IJPSR (2016), Vol. 7, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 June, 2016; received in revised form, 26 August, 2016; accepted, 13 September, 2016; published 01 December, 2016

PHYTOCHEMICAL EVALUATION AND HPTLC ANALYSIS OF *PHOENIX SYLVESTRIS* FRUIT EXTRACT AT TWO RIPENING STAGES

Gangaraju Rapaka *, Archana Bollavarapu and Raghavarao Tamanam

Department of Biochemistry, Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Phoenix sylvestris, HPTLC, CAMAG LINOMAT 5, unripened, ripened.

Correspondence to Author:
Gangaraju Rapaka

Research scholar,
Department of Biochemistry,
Andhra university, Visakhapatnam - 530003, Andhra Pradesh, India.

E-mail: gangaraju.r@yahoo.com

ABSTRACT: *Phoenix sylvestris* Roxb, commonly known as date sugar palm or wild date palm, grows naturally in the tropics and subtropics of the Indian subcontinent. The present study was aimed for phytochemical evaluation and to develop the high performance thin layer chromatography (HPTLC) fingerprint profile of methanolic extract of *P. sylvestris* at two ripening stages. The powdered plant material was extracted using methanol. Quantitative analysis of phytochemicals and HPTLC fingerprinting analysis were carried out. The quantitative analysis of the phytochemicals revealed the presence of various amounts of total phenolic, total flavonoids and total tannins. These phytochemicals were significantly higher in the unripened stage than ripened stage. The HPTLC fingerprinting analysis was carried for phenolic compounds by using Camag Linomat 5 instrument which revealed the presence of phenolic compounds in two stages of *P. sylvestris.* HPTLC data revealed that unripened extract has high quality phytochemicals than ripened extract. The phytochemical changes of *P. sylvestris* fruit ripening clearly explained its growth, development and ripening stages.

INTRODUCTION: The importance of wild plants in subsistence agriculture in the developing world as a food supplement and as a means of survival during times of drought, famine has been overlooked. The consumption of wild plants seems more common and widespread in food insecure areas where a wide range of species is consumed. Local people know about the importance and contribution of wild plants in their daily diet. Wild plant species continue to provide important energy and micronutrient needs during drought and social and political unrest.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(12).5067-71

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (12).5067-71

In spite of the role of edible wild plants in the bridging period of food shortages and providing dietary variety, very little attention has been given to the inventory and conservation of such species. Compositional knowledge of these plant materials could help in developing technological processes to make the plant material edible and more digestible.[1]

Phoenix sylvestris Roxb., commonly known as date sugar palm or wild date palm, grows naturally in the tropics and subtropics of the Indian subcontinent and is also cultivated for its edible fruits and to provide ingredients for sweets and beverages. Fruits of the plant are used to treat back pain, stomachache, toothache, headache, arthritis, pain of buttocks, fever, piles, nervous debility, and as a nervine tonic, restorative, sedative in ethnomedicine. [2, 3, 4, 5]

The present study was aimed to perform the Quantitative Analysis of phytochemicals of *Phoenix sylvestris* at two ripening stages. Fruit extraction has been done to identify the chemical constituents and HPTLC fingerprinting of fruit extracts has been performed which may be used as markers for quality evaluation and standardization of the drug.

MATERIALS AND METHODS:

Collection of Plant Material: Mature, unripened fruits of *P.sylvestris* were collected from Ramannapalem, West Godavari District, Andhra Pradesh.

Extraction: The pulp of the fruits were separated from the pit and grounded. The powdered pulp (230g) was macerated using 1000mL of methanol with occasional stirring at room temperature. The extract was then filtered using sterilized cotton filter. A rotary evaporator was used to remove the solvent at 40° C and 50 r.p.m under reduced pressure.[6]

Quantitative analysis of Total Phenolics: The amount of total phenolics in the extract was determined according to the Folin- ciocalteu procedure with slight modifications.[7] Samples (100µl) were introduced into test tubes, 2.5 ml of Folin ciocalteu reagent and 2ml of sodium carbonate (7.5%) were added. The tubes were mixed and incubated for 15 minutes at 45^0C. Absorption at 765 nm was measured. Total phenolic contents were quantified through a calibration curve obtained from measuring the absorbance of known concentrations of a Gallic acid standard. The results were expressed as mg of Gallic acid equivalents (GAE) per gram of extract. All samples were analyzed in triplicates.

