IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2018 Vol.:11, Issue:2

Phytochemical Screening of Ajwa Seed (*Phoenix dactylifera* L.) Extract by HPTLC Fingerprinting Method

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

IBRAHIM AFSAL V.T[1*], B. ARUL [2]

1. *Department of Pharmaceutics, Research scholar, Institute of Pharmaceutical sciences and Research centre, Bhagwant University, Sikar road, Ajmer, Rajasthan, India-305004.*

2. *Department of Pharmaceutics, Vinayaka mission College of Pharmacy, Salem, Tamil Nadu, India.*

Submission: 23 December 2017
Accepted: 30 December 2017
Published: 30 January 2018

Keywords: *Phoenix dactylifera* L., HPTLC, Rutin, Quercetin

ABSTRACT

Aim: The aim of this current investigation is to evaluate the various constituents present on ethanolic seed (Fruits of the date palm) extract of *Phoenix dactylifera* L. by HPTLC fingerprinting method. Materials and Methods: In the present study, an attempt has been made to develop simple, precise and accurate HPTLC method by using Rutin, Gallic acid and quercetin as a standard marker compound with the mobile phase of Toluene-Ethyl acetate-Formic acid Methanol (3:6:1.6:0.4). The detection of Rutin, Gallic acid and quercetin were performed at 254 nm respectively. Results: The ethanolic seed (Fruits of the date palm) extract of *Phoenix dactylifera* L. were subjected to generate HPTLC fingerprinting profile represented as the chromatogram. The solvent system used in the investigation was found to give compact spots for extracts at different Rf value. The detection of Rutin, Gallic acid and quercetin were performed at 254 nm respectively. Conclusion: From the present work we conclude that species of *Phoenix dactylifera* L. may be highly potential in biological activity due to preliminary screening of the samples revealed the presences of the high-value class of compound like the phenolic group as the major content in the seeds.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value [1, 2]. Hence, important chemical constituents from medicinal plants need to be investigated by HPTLC fingerprinting method. The plant *Phoenix dactylifera L.* belonging to the family of *Arecaceae*. Fruits of the date palm (*Phoenix dactylifera* L. Arecaceae) are very commonly consumed in many parts of the world and are a vital component of the diet in most of the Arabian countries. The date is one of the oldest known fruit crops and has been cultivated in North Africa and the Middle East for at least 5000 years (3). One of the miracles of Zamzam water is its ability to satisfy both thirst and hunger. More recently, in the last few decades, samples of Zamzam water have been collected by scientists and they have found certain peculiarities that make the water healthier, like a higher level of calcium [4].

MATERIALS AND METHODS:

Chemicals and reagents

All chemicals, reagents, and solvents used in the study were of analytical grade.

Plant material: The plant seeds materials were identified and authenticated by Dr. Pradeep, Botanist, Calicut University, Kozhikode. Voucher specimens were kept in our laboratory for future reference.

The extract/drug: The granulated dried seeds of *Phoenix dactylifera* L (500 g) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for using 450 ml of ethanol (95 % v/v) as the solvent. The extract was concentrated to dryness under reduced pressure and controlled temperature and dried in a desiccator (yield 75 g, 15 % w/w). The extract was suspended in Zamzam water and used for further experiments.

Preliminary phytochemical screening

The extract was screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests [5, 6].

Instrumentation

In the present work Camag HPTLC system equipped with Linomat 5 applicator, twin trough chamber (20x10cm, 0.2 mm thick) size, TLC scanner 3, Reprostar 3 with 12bit CCD camera for photo documentation, controlled by WinCATS- 4 software were used. All the solvents used were of high grade obtained from MERCK. All weighing was done on Precisa XB 12A digital balance.

Preparation of standards and sample solution:

2mg in 10 ml ➔ 0.2 mg /ml ➔ 200-µg/1000µl ➔0.2µg/µl

Mobile phase

The organic solvents such as toluene**:** ethyl acetate**:** formic acid**:** methanol

(3:6:1.6:0.4) was used as a mobile phase

Chamber used for mobile phase

Camag twin trough chamber (20 x 10 cm)

Chamber saturation

Chamber saturation was done for 5 minutes.

Stationary phase

TLC aluminum sheet precoated with silica gel 60 F254, (20x10cm) was used as stationary phase, obtained from MERCK.

