



Phytochemical Analysis of Seeds of *Phoenix dactylifera*

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(Received 11 April, 2016 Accepted 20 May, 2016)

(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Date Palm is a high energy value crop with a good nutritional value. Seeds of this plant provide a remedy to a lot of ailments and nutritional value to human. It is used in cold, fever, cystitis, edema, throat infection, bronchial catarrh, low sperm count and abdominal trouble etc. The present study involves extraction & photochemical analysis of *Phoenix dactylifera* L. belonging to the family Arecaceae for its medicinal value. A qualitative photochemical analysis of four solvent extracts chloroform, ethyl alcohol, ethyl acetate and petroleum ether was performed for the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, flavonoids, proteins, amino acids and diterpenes. Results reveal the presence of maximum bioactive compounds in ethyl alcohol than in ethyl acetate. Ethyl alcohol and ethyl acetate were good solvents for extraction of bioactive compounds while chloroform is a poor solvent. The presence of bioactive compounds in the seeds showed that the seeds of this plant serve as good sources of useful drugs.

Keywords: *Phoenix dactylifera*, Arecaceae, Phytochemical, bioactive compounds.

I. INTRODUCTION

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. Due to economic constraints, providing modern medical healthcare in developing countries such as India is still a far-reaching goal. The most important and commonly used drugs of modern medicine such as aspirin, quinine, taxol, digitoxin etc. have originated from plant source. Out of an estimated 250,000 higher plants, less than 1% has been screened pharmacognostically and pharmacologically. Therefore, it is prudent to look for options in herbal medicines or herbal extracts for diseases like stress, diabetes, inflammation and diuretics etc. An ethnomedicinal approach for diseases is a practical, cost-effective and logical for its treatment. The goals of medicine no matter to which group it belongs, are the same i.e. the welfare of the patient.

India enjoys the privilege of having time-tested traditional systems of medicines based on natural products. Plants based natural products have been in use for medicinal, therapeutic or other purposes right from the dawn of history. However, drugs used in traditional systems of medicine are all crude drugs in their natural state or their preparations. Until 19th century, even western medicine (allopathic) depends largely on crude drugs. During 20th century the progress in chemical techniques and with the growth of pharmaceutical industry, chemical (synthetic)

drugs replaced crude drugs gradually. However, contending with various mechanisms in human body to prevent their excessive action, they have a single mode of action and may cause several adverse reactions when given in large doses or over a long period. Alternatively, the result may differ with individuals. Thus, synthetic drugs can produce remarkable life-saving results in acute diseases but cannot be used often in the treatment of chronic diseases.

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals naturally occur in the medicinal plants, leaves, roots and vegetables that have defense mechanisms. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins, common sugars are included in primary constituents whereas terpenoid, alkaloids, phenol, tannin, saponin, flavonoids etc. are included in secondary metabolites.

Phoenix dactylifera [pind khajura] belongs to the family Arecaceae is also known as date palm, cultivated for its edible sweet fruit. Date fruits are a good source of low cost food and are an integral part of Arabian diet. Dates contain 20-70 calories each, depending on size and variety. Date seeds are soaked and ground up for animal feed. Their oil is used in soap and cosmetics. Date palm seeds contain 0.56-5.4% lauric acid.



Habit



Fruit



Dried fruit with seeds

The seeds are also burned to make charcoal for silversmiths and can be stung in necklaces. Date palm is dioecious, having separate male and female plants. Seeds of *Phoenix dactylifera* used in wounds, lesions, inflammation, laxative, expectorant, nutrient and prescribed in the case of asthma, gonorrhoea.

Recent studies revealed that consuming high amounts of fruits and vegetables will reduce the risk of a number of chronic diseases (Nicoli *et al.*, 1999). This is attributed to the presence of a group of phytochemicals, dietary fiber, natural antioxidants, and other bioactive compounds. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The antioxidative effect is mainly due to phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes (Pietta, 1998).