Quantitative analysis of Total Flavonoids: Total flavonoid content was measured by the aluminum chloride colorimetric assay. [8] About 100µl of plant extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml 5 % $NaNO_2$ were added. After 5 min, 0.3 ml 10% $AlCl_3$ was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and Absorbance of the mixture was determined at 510 nm versus the prepared blanks. The total flavonoid content was calculated using standard Quercetin calibration curve (range 40-200µg/ml) and the results were expressed as milligrams of Quercetin equivalents (QE) per gram of extract. All samples were analyzed in triplicates.

Quantitative analysis of Total Tannin: The Total tannin content was determined using the Folins-ciocalteu method with slight modifications.[9] Briefly, 100µl of plant extract was taken in test tubes, 1.5 ml of Folins- ciocalteu reagent and 0.5 ml of sodium carbonate (20%) were added. The absorbance of the sample was measured in a spectrophotometer at 725 nm. The total tannin content was calculated using standard tannic acid calibration curve (range 20-100µg/ml) and the results were expressed as milligrams of tannic acid equivalents (TAE) per gram of extract. All samples were analyzed in triplicates.

HPTLC Analysis: HPTLC was performed on silica gel 60 $f_{254,}$ 20X10 cm HPTLC plates (Merck, Germany), with ethyl acetate: methanol: formic acid: water [20:2.5:0.5:2 (v/v)] as a mobile phase. The standard (Quercetin, Chatechin, p- Coumaric acid, and Caffeic acid) solutions and two fruit extracts (5.0 µL of each concentration 1 mg/mL) were applied to the plates as 10 mm bands, sample application with CAMAG-Linomat IV automated spray on band applicator equipped with a 100 µL syringe and operated with following settings: band length 10 mm, application rate 10 sec/ µL, distance between 4 mm, distance from the plate side edge1.5 cm and distance from the bottom of the plate 2 cm. [10, 11] CAMAG TLC Scanner 3 was used to densitometrically to quantify the bands using WIN CATS software (Version 4 X). The scanner operating parameters were: (Mode: absorption / reflection; Slit dimension; 5 x 0.1 mm; scanning rate: 20 mm/s and monochromator band width: 20 nm at an optimized wavelength at 254 nm).

RESULTS AND DISCUSSION:

Quantitative analysis of phytochemicals: The total phenolics assessed using colorimetric Folin–ciocalteu phenol's method showed that the Unripened stage possessed a significantly higher amount of phenolics than a ripened stage with mean values of 8.76±0.41 and 5.39±0.72 mg GAE/g FW given in the **Table 1**. Phenolic compounds are crucial for plants growth and

reproduction, and are produced as a response to environmental factors (light, chilling, pollution, etc.) and to defend injured plants. [12] Our data are in agreement with previous studies indicating that the phenolic content in fruit of date palm decreases as ripening progresses. [13, 14, 15] Similar findings have been reported by Aziz *et al.*,[16] a general decline in total phenolic content in the pulp of banana fruit during ripening. An apparent gradual decrease in the total fruit phenolic concentrations, which is connected with an increased polyphenol oxidase activity during the ripening of the fruit.

TABLE 1: QUANTITATIVE ANALYSIS OF DIFFERENT PHYTOCHEMICALS OF *P. SYLVESTRIS* FRUIT EXTRACTS

Fruit pulp of *Phoenix Sylvestris*	Total Phenol (mg GAE/gm FW) Extract	Total Tannin (mg TAE/gm FW) Extract	Total Flavonoid (mg QE/gm FW) Extract
Ripened	5.39±0.72	10.54±1.84	24.93±1.12
Unripened	8.76±0.41	48.17±1.32	61.6±0.90

The Total Flavonoid content was found to be higher in unripened stage than ripened stage with mean values of 24.93±1.12 and 61.6±0.9 mg GAE/g FW. Flavonoids are well known for their antioxidant activity. Antioxidants are specific compounds that protect human, animal and plant cells against the damaging effects of free radicals (reactive oxygen species, ROS). The decline in flavonoid content over the course of maturation was also observed in the oil palm fresh fruit bunches.[17] The higher concentrations of flavonoid compounds in younger (unripened stage) fruit as compared to those in semi-ripe and fully-ripe fruits could be explained by the fact that in the later stages of ripening different phenolic acids might have condensed to form complex phenolic compounds such as tannins and lignin etc.[18]

