the biological antioxidant reduced glutathione and regulate neutrophil respiratory burst. (Ganeshpurkar and Saluja, 2017).

Buckwheat's rutin content varies depending on the species, harvesting circumstances, and growing environment. The most popular kind of buckwheat is common, which has the benefits of a large, easily dehulled seed and a pleasant flavour (Kreft *et al*., 2004). Despite this, Tartary buckwheat is recommended because it has significantly more rutin and other bioactive ingredients than regular buckwheat.

Using TLC and HPTLC, the rutin content of buckwheat varieties growing in different parts of Uttarakhand state was assessed to determine the best source of rutin. This study describes the quantitative measurement of rutin in *Fagopyrum tataricum* seeds for the first time using an HPTLC approach that is acceptable for routine usage, despite the fact that quantitative estimation of rutin has been reported by HPTLC in a variety of herbal extracts (Rodríguez *et al*., 2021).

2. Material and Methods

Plant material

Fagopyrum tataricum seed samples were gathered and seeded in the spring from twelve Uttarakhand seed sources, which included the whole Garhwal region and the high-altitude Kumaon region. *F. esculentum* seeds were purchased at the neighbourhood market in order to compare the phyto-constituents of the two species.

Reagents and other materials

All chemicals and solvents used were of analytical and HPLC grade. Rutin (Sigma-Eldrich), toluene, acetone, ethyl acetate, dichloromethane, formic acid, glacial acetic acid, methanol and silica gel F254 precoated TLC aluminium plates were procured from E-Merck.

Extraction of plant material, Preparation of standard and sample solutions

The plant extract was prepared using several solvents according to the plant's differential polarity. To extract plant material, the following solvents were utilised in increasing polarity order: petroleum ether, chloroform, methanol, and water. Ten milligrammes of rutin were precisely weighed into a ten millilitre volumetric flask, dissolved in five millilitres of methanol, and then diluted to ten millilitres using the same solvent (1 mg/mL). One gram of the finely ground powder was utilised to extract rutin from five grammes of seeds and two grammes of leaves of *Fagopyrum tataricum* using ten millilitres of methyl alcohol. The powder was then decocted using a Soxhelt extraction device for 60 minutes at 70 degrees Celsius.

Preliminary phytochemical screening: Preliminary phytochemical screening was done in order to reveal the presence of phytosterol, polyphenol, flavonoids and carbohydrates in the plant extracts.

Preliminary TLC analysis

All the 12 seed sources of *Fagopyrum tataricum* were examined for the presence of Rutin content using the silica-G as a stationary phase. Different combination of solvents were tested for the preparation of mobile phase as: 90% Chloroform + 10% Methanol, 80% Chloroform + 20% Methanol, 75% Chloroform +25% Methanol, Ethyl acetate + Methanol + Water, Ethyl acetate + Formic acid + n-Butanol + Water. Finally the mobile phase used for the separation of Rutin was of Ethyl acetate, n-butanol, formic acid, water in the ratio 5:3:1:1 respectively. Twenty minutes were given for the TLC chamber to saturate. After that, a solution of 95% methyl alcohol and 5% concentrated sulfuric acid was sprayed onto the TLC plate. After that, the plate was heated for ten minutes at 1100 C. The existence of rutin in the buckwheat seeds was then ascertained by computing the Rf values of various samples and comparing them to the Rf value of standard rutin.

HPTLC analysis

A Camag HPTLC system equipped with an automatic TLC sampler (Linomat 5), a TLC scanner 3 (WINCATS version 1.2.3) with a UV cabinet and a twin trough glass tank were used. The samples were applied using an automated TLC sampler in 5 mm bands at 10 mm from the X and Y axis and 5 mm spaces were left between adjacent bands.

3. Results and Discussion

Fagopyrum tataricum have a rich source of compound of neutraceutical value. Plants from this family are reported to have anti-inflammatory, anti-cancerous and antioxidant properties. Thus, the objective of this study was to determine qualitatively and quantitatively detection of evaluation of neutraceutical compound Rutin. In the present study investigation, the phytochemical constituents of the extract of different methanolic extracts of Fagopyrum was analysed and then the quantitative Rutin content was determined by HPTLC.
Seed samples were subjected to extraction of different phytochemical compounds with different polar solvents. The results of qualitative chemical investigation of these extracts have indicated the presence of following compounds that are tableted in Table 1.

Extracts mentioned in the table were found to possess steroids, flavonoids, alkaloids and carbohydrates. Out of the two extracts, methanolic extract was found to be rich in alkaloid, flavonoid, tanin and carbohydrates. While the aqueous extract was found to have very low concentration of these compounds. The methanolic extract was further used for the qualitative and quantitative determination of Rutin from these extracts.