Here is a need of constant search for more potent and cheaper raw material to feed the drug industry. However, very less research work was done on phytochemical analysis of seeds of *Phoenix dactylifera*. Hence the main objective of the study to analyze the phytochemicals present in the seeds.

II. MATERIALS & METHODOLOGY

(1) **Collection of the Material:** The seeds of the date palm were collected from the market of Bhopal, Madhya Pradesh. The seeds were dried at the room temperature. The dried seeds were powdered in a mixer blender.

(2) **Preparation of Seed Extracts:** Dried and powdered seeds were filled in thimble. 40 ml solvent was taken in the flask. Temperature was maintained at the boiling point of the respective solvent. Soxhlet exhaustion was continued till the solvent become colorless in tube. Extract was collected and dried at 40 degree centigrade in hot air oven. Dried extract was collected and stored in dark refrigerated conditions. (Elumali 2009).

(3) **Phytochemical Screening:** Phytochemical examinations were carried out for all the extracts as per the standard methods. (Harbone 1998 & Kokate 1996)

(a) **Detection of Carbohydrates:** Extracts were dissolved in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

-Fehling's Test: - Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

-Detection of Proteins and Aminoacids: Xanthoproteic Test:- Extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

(b) **Detection of Alkaloids:** Extracts were dissolved individually in dilute HCl. and filtered.

-Wagner's Test: - Filtrates were treated with Wagner's reagent (iodine in potassium iodide). Formation of brownish/ reddish precipitate indicates the presence of alkaloids.

-Hager's Test: - Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

(c) **Detection of Glycosides:** Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides.

-Legal's Test: - Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

(d) **Detection of Saponins:**

-Froth Test: - Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 mins. Formation of 1 cm layer of foam indicates the presence of saponins.

(e) **Detection of Flavonoids:**

-Alkaline Reagent Test: - Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

-Lead Acetate Test: - Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

(4) High Performance Liquid Chromatography (HPLC)

This technique was formally referred to as High-pressure liquid chromatography, it is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly different with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column.

III. INSTRUMENTATION

The schematic HPLC instrument includes a sampler, pumps and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it to the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates the signal proportional to the amount of sample component emerging from the column, hence allowing for the quantitative analysis of the sample components. A digital microprocessor is used and user software controls the HPLC instrument and provides data analysis.

Table 1: Selection of Separation Variable.

| Variable | Condition |
|----------------------|------------------------------------|
| Column | |
| Dimension. | 250mm x 4.60mm |
| Particle Size | 5 μ m |
| Bonded Phase | Octadecylsilane (C ₁₈) |
| Mobile Phase | |
| Acetonitrile | 50 ml |
| Methanol | 50 ml |
| Flow rate | 1ml/min |
| Temperature | 28°C. |
| Sample Size | 20 μ l |
| Detection wavelength | 254 nm |
| Retention time | 3.171 0.3 min |

Table 2: Phytochemical analysis of *Phoenix dactylifera*.

| Phytoconstituent | Name of the Test | Extract 1 Petroleum Ether | Extract 2 Chloroform | Extract 3 Ethyl Alcohol | Extract 4 Ethyl Acetate |
|------------------------------|--|---------------------------------|-------------------------|----------------------------|----------------------------|
| (1) Proteins and Amino Acids | (a) Xanthoproteic Test (H ₂ O) | -VE | -VE | +VE | -VE |
| (2) Carbohydrates | (a) Fehling's Test (HCL) | -VE | -VE | -VE | -VE |
| (3) Glycosides | (a) Legal's Test (H ₂ O) | -VE | -VE | +VE | +VE |
| (4) Saponins | (a) Froth Test (H ₂ O) | -VE | -VE | +VE | +VE |
| (5) Phenols | (a) Ferric Chloride Test (H ₂ O) | -VE | -VE | -VE | +VE |
| (6) Flavonoids | (a) Alkaline Reagent Test (H ₂ O) | -VE | -VE | -VE | -VE |
| | (b) Lead Acetate Test (H ₂ O) | -VE | -VE | +VE | +VE |
| (7) Alkaloids | (a) Wagner's Test (HCL) | -VE | -VE | -VE | -VE |
| | (b) Hager's Test (HCL) | -VE | -VE | +VE | +VE |
| (8) Diterpenes | (a) Copper Acetate Test (H ₂ O) | +VE | -VE | +VE | +VE |