Procedure

The Ethanolic seeds extract solutions were prepared. The TLC plate was activated by heating at 1200C for about 30 min prior to use. Ethanolic extract solution (2 µl), standard solution (0.2µg/µl) were applied in duplicate, as tracks 8, with a band length of 6.0 mm each on a precoated silica gel 60 F254 TLC plate, with Linomat V applicator using a Hamilton syringe (100µl).Mobile phase used toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4). No prewashing of the plate was done. Chamber saturation time was 5 minutes. The TLC plate

was kept for development to a migration distance of 77 mm. Post derivatization had been done with vanillin-phosphoric acid. The derivatized plate was dried in hot air oven at 60 ^{0}c for 5 minutes and scanned at 254 nm, band length 6.0 mm, slit dimension 6.00x0.45mm, micro, scanning speed (20mm/s) and source of radiation was Deuterium and Tungsten lamps respectively. The Rf and peak area of the spots were interpreted by using the software. The derivatized plate was photo documented under 254 nm light using Camag Reprostar 3, equipped with 12 bit CCD camera.[7,8]

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of fractions of *Phoenix dactylifera L.* shows the presence of steroids, alkaloids, flavonoids, glycosides, saponins, tannin and carbohydrate.

Table No 1: Preliminary phytochemical screening

Constituents	**Test**	***Phoenix dactylifera* L.,**
Carbohydrates	• Molisch Test • Fehling's Test • Benedict's test: • Barfoed's test:	+ + + +
Alkaloids	• Dragendroff's Test • Wagner's test • Mayer's Test • Hager's Test	+ + + +
Steroids and Sterols	• Liebermann Burchard test • Salkowski test	- +
Glycosides	• Legal's test • Baljet test • Borntrager test • Killer Killani test	+ + + +
Saponins	• Foam test	+
Flavonoids	• Shinoda test	+
Tri-terpenoids	In the test tube, 2 or 3 granules of tin+2ml of thionyl chloride solution and test solutions added.➔Pink color	+

HPTLC fingerprinting of *Phoenix dactylifera L.* seeds extract

The ethanolic seeds extract of *Phoenix dactylifera L.* were subjected to generate HPTLC fingerprinting profile represented as the chromatogram. The solvent system used in the investigation was found to give compact spots for extracts at different Rf values table 9-12

Table No 2: Observation of Rf values and % area of the chromatogram of rutin gallic acid at 254nm (std)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	22.8	0.02	89.9	8.13	0.07	1.4	4.57	4.57	Unknown*
2	0.08	0.3	0.13	147.1	13.30	0.21	14.2	1.26	14.26	**Rutin**
3	0.21	14.4	0.24	45.0	4.07	0.25	43.0	1.84	1.84	Unknown*
4	0.25	43.5	0.26	54.1	4.89	0.32	0.0	1.82	1.82	Unknown*
5	0.37	9.1	0.38	10.5	0.95	0.41	0.1	0.30	0.30	Unknown*
6	0.45	3.2	0.46	12.3	1.11	0.49	1.6	0.33	0.33	Unknown*
7	0.55	1.6	0.59	19.7	1.79	0.60	19.2	0.66	0.66	Unknown*
8	0.64	14.3	0.70	320.3	28.96	0.74	123.4	22.06	22.06	**Gallic acid**
9	0.74	124.4	0.84	391.1	35.36	0.96	0.1	53.99	53.99	**Quercetin**
10	0.97	0.8	0.98	15.9	1.43	0.98	3.7	0.18	0.18	Unknown*

