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SIMULTANEOUS HPTLC METHOD FOR DETERMINATION OF GALLIC ACID, VANILLIC ACID AND SYRINGIC ACID IN AGRO-INDUSTRIAL WASTE OF DATE FRUITS

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Keywords:

Phoenix dactylifera, Agro-industrial waste, Valorization, Phenolic acid, HPTLC

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ABSTRACT: *Phoenix dactylifera* is a medicinally important plant. Fruits are traditionally used for the nutritional purpose. Date seeds are the rich sources of phenolic acids which exhibits strong antioxidant property. HPTLC is a simple, versatile method and it provides visualization of separated constituents. The advantage of this method is this method requires a small amount of sample for analysis. The present work describes a simple, precise, and accurate HPTLC method for quantification of gallic acid, syringic acid, and vanillic acid present in seeds of date fruits. The analysis was done on CAMAG ATS4 automatic TLC sampler system. The chromatographic separation was carried out on precoated silica gel 60 F254 aluminum plates, using a mixture of toluene: ethyl acetate: formic acid (4:4:2 v/v) as mobile phase. The evaluation of spots was carried out at 254 nm using Camag TLC scanner 3. The aimed compounds were resolved satisfactorily with R_f 0.47, 0.53, and 0.60 for gallic acid, syringic acid, and vanillic acid, respectively. The developed method was validated by parameters linearity, accuracy, precision, and repeatability. The correlation coefficients for each marker were greater than 0.99, which meets the validation acceptance criteria. The precision was carried out by intra-day and inter-day precision. Repeatability was measured by scanning spots six times without changing the position of the plate. *Phoenix dactylifera* seeds are the rich source of phenolic acids, which attribute to antioxidant properties of *Phoenix dactylifera* seeds. The seeds are the cheaper source that could be useful in medicinal and nutritional areas due to the presence of bioactive phytoconstituents.

INTRODUCTION: The palm date tree (*Phoenix dactylifera*) has played an important role in providing valuable food for middle-east and North Africa region and all over the world [1]. Date fruits are considered for disease prevention through antioxidant and anti-inflammatory activity [2]. The phytochemical study suggests the presence of phenolic acids, alkaloids, flavonoids, fatty acids, tocol and sterols in fruits as well as in seeds [3].

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Date fruit contains 4.4 to 11.4% dietary fibers; the consumption of fiber-containing fruits reduces the chances of hypertension, hypercholesterolemia, obesity, and diabetes [4]. In ancient days date seeds were applied to wounds, lesions, inflammation and used as expectorant and laxative [5]. Annually tons of date seeds are produced by date processing industries that contain valuable chemical constituents.

It majorly consists of fatty acids including capric, lauric, myristic, myristoleic, palmitic, stearic, oleic, linoleic, linolenic, arachidic acid [6]. Date seeds also contain a considerable amount of phenolic acids. Phenolics have attracted much attention due to their superb properties as an antioxidant, anti-inflammatory or antitumor properties [7]. Gallic acid is well known for its anti-oxidative, anti-aging,

anti-carcinogenic, anti-allergic, anti-inflammatory, anti-viral, antibacterial, and anti-arteriosclerosis activities [8]. Vanillic acid and syringic acid are reported to suppress liver inflammation and show hepatoprotective activity; syringic acid is also evaluated for its antidiuretic activity [9, 10]. The present study aims to develop and validate simultaneous High-performance thin-layer chromatography (HPTLC) method for the determination of Gallic acid, syringic acid and vanillic acid present in the extract of *P. dactylifera* seeds. Phenolic compounds are multipurpose bioactive agents present in various plant parts, and these compounds exhibit an important role as anti-oxidants. In recent years, the focus on natural antioxidants increased all over the world to replace synthetic antioxidants because of their unwanted effects on human health.

We have tried to valorize agricultural waste by developing a simple, isocratic HPTLC method for simultaneous estimation of gallic acid, syringic acid and vanillic acid in date seeds. The present study aims to quantify phenolic compounds from *Phoenix dactylifera* seeds by using HPTLC. In our previous study, we have developed a novel high-performance liquid chromatographic method for simultaneous estimation of phenolic acid in date seeds [11]. HPTLC has the advantage of providing visualization of the separated constituents of the sample. It requires very little sample for analysis [12]. HPTLC being versatile, easy, and economical, it was though worthwhile to develop a novel HPTLC method for simultaneous quantification of gallic acid, syringic acid, and vanillic acid in date seeds.