(a) **Preparation of Standard Stock Solution.** 10 mg of Gallic acid was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

(b) **Preparation of Working Standard Solution.** From stock solutions of Gallic acid 1 ml was taken and diluted up to 10 ml with this solution 1.0 ml solution was transferred to 10 ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 10 $\mu\text{g/ml}$ concentration.

(c) **Qualitative estimation of Gallic acid in herbal extracts.** For analysis of the Extract, weight equivalent to 10 mg of extract was transferred to 10 ml separate volumetric flask and dissolved in methanol. The solution was shaking vigorously for 20 mins and filtered through Whatman filter paper no. 41, then volume was made up to mark with methanol. Inject the solution in HPLC with the help of fixed loop injector.

IV. RESULT AND DISCUSSION

Table shows results of phytochemical analysis of all the four solvent extracts. It reveals presence of carbohydrates, protein & amino acids, Alkloids, glycosides, saponins, phenols, flavonoids compounds in different solvents. In petroleum ether extract only diterapenes present.. In ethanol extract alkaloids, glycosides, saponins, flavonioids, diterpens, proteins & carbohydrates were present only phenol was absent. In ethyl acetate except carbohydrates & proteins all were present. Chloroform proved to be poor extractant for bio-active molecules.

Result of HPLC: HPLC analysis revealed that methanol fraction of *Phoenix* seeds contains a bioactive component gallic acid, one of the tannin components. The retention time of standard gallic acid was recorded to be 3.171 minutes and retention time of sample was found to be 3.17 minutes. This confirmed the presence of gallic acid (tannin) in herbal extract.

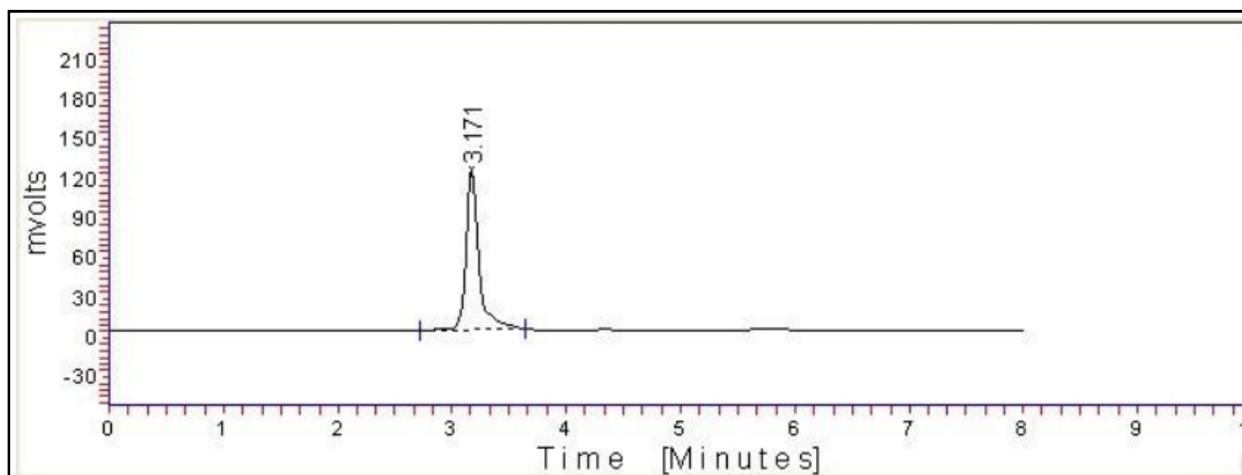


Fig. 1. Standard Chromatogram of Gallic acid.