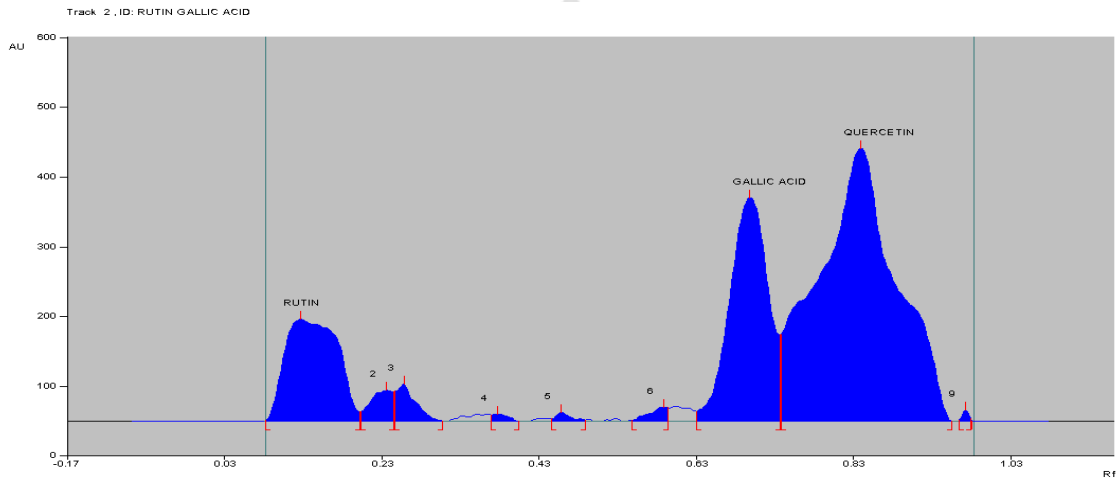

Fig. 1: Observation of Rf values and %area of the chromatogram of rutin gallic acid at 254nm (std)

Table No. 3: Observation of Rf values and %area of the chromatogram of Quercetin at 254nm (std)

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	4.5	0.02	117.0	13.98	0.07	40.7	3590.9	13.87	Unknown*
2	0.18	17.3	0.19	19.4	2.32	0.24	0.0	417.1	1.61	Unknown*
3	0.28	3.2	0.30	16.1	1.93	0.32	0.9	171.7	0.66	Unknown*
4	0.32	2.2	0.33	18.6	2.22	0.34	0.0	90.3	0.35	Unknown*
5	0.38	0.0	0.40	12.1	1.44	0.41	2.9	128.5	0.50	Unknown*
6	0.41	3.1	0.44	20.3	2.43	0.47	12.5	396.1	1.53	Unknown*
7	0.47	13.6	0.47	23.1	2.76	0.49	2.5	161.9	0.63	Unknown*
8	0.50	0.6	0.51	19.0	2.27	0.54	0.0	199.5	0.77	Unknown*
9	0.55	0.7	0.56	22.0	2.63	0.58	2.6	236.9	0.91	Unknown*
10	0.70	16.3	0.74	37.2	4.44	0.75	26.7	656.5	2.54	Unknown*
11	0.75	16.3	0.85	532.0	63.57	0.96	0.2	19845.0	76.64	**Quercetin**

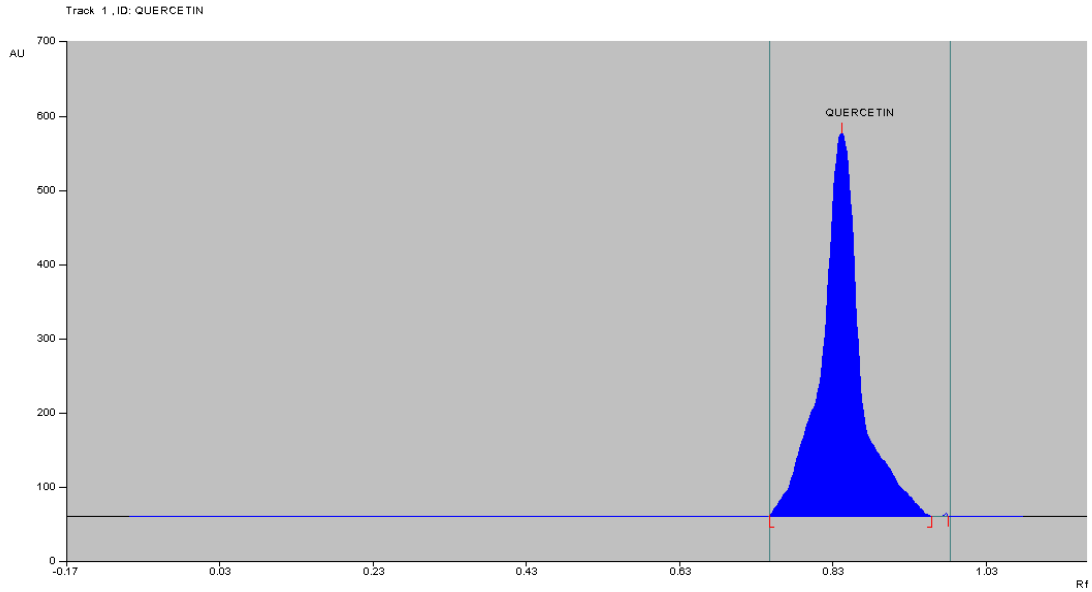

Fig. 2: Observation of Rf values and % area of the chromatogram of Quercetin at 254nm (std)

