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Qualitative and quantitative estimations of phytoconstituents from *phoenix sylvestris* roots

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ABSTRACT

Phoenix sylvestris is commonly known as Indian date and is native to India. It is traditionally important and known for its nutritional values throughout the world. It is a rich source of carbohydrate, phenols, amino acids, flavonoids, tannins, alkaloids, terpenoids, dietary fibers, essential vitamins and minerals. Different parts of the plant exhibit diverse medicinal properties such as being antipyretic, cardiotonic, laxative, diuretic and antioxidant. In the present study The root part was selected. Morphological characteristic were performed and transverse section was taken and observed under microscope. Extraction was done by using methanol as solvent in Soxhlet apparatus. Extract was subjected for different phytochemical constituent. Total flavonide and terpenoid were determined and it was observed that *Phoenix sylvestris* root contains 7.25% total flavonide and 0.25% terpenoide.

Keyword: *Phoenix sylvestris* root, Methanolic extract, Flavonide, Terpenoid.

INTRODUCTION

Since the inception of mankind, human beings have been using traditional plants for the management of various ailments. One traditional plant, *Phoenix sylvestris*, is widely known as Wild date palm. The date palm (*Phoenix sylvestris*) has been known and used for medicinal purposes since ancient times [4]. Date palm tree (*Phoenix sylvestris* L.) is considered as one of the oldest and main staple and ancient crops in Southwest Asia and North Africa. Besides, dates can be grown in Australia, Mexico, South America, southern Africa, and the United States, especially in southern California, Arizona, and Texas. Date palm tree belongs to Arecaceae family (Angiosperms, monocotyledon) consisting of about 200 genera and more than 2,500 species. Phoenix (Coryphoideaephoeniceae) is one of the genera with approximately 14 species which are native to the tropical or subtropical regions of southern Asia

or Africa, including Phoenix sylvestris L The name of the species dactylifera means "finger-bearing" which refers to the fruit clusters produced by this plant. Dactylifera is a grouping of the Greek word dactylus, means "finger," and the Latin word ferous, mean "bearing". Very recently the whole genome of date palm tree was re-sequenced yields insights into diversification of a fruit tree crop [1]. Dates are implicated to possess medicinal properties in addition to its nutritional value. Several studies have reported date fruit with a wide range of bioactivities, such as antioxidant activity due to the presence of phenolics, carotenoids and anthocyanins, antimutagenic, anti-inflammatory, antihyperlipidemic, antibacterial and antifungal activities. Date fruit consist of 70% carbohydrates, most of which is in the form of sugars. Because of this the fruits are a high source of energy and it is approximated that 100 g of the flesh can provide 314 kcal of energy. Drying of date decreases the water activity and this increases the sugar concentration. Because of this the shelf life of dry dates are high and are available for extended periods of time. The fruits are also used as a sweetener in the preparation of beer. The date pits which are a waste product have been used for centuries in the Arab world to make caffeine-free drink. Recently, date pit powders are also marketed and are a source of choice to people preferring a non-caffeinated coffee with coffee-related flavor. Date pits are also ground and added to the feed of domesticated animals and observations suggest they are devoid of any harmful effects. Date pits are rich in protein (5.1 g/100 g), fat (9.0 g/100 g), dietary fiber (73.1 g/100 g), phenolics (3942 mg/100 g), and antioxidants (80,400 μmol/100 g), and may be of use in enhancing the nutritional value of incorporated food products. The most important quality attributes to grade dates are color, flavor (sugar level), moisture (26–30%) and absence of defects such as insect, damage, cracks and surface damage. Date fruit is good source of high nutritional value food. In more details, carbohydrates forms 70% of date fruit and are mostly fructose and glucose in equal ratio while date proteins are rich in amino acids that contain acidic side chain but poor in methionine and cysteine, which their side chain composed of sulfur. Minerals in date fruits are calcium, iron, magnesium, selenium, copper, phosphorus, potassium, zinc, sulfur, cobalt, fluorine, manganese, and boron. The edible part of the date palm tree has been recognized to possess many medicinal properties when consumed alone or in mixture with other medicinal herbs. In recent years, a huge interest in the abundant health promoting properties of date fruits had led to many pharmacological studies (in-vitro and in-vivo) as well as the identification and quantification of different classes of phytochemicals. The date fruits are highly nourishing and may have numerous potential health benefits. The protective effects of fruits against chronic diseases are ascribed to bioactive non-nutrients called phytochemicals. Phytochemicals have gained increased interest among several investigators, including clinicians due to their antioxidant activity, cholesterol-lowering properties, and other potential health benefits such as chemoprevention of cancer, prevention of diabetes and cardiovascular diseases [3]. Numerous beneficial health effects have long been associated with date fruit, including antioxidant, anti-mutagenic and anti-inflammatory activity, and protection of the gastric mucosa against damaging effects of stomach acid [1]. Hepatoprotective activity has also been linked to date fruit, including reduced alkaline phosphatase levels. These effects have been linked to the presence of anthocyanins, ferulic acid, caffeic acid, quercetin and proanthocyanidins [6]. Every part of the date-palm has good use: The wood and leaves provide timber and fabric for houses and fences; the leaves are also used for making ropes, baskets etc; the stalk is used as fuel. The fruits famous for their delicious and sweet taste are taken directly or processed to produce vinegar, pickle, bakery items and flavours. The date seeds are used as cattle feed after they have been softened by soaking and crushing. The date-palms are usually un branched. It is interesting to note that the branching occurs only in the male plants! There are typically a dozen bunches of dates per tree. A bunch weighs about seven kilograms and has a thousand dates. The life of a date tree is over hundred and fifty years. There are about a thousand varieties of dates. Some of these are facing possible extinction. The date fruits vary in size, shape and colour. Each of these numerous varieties have different name in Arabic. According to the estimates of the World Food Agricultural Organization, there are over hundred million date-palms in the world, producing two million tons of dates each year. About 65% of these