MATERIALS AND METHODS: Phyto-constituents used in the study were Gallic acid, Syringic acid, and vanillic acid of purity 95% were procured from Chemdyes Corporation, Rajkot, India. Toluene, Ethyl acetate, formic acid are procured from Thermo Fischer Scientific, India Pvt. Ltd. Mumbai. A mobile phase was prepared by a mixture of toluene, ethyl acetate, and formic acid in ratio of 4:4:2 in a twin trough chamber. The stock solutions of gallic acid, syringic acid, and vanillic acid were prepared so as to give a concentration of 1 mg/mL in methanol. Suitable standard mixture solutions were prepared by dilution from the above stock solutions. For sample preparation, about 3 g seed powder was extracted with 100 ml of methanol by refluxing it for 20 min. The solution was filtered using Whatman filter paper. The final concentration of the sample solution was 300 µg/10µL.

Chromatography: The analysis was performed on a CAMAG ATS4 automatic TLC sampler system with CAMAG development chamber of dimension 20*10 cm and a TLC scanner 3. The TLC auto-sampler, fitted with a 100 µL syringe which is connected to inert gas, was used for a sample application. Development was conducted in the CAMAG development chamber. TLC Scanner 3 was used for quantification. Quantification was done using the UV/Vis densitometer in absorption mode (D_2 & W lamp) at 254 nm. The system was controlled using WINCATS (version 1.4.4.6337; Switzerland) planar chromatography software. HPTLC was performed on 8 cm × 10 cm silica gel 60 F254 plates from Merck. Samples and standards were applied to plates as 5 mm bands, 14 mm apart, 8 mm from the lower edge, and at least 12 mm from the left edge of the plates, by means of a CAMAG ATS4 automatic TLC sampler.

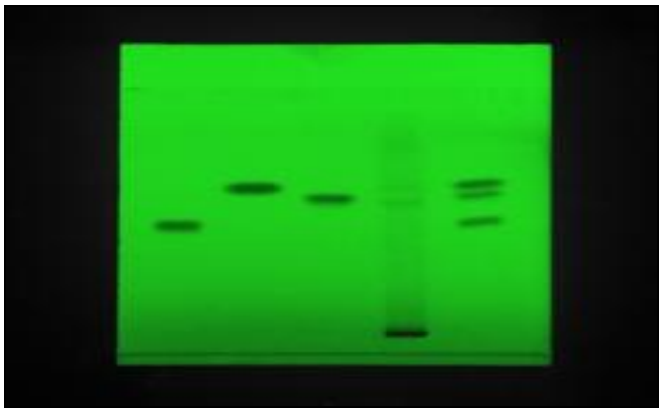

FIG. 1: DEVELOPED HPTLC PLATE FOR GALLIC ACID, SYRINGIC ACID AND VANILLIC ACID

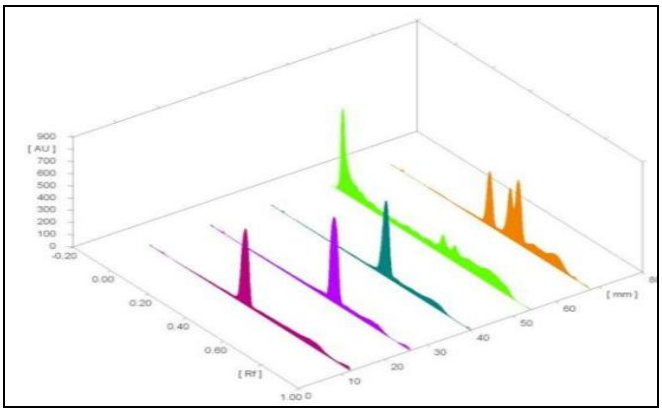

FIG. 2: 3D-OVERLAY AT 254 NM

Method Validation: The performance attributes of the optimized analytical method meet the requirements of the intended analytical application. The developed HPTLC method was validated according to ICH guidelines. The various validation parameters include linearity, accuracy, precision, and repeatability.

Linearity: Calibration curves of the three different markers were constructed individually by plotting the concentration levels with respect to the corresponding peak areas of each marker. The correlation coefficients for each marker were greater than 0.99, which meets the validation acceptance criteria. This concludes that the developed method is linear. The linearity was done in three replicates.

Accuracy: Accuracy is determined by means of recovery experiments in which the % mean recovery of each marker in the sample at three different levels (50%, 100%, and 150%) was determined. The analysis was performed in triplicates.

Precision: The precision parameter was assessed as 1) Intra-day precision 2) inter-day precision

Intra-Day Precision: Intra-day precision was found out by carrying out the analysis of the standards solutions at a concentration of 10-400 µg three times on the same day. The percentage relative standard deviation (RSD) was calculated.

Inter-Day Precision: Inter-day precision was found out by carrying out the analysis of the standard solution at a concentration of 10-400 µg for three different days, and the percentage RSD was calculated.

Repeatability: Repeatability is a measurement of the peak area was determined by spotting 300 µg/spot of gallic acid, syringic acid, and vanillic acid on a pre-coated TLC plate.

The separated spots were scanned six times without changing the position of the plate, and the percentage RSD was calculated.