The Total Tannin content was observed to be high in unripened stage than ripened stage with mean values of 48.17±1.32 and 10.54±1.84 mg TAE/g FW. The amount of total tannin content decreased significantly during fruit ripening. The decline tannin content may be due to increasing enzyme activity such as anthocyanin synthase (AS) and 3-glycosyl transferase (3GT) in the formation of anthocyanins.[19]

HPTLC finger printing: The HPTLC analysis of *Phoenix sylvestris* fruit pulp extract at 2 different ripening stages. The results showed using Ethyl acetate: methanol: formic acid: water (20:2.5:0.5:2 (v/v). The chromatogram showed the percentage area after scanning and visualizing the plates in absorbance mode at 256nm (after spraying with anisaldehyde sulphuric acid reagent). Methanolic fruit extracts of *P.sylvestris* used for HPTLC analysis. Four phenolic markers have been done in the same way for the comparison of phenolic compounds in the extract (shown in **Fig. 1** and **Table 2**) Three dimensional representation of HPTLC chromatogram shown in **Fig. 2.**

TABLE 2: HPTLC FINGER PRINT OF STANDARD PHENOLIC ACID MARKERS SHOWED MAJOR PEAKS AT UV 256 nm

Name of the track	Rf	Maximum Height	Area (%)
Catechine	0.28	470.8	89.06
p-Coumaric acid	0.28	455.1	87.94
Caffeic acid	0.43	411.9	83.26
Quercetin	0.29	707.1	92.78

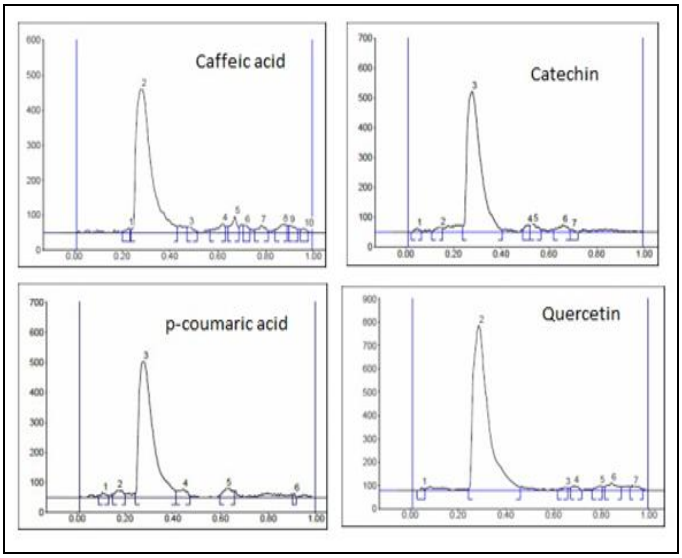

FIG. 1: HPTLC CHROMATOGRAM OF DIFFERENT STANDARD PHENOLIC MARKERS

The HPTLC finger print of ripened extract showed 7 peaks with different Rf values ranging from 0.06 to 0.73 at UV 256 nm (shown in **Fig 3**, **Table 3**). The major peak at Rf 0.32 constitutes 72.79% of the total area of the peak and peak at Rf 0.38 constitute 27.61%.

The HPTLC finger print of unripened extract observed 4 peaks with different Rf values ranging from 0.33 to 0.89 (Depicted in **Fig. 3, Table 4**) moreover, the major peak at Rf 0.33 constitutes 97.06% peak area at UV 256 nm.

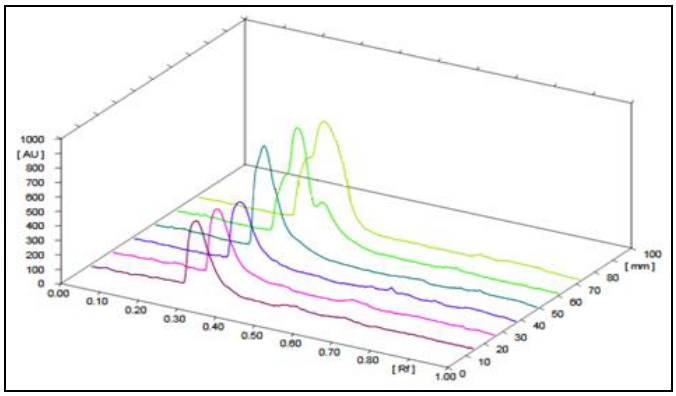

FIG. 2: THREE DIMENSIONAL REPRESENTATION OF HPTLC CHROMATOGRAM OF *P. SYLVESTRIS* METHANOLIC EXTRACTS WITH STANDARD PHENOLIC MARKERS MEASURED AT 256 nm

