



## Seed Mediated Synthesis of Silver Nanoparticles Using Phoenix Dactylifera & Its Anti-Inflammatory and Antioxidant Activity

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### ABSTRACT

**Introduction:** Phoenix dactylifera commonly known as date palm belongs to the palm family, Arcaceae. Earlier studies demonstrate that constituents of dates act as potent antioxidant, antitumor and anti-inflammatory and provide a suitable alternative therapy in various disease.

**Materials and methods:** Dates seeds are grounded, mixed with distilled water, boiled & filtered. Silver nitrate is added to the solution & subjected to a magnetic stirrer. Tests done for anti-inflammatory activity are BSA and EA assay and tests done for antioxidant activity are DPPH and H<sub>2</sub>O<sub>2</sub> assay.

**Results:** The anti-inflammatory & antioxidant activity of Phoenix dactylifera were roughly equal to the standard used in our study. Henceforth proving that they have high anti-inflammatory and antioxidant potential.

**Conclusion:** The study concludes that silver nanoparticles synthesized from the Phoenix dactylifera seeds exhibit good anti-inflammatory and antioxidant activity and hence can be used in the formulation of organic anti-inflammatory gels or as an adjuvant to NSAIDs.

**Keywords:** *Nanoparticles, Antioxidant, Anti-Inflammatory, Activity*

## INTRODUCTION

Because of their distinctive optical, physical, and chemical characteristics, silver nanoparticles (AgNPs) have recently become one of the nanostructures that is the subject of the most investigation. The size, shape, and structure of AgNPs can be altered to create nanoparticles with fascinating and distinctive characteristics. AgNP-based materials have a wide range of intriguing, difficult, and promising characteristics that make them appropriate for several applications in biosensing, diagnosis, imaging, catalysis, and drug delivery. Particularly, AgNPs' size-dependent special plasmonic properties make them more desirable for biological and diagnostic applications. (Kumar et al. 2021)

Previous studies have shown the critical role of metal based nanoparticles in the health sciences, including their anti-bacterial, antifungal and antiviral activity, as well as their potential use in the therapy of several diseases. Silver nanoparticles (AgNPs) are among the noble metal nanoparticles that are of particular interest because of their numerous uses in the fields of medicine, dentistry, drug delivery, tissue and tumour imaging, biolabeling, biosensing, optics, coating for solar energy absorption, catalysis, mirrors, photography, electronics, and the food industry. (Ansari and Alzohairy 2018) In the fields of nanoscience and nanotechnology silver nano particles which have distinctive optical and electronic properties and serve in a variety of capacities (such as catalysis, degradation of environmental pollutants, biosensors, cancer therapy and antibacterial activity) have attracted a lot of attention. Application of AgNPs in everyday life such as bio labeling, coating for solar energy absorption, catalysis, electronics and the food industry, have been documented. These applications include dentistry, medication delivery and tumor imaging. Silver nanoparticles have also been shown to have anti biofilm function and to stop pathogenic bacteria from growing. (Salleh et al. 2020)

Metal particles can be created using a variety of synthetic techniques, including photochemical, solvothermal, Sonochemical and spin coating processes. The need for producing safe and no toxic chemicals, as well as biocompatible and ecologically friendly solvents, has caused interest in biosynthesis to increase rather than physical and chemical synthesis. Furthermore, biological

synthesis is better than Physio chemical methods since it is more environmentally friendly and because it has been demonstrated to yield substantially more nanoparticles. The chemical and physical synthesis techniques that are still frequently employed today have thus been challenged by biological methods that employ aqueous extracts of plant components. For the green synthesis of metal nanoparticles up to this point, plant, plant extracts, plant tissue, fruits, microbes and marine algae have been utilized. Plant extracts may function as stabilizing as well as reducing agents during the manufacture of nanoparticles and this process is quicker and compatible with the large-scale synthesis of metal nanoparticles than previous approaches. (Sennuga 2011)

The date palm (*Phoenix dactylifera*), one of the earliest plants cultivated by humans, is significant to human daily life and has significant health consequences. Date fruit extracts have been shown to have health benefits, have used in the pharmaceutical business and can be used to create industrial goods based on natural compounds. (Al-Alawi et al. 2017) Additionally, dates include a number of other components, such as poly phenolic compounds with anti inflammatory, hepatoprotective and anti cancer activities. They also reportedly contain antioxidant, hypolipidemic and anti diabetic qualities. Our team has extensive knowledge and research experience that has translate into high quality publications (Samuel et al. 2021; Samuel et al. 2021; Gowhari Shabgah et al. 2021; Muthukrishnan 2021; Kanniah et al. 2020; Chellapa et al. 2020; Kumar et al. 2020; Ramesh Kumar et al. 2011; Ganapathy et al. 2022; Anita et al. 2020)

The aim of this study is to biosynthesis and characterise silver nanoparticles using the seed extract of *Phoenix dactylifera*.