are grown in the Arab countries. Let us have a closer look at this familiar fruit, which is also known as the *tree of life* and *king of the oasis*. The botanical name of the date-palm is *Phoenix sylvestris Linn*. In Arabic the date-palm is known as *Nakhl*, and the fruit is known as *Tamar*. We shall note the names in several other languages: *Tamar* (Hebrew), *Khajur* (Hindi, Urdu, and several Indian languages), *Khurma* (Persian, Urdu), *Kharjur* (Sanskrit), *Khejur* (Bengali), *Finik* (Russian), *Datil* (Spanish), *Date* (Italian), *Datteir* (French), *Daten* (German), *Datum* (Dutch), *Datas* (Portugese) and so on [4].

The Phoenix sylvestris consists of polyphenols like cinnamic acid and their derivatives in whichcoumaric acid, ferulic acid, and sinapic acids are major compounds. The darker color variety of thedate fruit has a higher concentration of polyphenols compared to the lighter variety. The date palm kernel contains phytohormones; thus, it produces a significant anti-wrinkle effect and can be used in antiaging products. Date fruits are rich in phenolic and also possess antioxidant activity. Date seeds can also be used in skin products, as they can yield moisturizing oil with different essential fatty acids [4].

MATERIALS AND METHODS

Plant Material

Root of *Phoenix sylvestris* was collected from Chandwad (District Nashik, M.S.) and authenticated from the department of botany, Dr. Babasaheb Ambedkar University, Aurangabad (Accession no.0716).

Morphology

The external morphology was studied according to reported methods. Macroscopic study was performed by using simple microscope. The colour, odour, taste, size and shape of flowers and roots were determined.

Transverse section & powder characteristics

The free-hand section of root were prepared from fresh plant material and finally stained with various staining reagents as per standard procedures. The disaggregation of plant material was performed by reported method. In brief, scales were disaggregated by means of boiling in an aqueous solution of NaOH (5 % w/v) for 5 min. After cooling and washing with water, pieces were treated with an aqueous solution of chromic acid (25 % v/v) for 30 min at room temperature. The section where cleared with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid (1:1) and toludine blue.[26]. Powdered drug were used for the observation of power microscopical character. The powdered drug were separately treated with phloroglucinol-hydrochloric acid (1:1) solution, acetic acid and iodine solution to determine the presence of the lignified fibres, Calcium oxalate crystals and starch grains respectively. A series of digital images captured using a Motic Digital microscope fitted with 1/3'' CCD camera imaging accessory and using Motic Images 2000 (1.3 version) analysis software. The micrometric data were generated from average of 30 measurements for each sample and expressed as lower limit mean+SD [8].