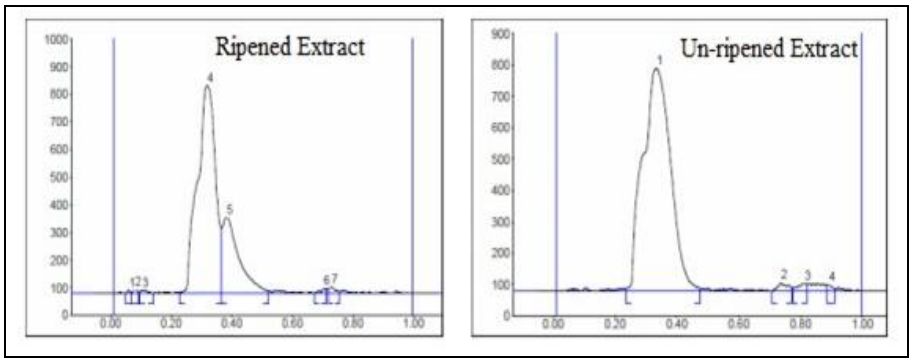

FIG. 3: HPTLC ANALYSIS OF *P. SYLVESTRIS* FRUIT EXTRACT AT TWO RIPENING STAGES

TABLE 3: HPTLC FINGERPRINT OF RIPENED FRUIT EXTRACT OF *P. SYLVESTRIS* AT 256 nm

Peak	Maximum Rf	Height	Area	% Area
1	0.06	11.9	80.0	0.18
2	0.08	16.8	151.0	0.34
3	0.10	11.0	193.0	0.44
4	0.32	753.1	32239.4	72.79
5	0.38	276.8	10995.7	24.79
6	0.70	19.0	254.7	0.58
7	0.73	22.8	374.7	0.85

TABLE 4: HPTLC FINGERPRINT OF UNRIPENED FRUIT EXTRACT OF *P. SYLVESTRIS* AT 256 nm

Peak	Maximum Rf	Height	Area	% Area
1	0.33	709.6	46092.0	97.06
2	0.74	28.6	618.6	1.30
3	0.81	23.1	510.1	1.07
4	0.89	20.7	267.2	0.56

The analysis and quality control of herbal medicines are moving a step ahead towards an integrated and comprehensive direction, in order to tackle the complex nature of herbal medicines.[20] After performing the HPTLC analysis, it can be suggested that extract contained different compounds. HPTLC analysis showed that the unripened stage has quality phytochemicals than the ripened stage. Quantitative analysis showed that unripened stage has high amount of phytochemicals than ripened stage. This indicates the variations in chemical constituents of both stages. Two ripening stages show nearly similar with the Rf value of Quercetin furthermore, these extracts may have Quercetin derivatives.

CONCLUSION: The present study is the first report on thorough phytochemical evaluation and comparative HPTLC fingerprint profiles of unripened and ripened methanolic extracts of *P. Sylvestris* HPTLC data revealed that unripened stage has high quality phytochemicals than ripened stage. The phytochemical changes of *P. Sylvestris* extract clearly explained its growth and development in ripening stages. These results provide important knowledge of changes in total phenolics, tannins and flavonoids content during fruit growth and maturity. Unripened fruit can be a good source of bioactive compounds. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat a variety of diseases. In addition, these data are very useful for determination of the fruit quality. HPLC analysis is needed for the further studies.