Table 1: Phytochemical analysis of *Fagopyrum tataricum* for two solvents

S. No	Test Performed	Methanolic Extract	Water Extract
1	**Test for Carbohydrate**		
	Fehling's Test	(-)	(-)
	Molisch's test	(-)	(-)
	Barford's Test	(+)	(+)
	Selivinoff's Test	(+)	(+)
	Benedict's test	(+)	(+)
2	**Test for Sreroids**		
	Salkowski Test	(+)	(-)
	Liberman-Buchard's test	(+)	(-)
	Hensen 'sTest	(+)	(-)
3.	**Test for Alkaloids**		
	Wagner's Test	(+)	(+)
	Hager's test	(+)	(+)
	Mayer's test	(+)	(+)
4	**Test for Phenolics and Flavonoids**		
	Vanillin-HCL test	(+)	(+)
	Ferric Chloride test	(+)	(+)
	Zinc Hydrochloric Acid reduction	(+)	(+)
5	**Test for Tanins**	(+)	(-)
6	**Test for sponins**	(-)	(-)

Phytochemical Screening of seed sample of *Fagopyrum esculaentum* and *Fagopyrum tataricum*

Various polar solvents were used to extract distinct phytochemical components from the seed samples. The presence of flavanoids, alkaloids, and carbohydrates was shown by the qualitative chemical analysis of these extracts (Table 2). Alkaloids, flavanoids, tannins, and carbohydrates were found to be abundant in the methanolic extract out of all the extracts. On the other hand, the concentration of these chemicals in the aqueous extracts was found to be quite low. The amount of rutin extracted from the methanolic extracts was quantified using these extracts as well. Tannins, flavonoids, alkaloids, and tannins were found in the plant's seeds according to a phytochemical examination. The tannins are found to be helpful in wound healing, whereas the alkaloids are known for their role in treatment of hypertension. The flavonoids are known to act as antioxidants. Antioxidants neutralized highly unstable and extremely reactive molecules called free radicals which attack the skin of human body everyday (Ishiguro *et al*., 2016). So the study there fore has provided the biochemical basis for ethno pharmacological uses of this plant in the treatment of various diseases and disorders.

Table 2: Quantitative estimation of total flavonoid content in *Fagopyrum tataricum* for different geographical locations of Uttarakhand

S. No.	Sampling Locations	Sample codes	Altitude (meters)	Total Flavonoid Content	
	Fagopyrum tataricum			Acetonic extract of Seed powder µg rutin/ 50mg DW	Methanolic extract of seed powder µg rutin/ 50mgDW
1.	Rudrapur	RDG	550	118.4±0.36	212.5±0.41
2.	Rudraprayag	RGG	610	120.2±0.43	204.9±0.36
3.	Lamgaon	LMG	985	139±1.46	237.6±0.22
4.	Almora	ALG	1646	186.25±0.14	230.6±0.42
5.	Khero	KHG	1700	186.25±0.14	243±.036
6.	Kosi	KOG	1783	193.1±0.44	260±0.22
7.	Joshimath	JOG	1875	250.9±0.16	272.5±0.28
8.	Munsiyari	MUG	2135	208±0.22	263.2±0.52
9.	Dhantoli	DHG	2250	251±0.17	260.25±0.57
10.	Hanumanchatti	HCG	2453	243.08±0.36	254.8±0.30
11.	Inderdhara	ING	3133	212.6±0.50	245.8±0.08
12.	Vinayak	VNG	3268	201.2±0.33	231±0.25
13.	Gajkoti	GJG	3450	202±0.44	242±0.22
14.	Mukba	MKG	3600	210±0.54	227.5±0.60
15.	Mana	MNG	3650	209.7±2.25	217.7±0.52
	Fagopyrum esculentum				
16.	Kuttu			198.1±0.47	221.2±0.22

Investigation of Rutin by Thin Layer Chromatography

Rutin is a biflavonoid and phenolic antioxidant and may have antioxidant anti-inflammatory anti carcinogenic, cytoprotective and vaso protective activity. Many, if not, most of the Rutin possible activities can be accounted for, in part, by Rutin andtioxidant activity. Rutin is a phenolic antioxidant and has been demonstrated to scavenge super oxide radicals. Rutin can chelate metal ions as Ferric cations. Ferric cations are involved in the so-called Fenton reaction, which generates reactive oxygen species. Rutin may also moderate the respiratory burst of neutrophils. So due to the presence of Rutin in buckwheat seed, in considerable amount, this plant can be used for ethano-pharmacological uses for the treatment of various diseases and disorders.

