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PHYSICOCHEMICAL AND SPECTROSCOPIC PROFILES FOR IDENTIFICATION OF SEED (PITS) OF PHOENIX SYLVESTRIS ROXB.

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ABSTRACT

The present study aimed to provide a number of physicochemical and spectroscopic profiles of seeds (pits) of *Phoenix sylvestris* (Family: *Palmae)* for correct identification of the material. The powdered material was subjected to proximate analysis (moisture contents, total ash and total extractives), qualitative and quantitative phytochemical analyses and FTIR spectroscopy. Qualitative phytochemical analyses indicated the presence of steroids, triterpenoids, phenols and tannins, whereas quantitatively the material was found to contain 3.04% glycosaponins, 4.35% proteins, 77.95% carbohydrates and 9.0% lipids. The powder produced brown, green and black fluorescence under UV light, before and after treating with certain reagents. FTIR spectrum indicated the presence of OH, CH, C=O and C-O-C. The findings of this study may be helpful for the correct identification of the pits of *Phoenix sylvestris*.

Keywords: Proximate analysis; Phytochemical analysis; *Phoenix sylvestris;* spectroscopic profiles

INTRODUCTION

Phoenix sylvestris (family: *Palmae*) is locally known as Khajur, Silver date palm, Wild date palm, Date sugar palm and Toddy. The family consists of about 200-210 genera and 2800 species, which are mostly found in tropical and subtropical regions of the world. In Pakistan, this family has 16 genera and 18 species (Malik, 1984). The fruit of the plant is consumed, while seeds (pits) are thrown as a waste (Hamada *et al*., 2002; Besbes *et al*., 2004). The pits yield is about 10% of the total weight of fruit, and during the past few years certain efforts have been made to utilize this waste material in an effective way to produce useful products. In the same context, date pits have been converted to an effective adsorbent by employing different physical and chemical processes (Banat *et al*, .2003).

Raw date pits and activated carbon developed from these pits have been reported to adsorb heavy metals, dyes, 2,4-dinitrophenol, textile and tannery effluents (Al-Ghouti *et al*., 2010; Banat *et al*., 2003; Al-Mutairi, 2010; Yakubu *et al*., 2008). The adsorption capacity of adsorbent developed by $ZnCl_2$ has been explored for paracetamol in simulated intestinal and simulated gastric fluid (Khan *et al*., 2012). Likewise, the carbon of date pits activated by H_3PO_4 has been investigated for adsorption capacity for paracetamol (Asif *et al*., 2014). The adsorption capacity of H_3PO_4 –activated carbon was found to be higher than that of the $ZnCl_2$-activated carbon. This indicates the importance of this bio-waste and to enhance its usage, correct identification of the plant species is important to have standardized adsorbents. Therefore, the present study was undertaken to provide physicochemical and spectroscopic profiles that could be used for the identification of *Phoenix sylvestris.*

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MATERIAL AND METHODS

Plant material

Date fruit, purchased from the local market and was authenticated from Prof. Dr. Zaheer ud Din Khan (Botanist), Botany Department, Govt. College University, Lahore, Pakistan, wherein a voucher specimen was deposited vide reference No. 957. The pits were separated, washed, dried in an oven at 80^0C for 2 h and pulverized. The powder was sifted using a sieve of 120 mesh size.

Chemicals and solvents

The chemicals and solvents of analytical grade of E. Merck, procured from the local market included triton-X, bovine serum albumin (Fraction V), sodium carbonate, Folin- Ciocalteau reagent, copper sulfate in 1% potassium sodium tartrate, potassium bromide, monobasic potassium phosphate, NaOH, HCl and H_2SO_4,HNO_3 and ethanol. Other materials were ash less filter papers (Whatman), paracetamol (China) and in-house prepared distilled water.