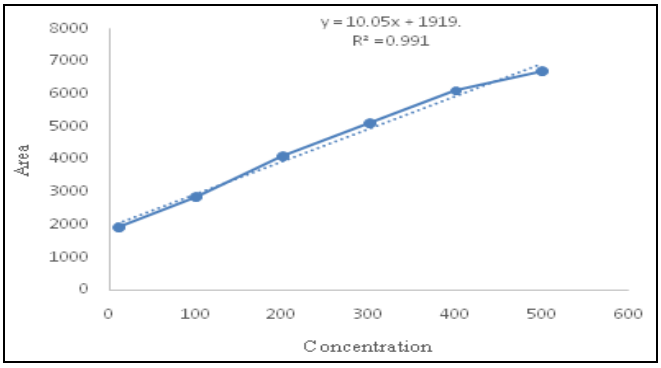

FIG. 3: CALIBRATION CURVE OF GALLIC ACID

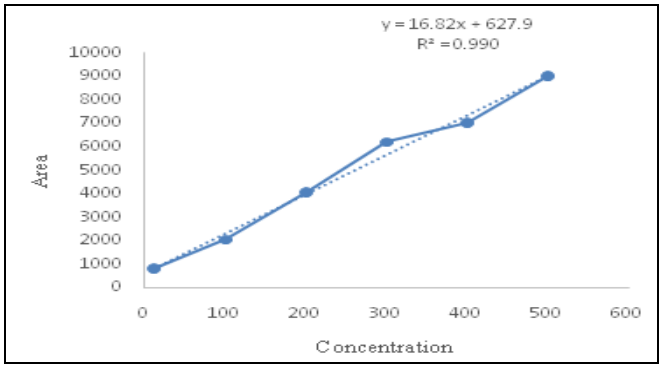

FIG. 4: CALIBRATION CURVE OF SYRINGIC ACID

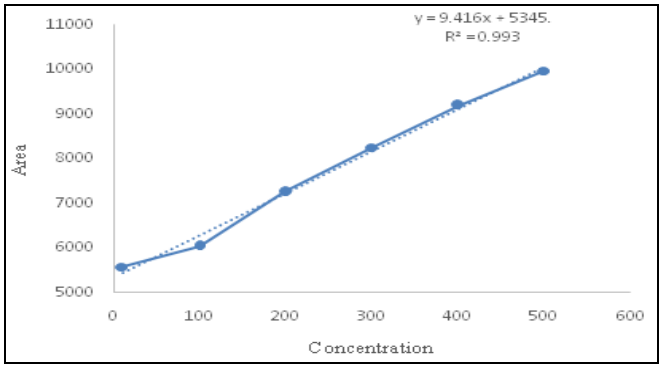

FIG. 5: CALIBRATION CURVE OF VANILLIC ACID

RESULTS: The content of the phytoconstituents in the formulation determined by the developed HPTLC method is summarized in **Table 1**. The results of all validation parameters are presented in **Table 2**.

TABLE 1: QUANTIFICATION OF MARKERS

Sample	Marker	% Content
Date seed extract	Gallic acid	0.0015
	Syringic acid	0.0005
	Vanillic acid	0.001

TABLE 2: THE RESULTS OF HPTLC METHOD VALIDATION PARAMETERS

S. no.	Parameters	Levels	Values		
			Gallic acid	Syringic Acid	Vanillic Acid
1	Linearity range (µg/band)	-	10-500	10-500	10-500
2	Equation of regression line	-	1919.345=10.056x+19	5345.614=9.4162x+53	627.9905=16.
			19.3	45.6	823x+627.94
3	Correlation coefficient (R^2)	-	0.9911	0.9906	0.9932
4	Accuracy (% Recovery)	50%	99	98	99
		100%	98	99.5	99
		150%	101	100	100
5	Intra-day precision(% RSD of area)	-	0.50	0.45	0.51
6	Inter-day precision (% RSD of area)	-	0.48	0.45	0.53
7	Repeatability	-	0.57	0.63	0.66

CONCLUSION: The present research work aims to valorize agricultural waste of date fruits. The enormous amount of date seeds that are thrown into the environment hold immense potential as raw material for bio-processing and augmentation of new range of products. Date seeds are the cheapest source of phenolic acids and can be used as a source of herbal antioxidants.

The developed HPTLC technique is reported for the first time for the quantification of phenolic compounds in date seeds. The advantage of this technique is, it requires a very small quantity of sample. The results imply that the technique is selective and re-producible for the simultaneous quantification of phenolic compounds present in the date seeds. The present novel HPTLC method will contribute to an accurate quantitative estimation of phenolic compounds in the date seeds which have not been quantified earlier. It concludes that the developed method offers several advantages like Simple, accurate, reproducible, robust, and cost-effective.

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CONFLICTS OF INTEREST: The author declares no conflicts of interest.

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