The methanolic extract which was obtained after Soxhelt extraction followed by distillation was subjected to Thin Layer Chromatography to check whether the Rutin is present or not. Initially the standard was allowed to run alone to notice the RF value of standard Rutin.

Different solvent systems were used to see that which solvent system is best for the separation of Rutin. First of all chloroform- methanol solvent system was used in different combinations. In chloroform- methanol solvent system it was observed that the Rf value of Rutin standard in methanol- chloroform water system having ratio 80:20 was higher than that of the other combination showed in the Figure 1.

Other solvent systems were also used which includes: Ethyl Acetate- methanol water system and Ethyl Acetate- formic acid- n butanol- water. Finally, it was observed that the best solvent system was Ethyl Acetate- formic acid- n butanol- water having a Rf of 0.47.

After the standardization of mobile phase, all the samples were allowed to run on the TLC plate along with the mobile phase. All the 12 samples were allowed to run along with the standard Rutin. After the completion it was found that only 3 samples having Id's VV, JJ and MM showed the presence of considerable amount of Rutin while the other extract should vary minute or no amount to be further used for HPTLC for their quantitative determination. The dark yellow spot in the relation of standard Rutin was seen after spraying with 5% H2S04 and 95% ethanol.

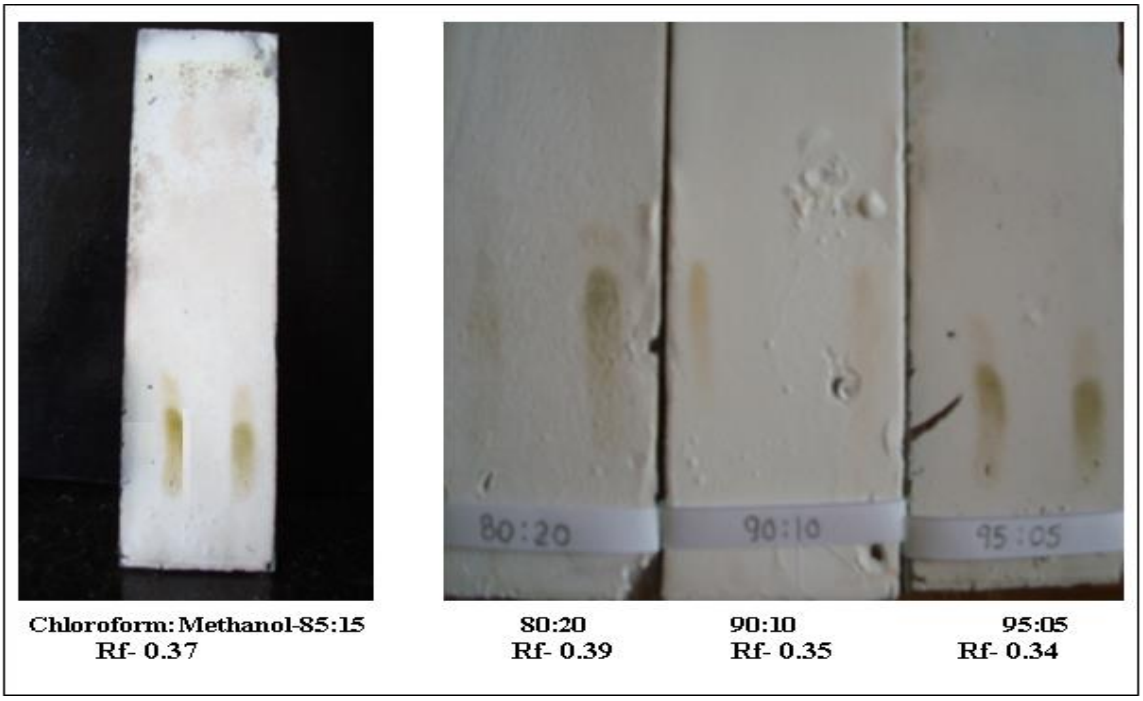

Figure 1: Qualitative determination of rutin content at different solvent combinations

Qualitative determination by HPTLC

Samples which are allowed to run through TLC plate, only 3 out of 12 were qualified for further investigation. These 3 samples were then subjected to Planner Chromatography (HPTLC). The 3 samples were applied as bands on different tracks at different concentration. Before the application of sample a calibration curve of standard Rutin was established at different concentration a straight line graph was obtained.

After the establishment of calibration curve the three samples were applied at different concentration on different track along with the different concentration of standard Rutin.