Physicochemical analyses

Proximate analysis (WHO, 1998; USP, 2005)

Moisture contents

A tarred china dish containing 2 g powder was kept in an oven for drying at 105ºC for 30 min. Then, it was removed and allowed to cool to the room temperature in desiccators. Finally, it was weighed to calculate the moisture contents, which were expressed as mg/g powder.

Total ash

Clean china dish was heated in oven at 100ºC for 10 min and allowed to cool to a room temperature. The dish was weighed and 2 g sample was taken in it for incineration in furnace by slowly increasing the temperature to 675 ± 25 ºC. Once the material got free from carbon, the china dish was kept in desiccators to cool to room temperature, and weighed to calculate ash contents that were expressed as mg/g powder.

Acid insoluble ash

Total ash was boiled for 5 min in 25 mL dilute HCl and the mixture was filtered through ash-less filter paper. The residue material was washed by warm distilled water. Then, the filter paper was dried and burnt in a tarred china dish until free from carbon. The china dish was cooled to room temperature in desiccators and weighed to calculate acid insoluble ash.

Acid soluble ash

The contents of acid soluble ash were determined by subtracting the amount of acid insoluble ash from the total ash, dissolved in 25 mL HCl. The contents of acid soluble ash were expressed as mg/g powder.

Water insoluble ash

The total ash was boiled in 25mL of water for 5 min and the mixture was filtered through ash-less filter paper. The remaining material was washed with warm water, and filter paper was allowed to dry and then burnt in crucible to a temperature not exceeding 450ºC. The crucible was weighed to calculate contents of water soluble ash.

Water soluble ash

The contents of water soluble ash were calculated by subtracting the contents of water insoluble ash from the total ash dissolved in 25 mL water. The contents of water soluble ash were expressed as mg/g powder.

Sulphated ash

Two gram powdered sample was taken in a tarred china dish, moistened with H_2SO_4 and heated till emission of white fumes ceased. Then was ignited in furnace at 500 – 600ºC and allowed to cool in desiccators, and again moistened with the acid and ignited. The process was repeated until constant weight was achieved. The contents of the ash were determined and expressed as mg/g powder.

Extractive values

Alcohol soluble extractives (at room temperature)

Five grams powder was allowed to macerate with 100 mL of 95% ethanol in a beaker for 24 h with constant stirring. The mixture was filtered and 25 mL filtrate taken in china dish was evaporated to dryness and the residue was dried at 105ºC. The china dish was allowed to cool to room temperature and weighed. The alcohol soluble extractives were calculated and expressed as mg/g.

Alcohol soluble extractives (at 60ºC)

Five grams powdered date pits were allowed to macerate with 100 mL of 95% ethanol in a beaker at 60ºC for 24 h with constant stirring. The mixture was filtered and 25 mL filtrate taken in china dish was evaporated to dryness and the residue was dried at 105ºC. The china dish was allowed to cool to room temperature and weighed. The alcohol soluble extractives were calculated and expressed as mg/g.

Water soluble extractives (at room temperature)

Five grams powdered date pits were allowed to macerate with 100 mL chloroform-water in a beaker for 24 h with constant stirring. The mixture was filtered and 25 mL filtrate taken in china dish was evaporated to dryness and the residue was dried at 105ºC. The china dish was

allowed to cool to room temperature and weighed. The water soluble extractives were calculated and expressed as mg/g.

Water soluble extractives (at 60ºC)
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Quantitative analysis
Estimation of total glycosaponins
One gram extract was dissolved in 50 mL methanol and refluxed for 30 min. The mixture was filtered and the filtrate was collected. With the residue, the procedure was repeated twice and the filtrates were combined and concentrated to 10 mL using rotary evaporator. The concentrate was added drop wise in 50 mL acetone in a tarred beaker. The precipitate was dried in oven at 100ºC, allowed to cool and weighed to determine total glycosaponins using the following equation (Hussain *et al*., 2008).
Glycosaponins = (Weight of precipitate / Weight of sample) X 100

Estimation of total lipid contents
Fifteen grams of date seed powder was extracted with petroleum ether at 40-60ºC using soxhlet apparatus. The material was allowed to macerate for 12 h in a thimble and the extraction was performed for 24 h. The extract was filtered and the filtrate was dried *in vacuo* at 40ºC in a tarred flask. The flask containing the residue was weighed to calculate the lipid contents that were expressed as mg/g of powder (Besbes *et al*., 2004).