Fluorescence analysis

Fluorescence study of powdered drug of root of Phoenix sylvestris was performed by following a standard procedure[28]. In this study the powdered root was treated with various acidic and basic solvents and observed in UV visible chamber under short and long wavelength region simultaneously [24, 25]. Fluorescence is an important factor which reveals various chemical constituents show fluorescence in the visible range in day light. Uv light gives fluorescence to many natural products such as berberin (an alkaloid) which do not generally produce fluorescence in day light. If the active constituents are not themselves fluorescent, they can be converted into fluorescent derivatives by using reagents. Hence it plays an important role for the pharmacognostcal parameter for the evaluation of crude drugs [29].

Extraction of root of phoenix sylvestris

50 g of powdered root of *Phoenix sylvestris* was packed in white cotton bags and then extracted successively using the following solvents in a Soxhlet apparatus. The solvents used for extraction were petroleum ether (60-80 ^{0}C), chloroform and methanol.

Each time before extracting with the next solvent, the powdered material was dried in an air oven below 50 ^{0}C. The extractive values and preliminary phytochemical studies were carried out on these successive extracts.

Qualitative estimation

In the present study, the qualitative chemical tests were performed on different successive extracts of *Phoenix sylvestris.* For the phytoconstituents such as alkaloids, glycosides, tannins, resins, carbohydrates, etc. The results obtained on examination of successive extracts are indicated in table 03 [36, 37].

QUANTITATIVE ESTIMATIONS

Estimation of total phenolic content

To determine total phenolic content from the methanolic extract of root of *Phoenix sylvestris*, calibration curve of standard gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and mg/ml of methnolic extract of root *Phoenix sylvestris* was prepared simultaneously. Each sample was mixed with 0.25 ml of Folinciocalteu reagent and 1.25 ml sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period the blue color ease measured at 725 nm on UV visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as gallic acid equivalent by using following formula.

T=C.V/M

Where T= Total content of phenolic compound in mg per gram of plant extract.

C= Concentration of gallic acid established from the calibration curve mg/ml.

V=Volume of extract in milliliter

M= gram weight of plant extract. [38, 39]

Estimation of total flavonoid content

An aliquot (l ml) of standard solution of quercetin (20, 40, 60, 80,100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% NaNO2 into it. After 5 minute 0.3 ml of 10% AlCl3 was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions.

Mixed well and taken the absorbance at 358 nm against blank. From the obtained standard curve of quercetin the total flavonoids content of methanolic extract of root of *Phoenix Sylvestris* was determined.

RESULTS AND DISCUSSION

Morphology

External Morphology was studied by according to reported methods. The obtained results are as follows.

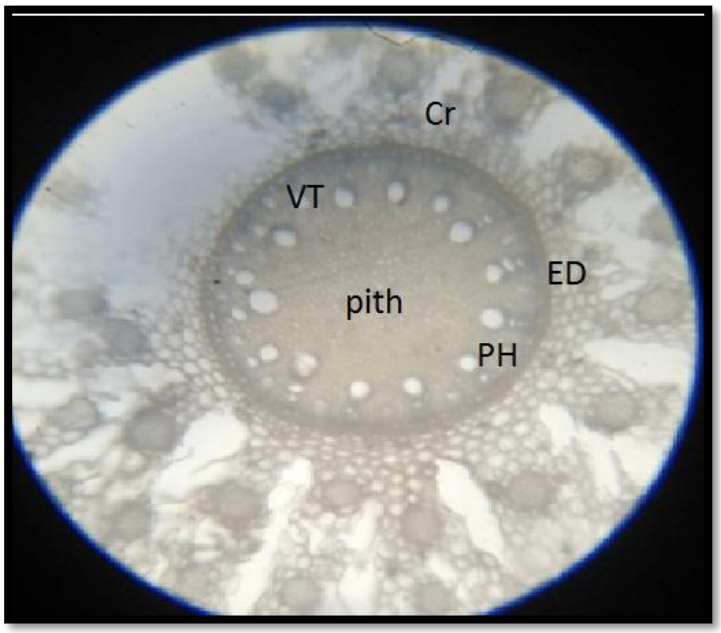
Figure 01: Roots of *Phoenix sylvestris*