Thereafter, the stock solution was prepared for both the standard and extracts. Sample preparation was done by dissolving the extract in HPLC grade methanol. The three samples which showed the presence of Rutin were subjected to HPTLC along with the standard Rutin. The peak at 254 nm was considered as the peak of Rutin. On the basis of this peak the Rutin content in exacts was calculated. After the running of samples, the HPTLC plate was scanned with CAMAG TLC Scanner 3 at 254 nm wavelength and the results were analysed by using Wincats Planner Chromatography software (Table 3).

After the completion of HPTLC, the percentage of Rutin present in extracts was calculated both via area and via height. When the percentage was calculated via height, the value was found to be 10.452 and 17.4034 for JOG and VNG respectively (Figure 2). But when the percentage was calculated via area, the calculated value was 3.583 and 14.3814 for JOG and VNG respectively. For the third sample MM, amount was less than 200 ng which was very less to be detected by HPTLC so finally out of 12 samples the highest percentage of Rutin was found in the samples having ID JOG collected from Joshimath, Uttarakhand (Figure 3).

Tartary buckwheat *Fagopyrum tataricum* flower contains large amount of Rutin and Quercetin which are polyphenols known to be functional factors in food (Suzuki *et al*., 2023). It is now well known to many recent researchers and that Rutin and Quercetin have many benefits beneficial effects on human health Many researchers have reported that Rutin antagonize the increase in capillary fragility, reduces high blood pressure, decreases the permeability of the vessels and reduces the risk of arteriosclerosis and has high antioxidant activity (Suzuki *et al*., 2014). Quercetin has high antioxidant activity exhibit's anti allergenic activities (Wieslander and Norback, 2021; Nishimura *et al*., 2016), has anti carcinoma activities reduces blood glucose levels and increase antibacterial activity in concert with other flavonoids.

However, the quantities of these polyphenolics or polyphenols very considerably among the variety of strains of tartary buckwheat flour therefore the development of an accurate and convenient chemical analysis would be helpful to the breeders who want to develop a new variety of flower having higher quality as functional food currently High-Performance Liquid Chromatography and near-infrared analysis are commonly used for these breeding programs

Table 3: HPTLC parameters of *Fagopyrum tataricum* from three geographical locations of Uttarakhand

Sample ID	Track No	Application Volume (in µl)	Application Position (in mm)	Rf	Peak Height	Calculated Amount	Peak Area	Calculated Amount
RUTIN	1	700	15.0	0.48	142.77	-	7144.00	-
RUTIN	2	600	26.3	0.47	115.73	-	5219.70	-
VNG	3	500	37.6	0.45	42.11	256.86	1412.65	224.15
JOG	4	500	48.9	0.45	56.05	320.27	2073.43	282.85
MKG	5	500	60.2	0.42	12.82	< 200	369.48	< 200
RUTIN	6	500	71.5	0.46	91.08	-	4440.16	-
RUTIN	7	400	82.8	0.45	75.15	-	3416.52	-
VNG	8	500	94.1	0.45	34.10	220.43	1281.23	211.47
JOG	9	500	105.4	0.44	37.82	237.37	1387.17	221.88
MKG	10	500	116.7	0.42	14.48	< 200	358.13	< 200
RUTIN	11	300	128.0	0.44	53.05	-	2273.33	-
RUTIN	12	200	139.3	0.43	29.66	-	1236.72	-
VNG	13	500	150.6	0.45	32.15	211.58	1248.51	209.56
JOG	14	500	161.9	0.44	38.98	242.62	1399.56	222.98
MKG	15	500	173.2	042	13.35	< 200	310.81	<200
VNG					**Average**	**229.62**		**215.39**
					CV %	**10.452**		**3.583**
JOG					**Average**	**266.76**		**242.57**
					CV %	**17.403**		**14.381**
MKG					**Average**	**NC**		**NC**
					CV %	**NC**		**NC**