Estimation of protein contents
Fifteen grams of date seed powder was macerated with 100 mL distilled water containing few drops of triton-X for 10 h, and the extract obtained was used for the estimation of total protein (Lowry *et al.,* 1951). Ten millilitres of extract was taken in centrifuge tube and centrifugation was carried out at 2700 rpm for 10 min. The supernatant (0.1 mL) was added in a tube and distilled water was added to make the volume 1 mL. Then 3 mL reagent C was added that was prepared by mixing 50 mL of reagent-A (2% Na_2CO_3 in 0.1N NaOH) and 1 mL of reagent-B (0.5% $CuSO_4$ in 1% potassium sodium tartrate). After adding 0.2 mL of Folin-Ciocalteau's reagent, tube was incubated for 30 min at room temperature. The mixture was analyzed by UV/visible spectrophotometer at 600 nm against a blank that was prepared by combining all the reagents and water in place of the sample. Bovine serum albumin (Fraction V) solution in a concentration range 12.50-100 µg/mL was treated like the sample that to draw the calibration curve. All the samples and the standards were analysed in triplicate and total protein contents were calculated from the calibration curve using linear regression.

Estimation of carbohydrates
Carbohydrate contents were calculated using the method described by (Al-Hooti *et al,*. 1998; Barminas *et al,*. 1999) as the difference of mean values, i.e., 100-(Sum of percentages of moisture, ash, protein and lipids).

FTIR analysis
FTIR spectra were recorded using FTIR Spectrometer (Thermo Nicolet, USA) equipped with software OMNIC version 6.0 a. One mg of the crude drug powder and 100 mg KBr were ground together and the mixture was transferred to a die. The die was then compressed in hydraulic press to produce discs which were used to obtain FTIR spectra in mid IR range 4000-400 cm^{-1}.

Fluorescence analysis
Fluorescence characters of the powdered date pits, alone and in combination with some reagents, under ordinary light and UV light (UV366 nm) were determined and the results are discussed in table 2. The powdered sample after treating with certain chemicals showed the visible variations in colours that may help in the proof of identity and development of monograph of the plant (Ravichandra and Paarakh, 2011).

RESULTS AND DISCUSSION

Physical appearance
The shape of dates and date pits (seeds) are shown in Figure 1. The fruit is about 2.5 cm long, orange yellow in colour, round at the ends, sweet in taste and edible. Seeds are woody and longitudionally grooved from one side (Malik, 1984).

Proximate analyses
The results of proximate analyses such as moisture contents, ash values and total extractives are shown in Table 1. The powder contained 6.4% of moisture, 2.3% total ash, 0.2% acid insoluble ash, 1.15% water soluble ash and 4.15% sulphated ash. Estimation of moisture contents in crude drug assisted in reducing errors in determining the actual weight of the material. The low percentage of moisture contents advocated the stability

of plant material against certain microbial growth (Folashade *et al*., 2012). The extractive values of drug in water and alcohol provide information regarding percentage yield of extracts in both hot and cold conditions. The extractive values were found to be higher with both the solvents under hot condition as compared to that of extraction at cold condition (room temperature). Physiological and non-physiological ash provides information about the quality of raw material. Plant material produces physiological ash whilst non-physiological ash is the

remnant of exogenous material like gravel and soil. The acid insoluble ash indicates the presence of silica, silica containing materials and soil in herbal materials (WHO, 1998). The low value of sulphated ash contents indicated that care had been taken in drying of the date pits.