Table 01: Result of Morphological study of *Phoenix sylvestris* root

Test Parameters	Observation
Colour	Whitish Brown
Odour	Characteristic
Test	Sweet
Shape	Long irregular

Transverse section of root of Phoenix sylvestris

The microscopy of root of *Phoenix sylvestris* was performed by using monochromatic staining agent like Pholoroglucinal and HCL in proportion of 1:1. It reveled presence of typical root characteristics as central pith, few layers of cork cell followed by cortex. Lignified vascular tissues were also observed. In epidermis few layers of cork is observed and in endodermis cortex, vascular tissue, and pith were observed.



Cr= Cortex, PH= Phloem, ED= Endodermis, VT= Vascular tissue

Figure 02: Transvers section of *Phoenix sylvestris* root

Fluorescence analysis

Present fluorescence analysis on powdered root of *Phoenix sylvestris* Shows characteristic colors with reagents like alcohol, 10% NaOH, and 10% HNO3, and 50% H2SO4. The result is depicted in table no 2.

Table 02: Fluorescence analysis of root

Fluorescence study Reagents	Long UV light	Short UV light
Powder as such	Yellow	Yellowish green
Powder in distilled water	Yellow	Yellowish green
Powder in Absolute alcohol	Pale green	green
Powder In 10% NaoH	Green	Dark green
Powder in 10% HNO_3	Light green	green
Powder in 50% H_2SO_4	Yellow	Pale yellow

QUALITATIVE ESTIMATION OF PHYTOCONSTITUENT

Table 03: Result of Phytochemical tests of extracts

Sr.No	Constituents	Tests	Pet. Ether	Chloroform	Methanol
1.	ALKALOIDS	Mayer's test	-	-	-
		Dragordraff's test	-	-	-
		Hager's test	-	-	-
		Wagner's test	-	-	-
2.	STEROLS	Libermann's Burchard test	-	-	+
		Salkowski's -	-	+	+
3.	CARBOHYDRATES AND GLYCOISDES	Molisch's test	-	+	+
		Fehling's test	-	+	+
		Benedict's test	-	+	+
		Borntrager's test	-	+	+
4.	FIXED OILS AND FATS	Spot test	-	-	-
		Saponification test	-	-	-
5.	PHENOLIC COMPOUND	$FeCl_3$ test	--	-	+
6.	PROTEIN AND AMINOACIDS	Biuret test	-	-	-
		Ninhydrin test	-	-	-
		Xanthoprotein test	-	-	-
		Millon's test	-	-	-
7.	TRITERPINOID AND SAPONINS	Foam test	-	-	+
		Haemolysis test	-	-	+
8.	TANNINS	Gelatin test	-	-	-
		$FeCl_3$ test	-	-	-
9.	GUMS AND MUCILAGE	Precipitation with 90% alcohol	-	-	-
10.	FLAVONOIDS	Aqueous NaOH	+	+	+
		Conc. H_2SO_4	+	+	+

QUANTITATIVE ESTIMATION

Total Phenolic content

For quantitative estimations, total phenolic content, total flavonoid content were determined from methanolic extract of root of *phoenix dyctelifera.*

Each quantitative estimation was done by using standard samples. Results of each quantitative parameter are given below.