ACKNOWLEDGEMENTS: The authors are highly thankful to DST-PURSE Programme, Andhra University, Visakhapatnam for providing the instrumentation and experimental infrastructure facility.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

REFERENCES:

1. Salvi J and Katewa S S: Preliminary assessment of nutritional value of palm heart of *Phoenix sylvestris* (Roxb.). International Food Research Journal 2001; 21 (5): 2051-2054.
2. Ghani A. Medicinal Plants of Bangladesh: Chemical Constituents and Uses. Dhaka: Asiatic Society of Bangladesh. 2003.
3. Acharya E and Pokhrel B: Ethno-medicinal plants used by Bantar in Bhaudaha, Morang, Nepal. Our Nat. 2006; 4, 96–103. doi: 10.3126/on. v4i1. 508.
4. Harney N V: Ethno medicinal plants diversity of Bhadrawati Tahsil of Chandrapur district, Maharashtra, India. Int. J. Sci. Res. Pub. 2013; 3:8.
5. Manikandan S: Ethno medicinal flora of Ivanur Panchayatin Cuddalore district, Tamil Nadu, India. Int. Res. J. Plant Sci. 2013; 3, 39–46.
6. Shafiullah Shajib Md, Saleha Akter, Tajnin Ahmed and Mohammad Zafar Imam: Antinociceptive and neuropharmacological activities of methanol extract of *Phoenix sylvestris* fruit pulp. Frontiers in Pharmacology, 2015; 6.
7. Shoib A Baba and Shahid A Malik: Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume Journal of Taibah University for Science, 2015; 9, 449–454.
8. Sharma Ira, Mathur Manisha, Singh G P and Rishi Anirudha: Quantitative estimation of phenolic and flavonoid content and antioxidant activity of various extracts of different parts of *Plumbago zeylanica* Linn. Int. J. Drug Dev. & Res., April - June 2014; 6 (2): 136-140.
9. Bhavana G P, Chandrika R and Thara Saraswathi K J: Quantitative determination of secondary compounds in populations of *Eryngium foetidum* L. from India, INT J Curr Sci, 2013; 9: E 24-28.
10. Wagner H and Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin: Springer; 2001.
11. Males Z, Plazibat M, Bilusiae V, Vundae Z I, Hazler KP: Thin-layer chromatographic analysis of flavonoids, phenolic acids, and amino acids in some Croatian Hypericum Taxa. J. Planar. Chromatogr. 2004; 17:280–285.
12. Valentine I K, Maria V K, Bruno B: Phenolic cycle in plants and environment. J. Mol. Cell Biol., 2003; 2: 13-18.
13. Awad M A, Al-Qurashia A D and Mohamed S A: Antioxidant capacity, antioxidant compounds and antioxidant enzyme activities in five date cultivars during development and ripening. Sci. Hortic. 2011; 129:688–693.
14. Eid M N S, Al-Awadi B, Vauzour D, Oruna-Concha M J and Spencer J P E: Effect of cultivar type and ripening on the polyphenol content of date palm fruit. J. Agric. Food Chem. 2013; 61:2453–2460.
15. Hammouda H, Ch_erif J K, Trabelsi-Ayadi M, Baron A and Guyot S: Detailed polyphenol and tannin composition and its variability in Tunisian dates (Phoenix dactylifera L.) at different maturity stages. J. Agric. Food Chem. 2013; 61:3252–3263.
16. Aziz A B A, Abdul Wahab F K and Elghandour M A: Effect of different storage temperature on phenolic compounds in banana and mango fruit. Hort. Sci. 1976; 4:309–315.
17. Hazir M H M, Shariff A R M and Amiruddin M D: Determination of oil palm fruit bunch ripeness-based flavonoids and anthocyanin content. Ind. Crops Prod.2012; 36:466–475.
18. Ben-ahmed C, Ben-rouina B, Sensoy S, Boukhriss M: Saline water irrigation effects on fruit development, quality, and phenolic composition of virgin olive oils, CV. Chemlali. J. Agric. Food Chem. 2009; 57, 2803–2811.
19. Robbins M P, Bavudage A D, Strudwicke C and Morris P: Genetic manipulation of condensed tannins in higher plant. Plant Physiol.1998; 116 1133–1144.
20. Rakesh S. Shivatare, Dheeraj H. Nagore, Sanjay U. Nipanikar, 'HPTLC' an important tool in standardization of herbal medical product: A review. Journal of Scientific and Innovative Research; 2013; 2 (6): 1086-1096.

How to cite this article:
Rapaka G, Bollavarapu A and Tamanam R: Phytochemical evaluation and HPTLC analysis of *Phoenix sylvestris* fruit extract at two ripening stages. Int J Pharm Sci Res 2016; 7(12): 5067-71.doi: 10.13040/IJPSR.0975-8232.7(12).5067-71.