Figure 1 Pictures of fruit and seeds (pits) of *Phoenix sylvestris*

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Qualitative analysis helps in identification and characterization of plant materials to produce standardized products (Folashade *et al*., 2012). The results of qualitative analysis of both aqueous and ethanol extracts of seeds of the plant are given in Table 2. These results showed the presence of some of the phytochemicals in both of the extracts, whereas some of the tests gave negative results for aqueous extracts and positive test for alcoholic extracts. These characteristics may be beneficial for the authentication of the raw material of the plant.

The results of quantitative analysis of date pits are given in Table 3. The seeds contain fatty material higher than that of proteins. The seeds are reported to be rich in fatty acids and oil which is resistant to oxidation (Devshony *et al*., 1992; Besbes *et al*., 2004). The seeds contain 77.95% carbohydrates, which indicates that these may be used as a feed for animals and a raw material for the preparation of adsorbents.

The behaviour of the material under UV light gives useful information for identification purposes. This behaviour is studied with and without using reagents such as sodium hydroxide and hydrochloric acid. This is the first report about the florescence characters of the powder of date pits. The results of the fluorescence properties of powder of date pits are given in Table 4.). The powder colour (light brown) was similar in both visible and UV light. For the powdered pits before and after treating with certain reagents was carried out in UV (366nm) and day light.

Fluorescence analysis

The fluorescence analysis is one of the most important features in pharmacognostic studies. The powder of seeds of *Phoenix sylvestris* with treating with certain chemicals and without treating any chemical when observed in UV light and day light provide distinguished color changes that help in pharmacognostic evaluation. The powder moistened with 1 N aqueous and alcoholic NaOH appeared to be reddish brown in visible light, where as the appearance of both reagents under UV light was different from each other. The treatment with the acids such as HCl, HNO_3 and H_2SO_4 under visible light was as light brown, reddish brown and reddish brown, respectively. Under UV light HCl treated powder gave dark green appearance, whereas the other two acids gave black appearance. These profile may be helpful in identification of the powder of date pits of the plant..

Table 1 Proximate analysis of seeds (pits) of *Phoenix sylvestris*

Sr. No	Parameters	% age	(mg/g)
01	Moisture content	6.4	64.00
02	Total ash	2.3	23.00
03	Acid insoluble ash	0.2	2.00
04	Water soluble ash	1.15	11.50
05	Sulphated ash	4.15	41.50
06	Water extract (hot)	11.92	119.20
07	Alcohol extract (hot)	13.92	139.20
08	Water extract (cold)	12.64	126.40
09	Alcohol extract (cold)	10.40	104.00

Table 2 Qualitative assessment of phytochemical constituents of seeds of *Phoenix sylvestris*

Phytochemical group	Name of test	Ethanol extract	Aqueous extract
Terpenoids	Salkowaski test	-	+
	Liebermann's test	+	-
Sterols	Salkowaski test	-	-
	Liebermann's test	+	-
	Sulphur test	+	-
	Legals's test	+	+
Glycosides	Bromine water test	+	+
	Keller Killani test	-	-
	Legals's test	+	+
Flavonoids	Ferric chloride test	+	+
	Alkaline reagent test	+	+
	Lead acetate test	-	-
	Zn-HCl reduction test	-	-
Alkaloids	Mayer's test	+	-
	Wagner's test	+	-
	Hager's test	+	-
	Dragendorff's test	+	-
Proteins	Millon's test	-	+
	Ninhydrin test	-	-
	Biuret test	-	-
	Xanthoproeteic test	-	-
Carbohydrates	Barfoed's test	-	+
	Molisch's test	+	+
	Benedicts's test	+	+
Saponins	Foam test	+	+
	Bromine water test	+	+
	Legals's test	+	+
Lipids	Soap formation test	+	-

+ (Presence), - (Negative)

Table 3 Quantitative analysis of seeds of *Phoenix sylvestris*

Sr. No	Parameters	% age	(mg/g)
1	Total fat	9.00	90.00
2	Total proteins	4.35	43.50
3	Total carbohydrates	77.95	779.5
4	Total glycosaponins	3.04	30.40