Table No 4: Observation of Rf values and %area of the chromatogram of ethanolic seeds extract of *Phoenix dactylifera* L

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	7.4	0.01	198.7	27.84	0.04	140.3	4766.7	23.36	Unknown*
2	0.04	140.6	0.05	151.0	21.15	0.10	65.3	4134.6	20.27	Unknown*
3	0.11	65.2	0.12	76.3	10.69	0.15	66.2	1879.1	9.21	**Rutin**
4	0.15	66.7	0.19	101.1	14.16	0.25	25.0	4180.9	20.49	Unknown*
5	0.26	24.2	0.30	34.4	4.82	0.36	10.9	1395.4	6.84	Unknown*
6	0.41	7.6	0.45	38.7	5.43	0.52	2.5	1295.3	6.35	Unknown*
7	0.80	0.0	0.84	44.4	6.22	0.86	32.3	1019.6	5.00	**Quercetin**
8	0.86	32.5	0.89	52.2	7.31	0.92	11.7	1274.6	6.25	Unknown*
9	0.92	11.8	0.94	17.0	2.38	0.98	0.6	456.0	2.23	Unknown*

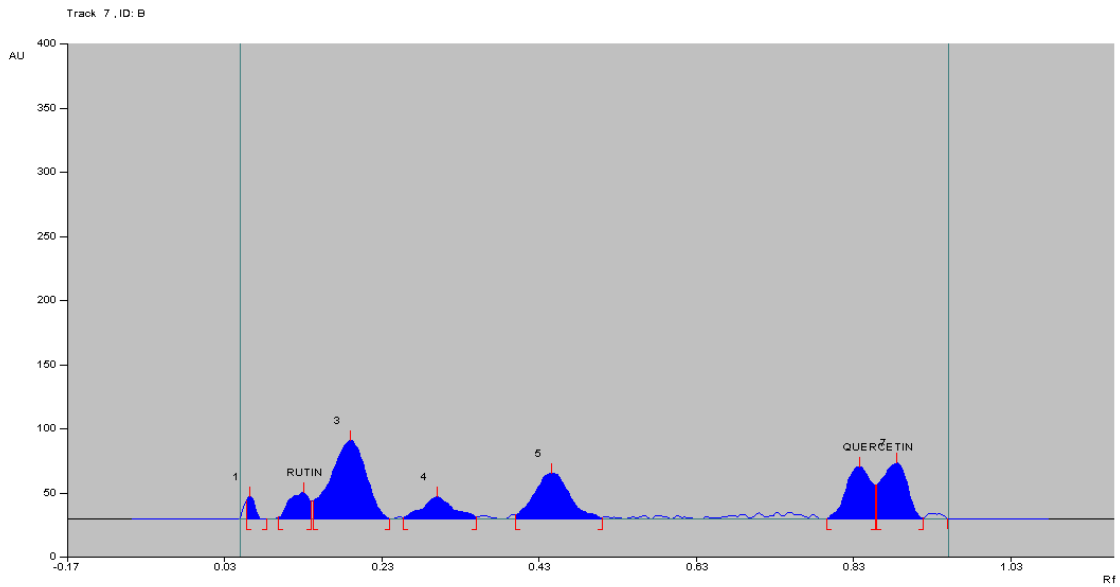

Fig. 3: Observation of Rf values and % area of the chromatogram of ethanolic seeds extract of *Phoenix dactylifera* L