Table 04: Absorbance observed in estimation of Total Phenolic content from root

Sr. No.	Standard (Gallic acid mg/ml)	Absorbance			Mean
		I	II	III	
1	20	0.275	0.273	0.278	0.275
2	40	0.56	0.56	0.57	0.563
3	60	0.887	0.888	0.884	0.886
4	80	1.188	1.190	1.185	1.187
5	100	1.458	1.460	1.457	1.458
RPS	Unknown	1.042	1.042	1.043	1.042

(RPS = Root of *Phoenix sylvestris*)

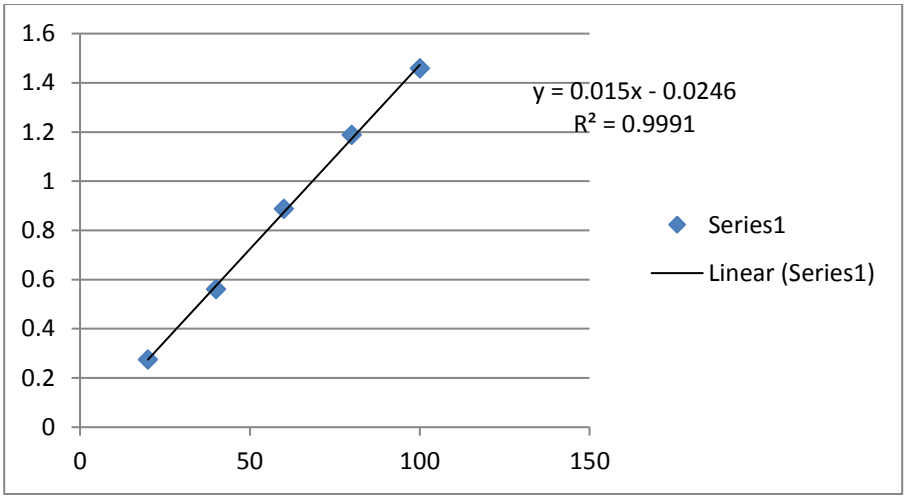


Figure 03: Calibration curve of standard Gallic acid

Determination of total concentration of Phenolic compound content was calculated on the basis of standard calibration curve of Gallic acid. Total Phenolic content from root of PS was calculated as 7.10 %.

Total flavonoid content

Table 05: Absorbance observed in estimation of Total flavonoid content from root PD

Sr. No.	Gallic acid (µg/mL)	Absorbance			Mean
		I	II	III	
1	20	0.053	0.055	0.054	0.054
2	40	0.133	0.132	0.133	0.132
3	60	0.223	0.226	0.224	0.224
4	80	0.309	0.311	0.308	0.309
5	100	0.377	0.374	0.379	0.376
RPS	Unknown	0.282	0.282	0.281	0.281

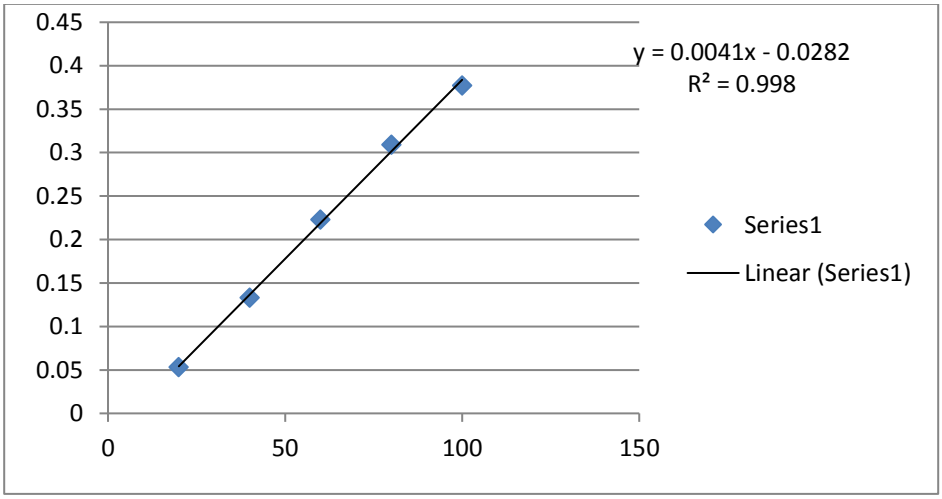


Figure 04: Calibration curve of standard Quercetin

CONCLUSION

Natural products can be good remedies because they are inexpensive and easy to access. Date palms possess high nutritional and therapeutic value with significant antioxidant, antibacterial, antifungal, and anti-proliferative properties. In above study root part of *Phoenix sylvestris* was selected because very rear work has been done on root. Methanolic extract was subjected for phytoconstituent and it was observe that the root contains flavonoid and terpenoid.

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