FTIR Spectroscopy

The FTIR spectrum of raw date seed powder is shown in Figure 2. This spectrum indicated the characteristic bands at 3400 cm^{-1} (O-H stretching), 3000-2800 cm^{-1} (C-H stretching of methyl and methylene groups),1744 cm^{-1} (un-conjugated C=O stretching), 1300-1000 cm^{-1} (various types of vibrations due to C=O, C-H, C-O and 1158 cm^{-1} (C-O-C, stretching). The spectrum of raw date pits was found to be analogous to that reported previously (Al-Ghouti*et al.*, 2010).

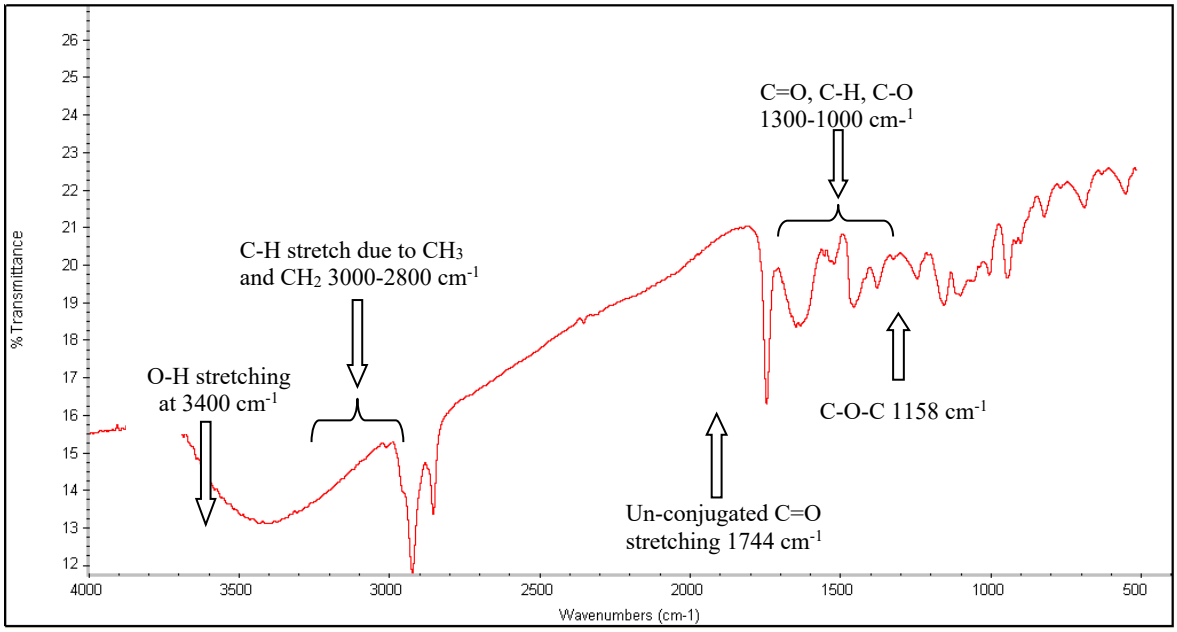

Figure 2 FTIR spectrum of powdered seeds (pits) of *Phoenix sylvestris*

Conclusion

The findings of the present study provide valuable information and passport data for the identification and authentication of seeds of *Phoenix sylvestris,* which is an emerging candidate in the queue of adsorbents. Physicochemical, phytochemical and FTIR spectroscopic profiles obtained from the present study may be used to produce standardized products from seeds of the plant.

CONCLUSION

The findings of the present study provide valuable information and passport data for the identification and authentication of seeds of *Phoenix sylvestris,* which is an emerging candidate in the queue of adsorbents. Physicochemical, phytochemical and FTIR spectroscopic profiles obtained from the present study may be used to produce standardized products from seeds of the plant.

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