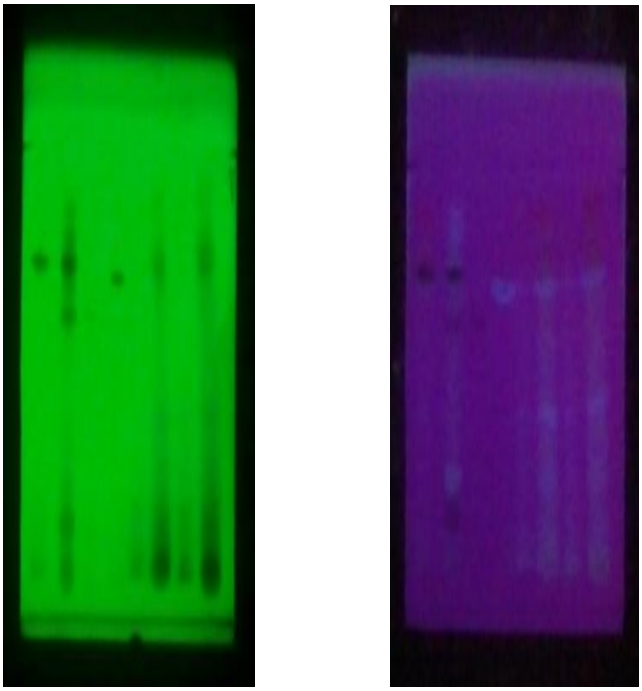

Fig. 4: Photo documentation of Standard and sample

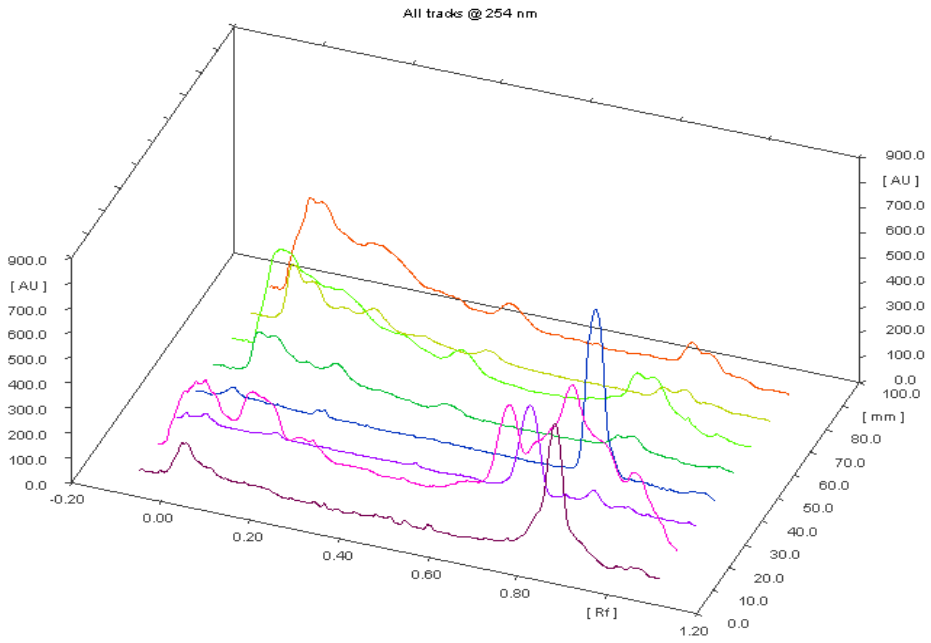

Fig. 5: 3D HPTLC Chromatography (Standard and seeds extract)

REFERENCES

1. Sandhu, D.S., Heinrich, M The use of health foods, spices and other botanicals in the Sikh community in London. Phytotherapy Res., 2005 19,633-42

2. Gupta, M.P., Solis P.N., Calderon, A.I., Guionneau-Sinclair, F., Correa, M., Galdames, C., Guerra, Espinosa, A., Alvenda, G.I., Robles, G., Ocampo, R Medicinal ethnobotany of the tribes of Bocas del Toro, Panama. J Ethnopharmacol, (2005) 96, 389-401

3. M Al-Farsi, C Alasalvar. M.Al-Abid, K Al-Shoaily, M Al-Amry. and F Al-Rawahy. "Compositional and functional characteristics of dates, syrups, and their by-products". Food Chemistry, 104, 943–947, (2007).
4. http://islamqa.info/en/6831
5. Kokate CK. In: Practical Pharmacognosy, Preliminary Phytochemical Screening, first ed., Vallabh Prakashan, New Delhi, 1986; 111.
6. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol. 1983;32:1141–1148
7. Rajasekaran.A, Arivukkarasu.R., and Archana.D.* HPTLC Method for estimation of Gallic acid and Rutin in Haritaki -An Ayurvedic Formulation. 2011; Vol. 3, No.2, pp 986-999.
8. P.Deepa*, S.Murugesh, R.Arivukkarasu HPTLC analysis of *Dolichandrone atrovirens* (*Sprague*) plant bark.2013; vol3(1) 1019-1029