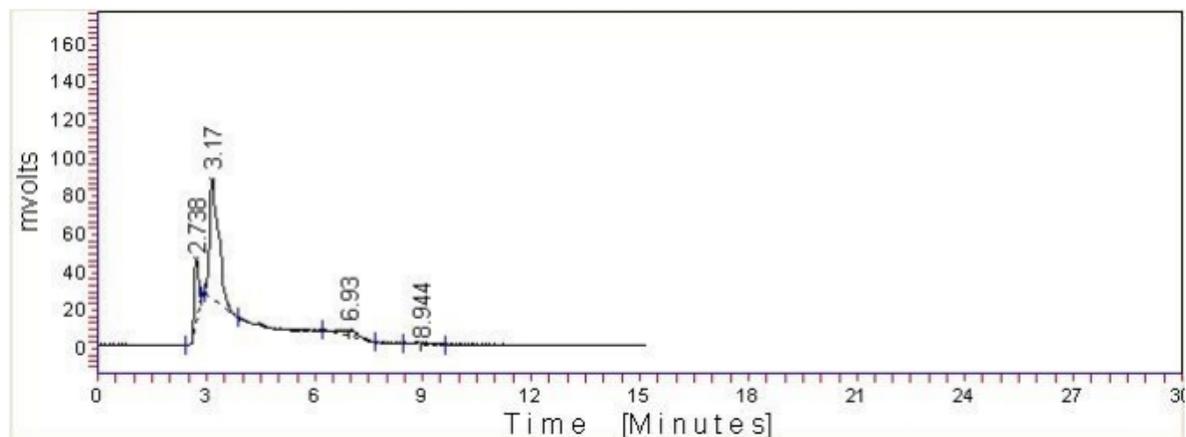


Fig. 2. Sample Chromatogram of extract.

DISCUSSION

The pharmacological studies conducted by N. Vyawahare *et al* 2008 on *Phoenix dactylifera* indicate the immense potential of this plant in the treatment of conditions such as diarrhoea, gastric ulcer, skin disorders, cardiovascular disorder inflammatory ailments, liver and kidney disorders, microbial and viral infections, cancer etc. Several studies indicate that consumption of fruits and vegetables is associated with reduced risk of several chronic diseases. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa *et al.*, 2007 and Mensah *et al.* 2012.

JS Negi *et al* 2011: These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, anticholinergic, antileprosy activities etc. (J.S Negi *et al.*) *Phoenix dactylifera* plant possesses tannin which have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are also present in this medicinal plant as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (DA Rio). Similar results were obtained in the present study. It was evident from the HPLC analysis that gallic acid was the main tannin present in the seeds have many medicinal properties. It is anticarcinogenic, antimicrobial, antimutagenic and anti-inflammatory agents (Choubey *et al*, 2015).

Ethanol extract of seeds of *Phoenix dactylifera* contains saponin, terpenoids, phenolic compounds and glycosides (D.V Delphin *et al* 2014). Similar results were observed in the present study. Phenols and flavonoids are very important plant constituents because of their antimicrobial activity (R. Taso *et al* 2004). Our results presented in this paper also confirmed that alcohols are better solvents of extraction of Phenols and flavonoids. Phenolic acids and flavonoids are generally better extracted using alcohols, water or a mixture of water and alcohols.

CONCLUSION

Phytochemical screening of seeds of *Phoenix dactylifera* was done. Results revealed the presence of alkaloids, saponins, glycosides, phenols flavonoids, diterpenes, proteins and amino acids. The phytochemicals present in palm seeds extract have well known curative activity against several human pathogens and therefore, could suggest the use of it for treatment of various diseases. Further studies are

needed for the characterization of individual phenolic, alkaloid and flavanoid compound to elucidate the mechanism underlying bioactive principles and the existence of possible synergism, if any among these compound.

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