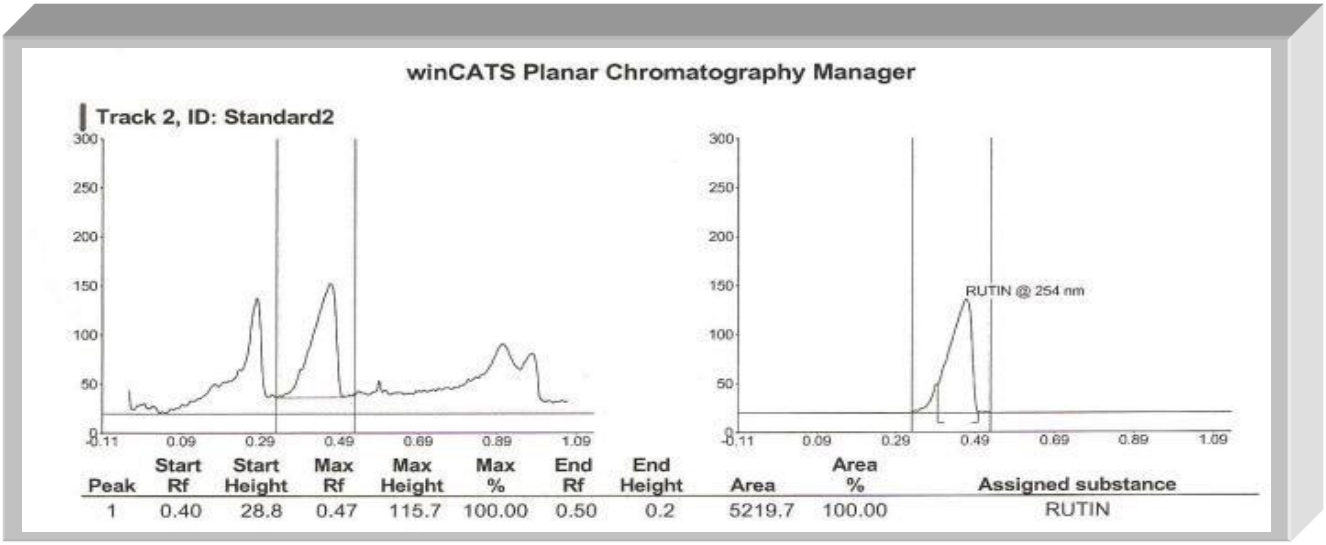

Figure 2: HPTLC profile for the detection and quantitative estimation of Rutin content of *Fagopyrum tataricum* form Joshimath

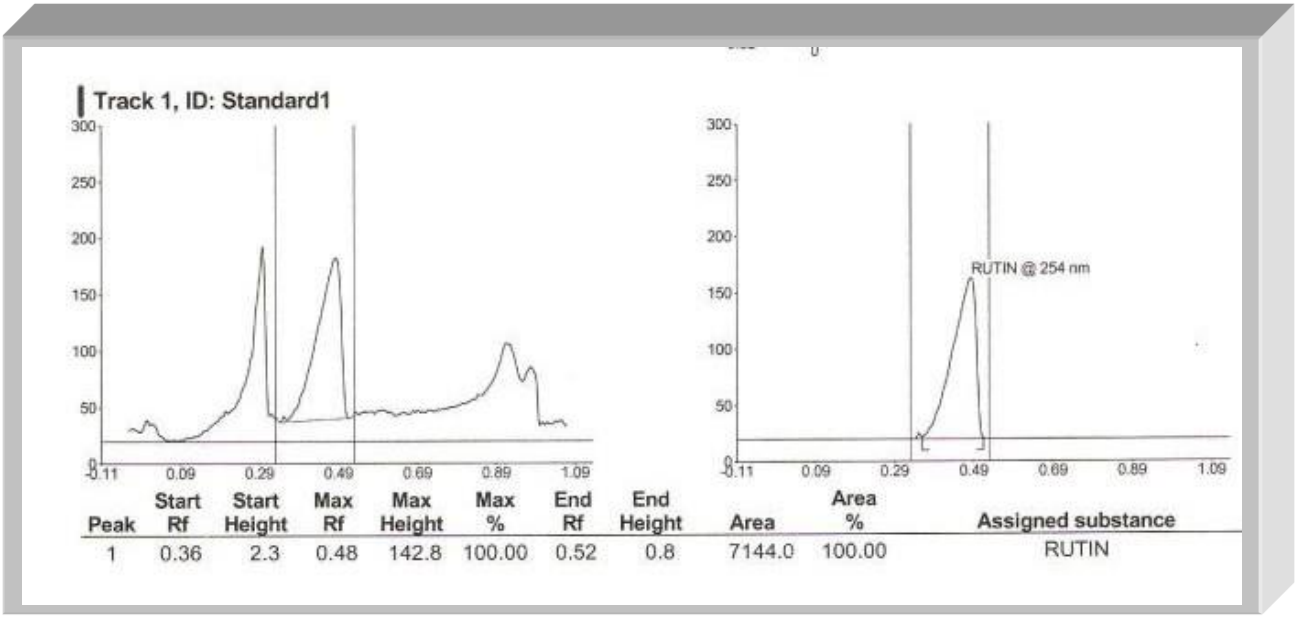

Figure 3: HPTLC profile for the detection and quantitative estimation of Rutin content of *Fagopyrum tataricum* form Vinayaka

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