Green Biosynthesis, Characterization and Antioxidant Activity of Phoenix dactylifera

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ABSTRACT

Phoenix dactylifera, commonly known as date or date palm, is a flowering plant species in the palm family, Arecaceae, cultivated for its edible sweet fruit. The date fruits are excellent source of carbohydrates, dietary fibres, protein, lipids, some vitamins, minerals and bioactive compounds. The date seeds are used in the cosmetics and pharmaceutical industries. The present study was focused on phytochemical screening and antioxidant potent of *Phoenix dactylifera.* The result reveals that all secondary metabolites present in the sample are completely extracted and observed in ethanolic, methanolic extracts when compared to the aqueous extract which is a good antioxidant source. 100µg/ml of the seed extract showed the highest antioxidant activity with 83.5% of inhibition. The study was also extended for the synthesis and characterization of nanoparticles. The UV-Visible spectroscopy shows Plasmon peak at the range of 470nm.

KEYWORDS: *Phoenix dactylifera*, phytochemical screening, antioxidant activity, nanoparticles, UV-Visible spectroscopy

INTRODUCTION

A global estimation of 40,710 new cases and 28,920 deaths due to liver and intrahepatic bile duct cancers in 2017 highlights the need for improvement in cancer management strategies[1]. The imbalance between reactive oxygen metabolites (ROM) production and antioxidant defence results in "oxidative stress" which deregulates the cellular functions leads to various pathological conditions including cancer[2].Inflammation caused due to tissue damage through different stimuli like irritants, pathogen or due to physical injury. Antioxidant ability can help to fight against different disease conditions like organ damage, inflammation and cancer. Natural antioxidants are safer and healthy than synthetic antioxidants used in food materials[3]. The incidence of high mortality and associated side effects following chemotherapy or radiotherapy increase the demand for alternative medicine for the cancer treatment.

Medicinal plants are the tremendous source of antioxidants and phytochemicals which have the ability to treat liver aliments and inflammation[4,5]. To decrease the side effects by the synthetic drugs, researchers now focusing on developing the herbal based remedy[6]. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. 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[7]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents.

Phoenix dactylifera

Phoenix dactylifera [Pind khajura] belongs to the family *Arecaece* 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 palm seeds contain 0.56-5.4% lauric acid. Seeds of *Phoenix dactylifera* used in wounds, lesions, inflammation, laxative, expectorant, nutrient and prescribed in the case of asthma, gonorrhea. Their oil is used in soap and cosmetics.

MATERIALS & METHODS

Collection of the Material

The seeds of the date palm *Phoenix dactylifera* were collected from the local market, Coimbatore District, Tamil Nadu. The seeds were dried at the room temperature. The dried seeds were powdered in a mixer blender.

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 respective solvent. Soxhlet exhaustion was continued till the solvent become colorless in tube. Extract was collected and dried at 40ºC in hot air oven. Dried extract was collected and stored in dark refrigerated conditions[8].

PHASE I

Phytochemical Analysis

Phytochemical examinations were carried out for all the extracts as per the standard methods[9,10].

a) 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

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

c) Detection of Saponins:

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

d) Detection of Tanin's:

- **Ferric chloride Test:** Extract was treated with dil. $FeCl_3$. Dark green or blue green shows the presence of tannins

e) Detection of Phenols:

- **Ferric chloride Test:** Extract was diluted with water followed by a few drops of 10% aqueous ferric chloride solution. Formation of blue or green colour indicates the presence of phenols

f) 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

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

- **Benedict's Test:** Extracts were treated with Benedict's reagent and then heated gently, orange red precipitate indicates the presence of reducing sugar

h) Detection of Terpenoids:

- **Salkowski Test:** Extracts were treated with chloroform and concentrated H_2SO_4. Formation of reddish brown colour indicates the presence of terpenoids

i) 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

PHASE II

Biosynthesis of silver nanoparticles

The aqueous solution of 0.01 M silver nitrate was prepared and used for the synthesis of silver nanoparticles. The ethanolic extract of *Phoenix dactylifera* was mixed with 0.01 M silver nitrate solution (1:9 ratio). The solution turned into brown colour indicating the formation of silver nanoparticles. Then solution

incubated for 24 hours, the resulting solution was centrifuged and the mixture was collected after discarding the supernatant. The collected silver nanoparticles were allowed to dry. The characterization of the synthesized silver nanoparticles was analyzed by UV-Vis Spectroscopic analysis method.

PHASE III

Antioxidative study

The seed extract of *Phoenix dactylifera* was used for radical scavenging activity. The antioxidant activity was determined by DPPH scavenging assay[11]. Various concentration of the extract was made upto 1.0ml with methanol and added the methanolic solution of 0.5ml DPPH. The mixture was incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 517nm. The percentage inhibition was calculated by comparing the results of the test with those of controls not treated with the extract, as per the following formula: Inhibition (%) = Control-Test/Control x 100

RESULTS AND DISCUSSION

PHASE I

Phytochemical Analysis

Phytochemicals naturally occur in the medicinal plants, leaves, roots and vegetables that exert 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.

Table 1 depicts the results of phytocomponents present in ethanolic, methanolic and aqueous extracts of *Phoenix dactylifera*.

S.No	Phytoconstituent	Name of the test	Extract 1 Ethanol	Extract 2 Methanol	Extract 3 Aqueous
a.	Flavonoids	Alkaline Reagent Test	**+ve**	**-ve**	**-ve**
b.	Alkaloids	Wagner's Test	**+ve**	**-ve**	**-ve**
c.	Saponins	Froth Test	**+ve**	**+ve**	**+ve**
d.	Tanins	Ferric chloride Test	**+ve**	**+ve**	**-ve**
e.	Phenols	Ferric chloride Test	**+ve**	**+ve**	**+ve**
f.	Proteins & Amino acids	Xanthoproteic Test	**+ve**	**-ve**	**+ve**
g.	Carbohydrates	Benedict's Test	**-ve**	**-ve**	**+ve**
h.	Terpenoids	Salkowski Test	**+ve**	**+ve**	**-ve**
i.	Glycosides	Legal Test	**+ve**	**+ve**	**-ve**

The result reveals that all secondary metabolites residing in the *Phoenix dactylifera* are completely extracted and observed in ethanolic, methanolic extracts when compared to polar aqueous extract. This shows that secondary metabolites are highly soluble in non-polar organic extracts. This is in support with the screening of phytochemicals in ethanolic extract of *Cassia fistula*[12]. Increased levels of phytocomponents are an indicative measure of high redox potentials acting as reducing agents.

PHASE II

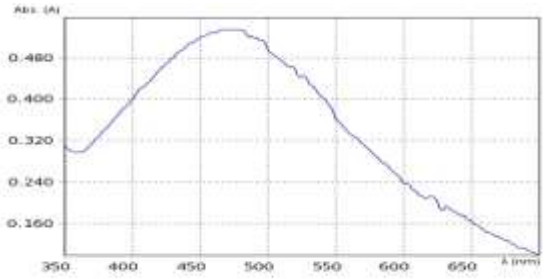
Rapid green synthesis in ethanolic extract of *Phoenix dactylifera*

The study was extended to characterize the nanoparticles synthesised from the ethanolic extract of *Phoenix dactylifera* using silver nitrate. The synthesised nanoparticles were characterised using UV – VIS spectrophotometer. High band intensities and peaks found in UV studies at the range of 470 nm is an indication of suitable surface plasmon resonance of the silver particles synthesized.

Silver nanoparticles have been the particular focus of plant based synthesis. Extracts of a diverse range of plant species have been successfully used in making nanoparticles. Biological methods can be adopted for the

synthesis of silver nanoparticles using micro-organisms[13] and plant extracts[14,15] have been suggested as potential environmental-friendly alternatives compared with physical and chemical methods.

Fig 1 shows the UV-Vis spectrum of silver nanoparticles showing surface Plasmon peak at the range of 470 nm

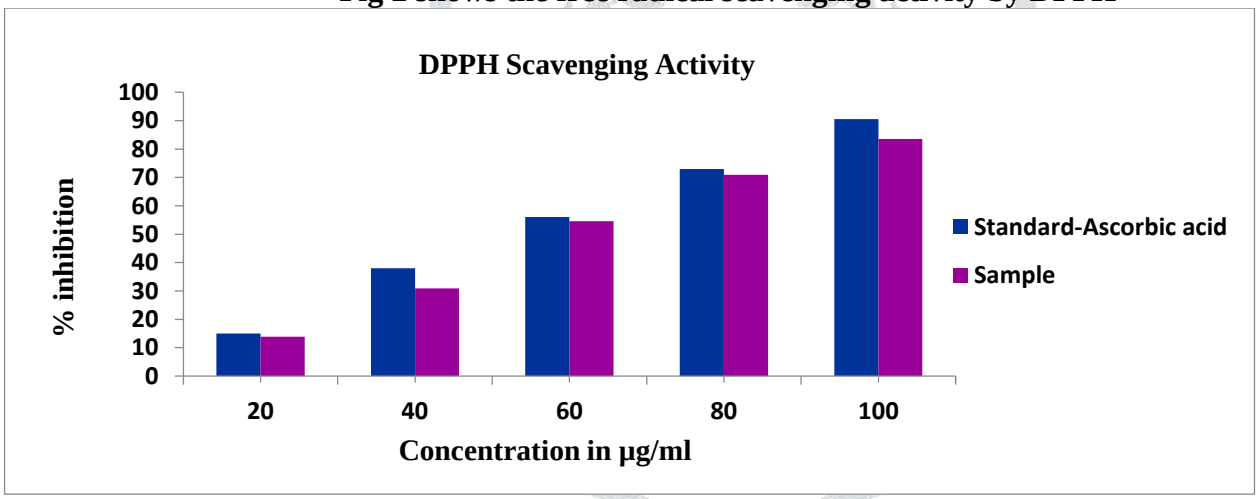


PHASE III

Antioxidative study

Antioxidative potentials of *Phoenix dactylifera* was assessed by the DPPH scavenging method. Diphenyl picrylhydrazyl (DPPH) is a nitrogen-centred free radical. It reacts similar to the peroxyl radical. Its reactive rates correlate directly with antioxidant activity. Higher the rate, more effective the antioxidants. Antioxidants tested on DPPH were also found extremely effective in cell systems of oxidative stress used to test anticancer agents[16].

Fig 2 shows the free radical scavenging activity by DPPH



The scavenging capacity of *Phoenix dactylifera* extract was found to be increased with increase in concentration. The antioxidative effect is mainly due to phenolic components such as flavanoids and terpenoids. *Phoenix dactylifera* possess significant amounts of these phenolic components which might be responsible for its antioxidative effect. Renuka and Jeyanthi, 2017 had reported free radical scavenging and antioxidant activities of *Momordica charantia* seed extracts by DPPH, Nitric oxide, Hydroxyl radical and Superoxide radical scavenging activities[17].

CONCLUSION

The *Phoenix dactylifera* seeds possess several phytocomponents. Nanoparticles synthesized from the seeds showed high band intensity which is observed in the plasmon surface might explore the potential therapeutic activity. The study can be further extended and explored for biomedical application emphasizing cancer therapeutics.

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