## MATERIALS AND METHODS

The date seeds were finely grinded and mixed with distilled water. It was then filtered and boiled for fifteen minutes. AgNO<sub>3</sub> solution was then added to it and it was kept in a shaker for twenty minutes. The colour of the solution now turns from dusky brick red to clean reddish-orange. It is then kept in the centrifuge for 24 hours. After this step we pour the supernatant into the sink and obtain the settled particles in pellets.

For the anti inflammatory activity the first test done is BSA assay or Bradford protein assay

It was tested by the following convention proposed by Muzushima and Kabayashi with specific alterations (Pratik Das et al.,2019). 0.05 mL of the extract of various fixation (10µL,20µL,30µL,40µL,50µL)was added to 0.45 mL bovine serum albumin(1% aqueous solution) and the pH of the mixture was acclimated to 6.3 utilizing a modest quantity of 1N hydrochloric acid. These samples were incubated at room temperature for 20 min and then heated at 55 °C in a water bath for 30 min. The samples were cooled and the absorbance was estimated spectrophotometrically at 660 nm. Diclofenac Sodium was used as the standard. DMSO is utilized as a control. Percentage of protein denaturation was determined utilizing following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

Second test for anti inflammatory activity was EA assay or egg albumin assay, wherein A 5ml solution was made which consisted of 2.8ml of freshly prepared phosphate buffered saline of pH - 6.3, 0.2 ml of egg albumin extracted from hens egg. Specific concentrations were prepared separately for the extract as (10µL,20µL,30µL,40µL,50µL). Diclofenac sodium was used as the positive control.. Then the mixtures were heated in a water bath at 37°C for 15 minutes. After which the samples were allowed to cool down to room temperature and absorption was measured at 660 nm.

For the anti oxidant property the first test done was DPPH or 2,2 diphenyl-1-picryl hydrazyl assay. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of the extract

was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Ascorbic acid was used as standard. The percentage of inhibition was determined from the following equation,

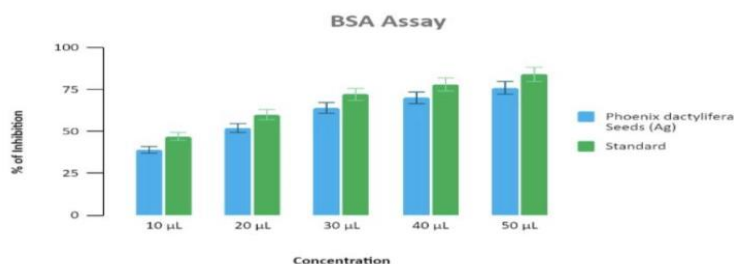
$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

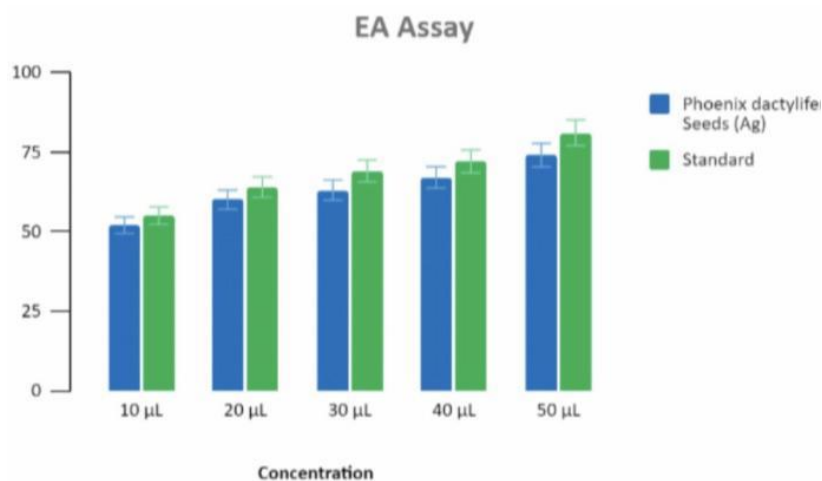
Second test done for anti-oxidant activity was H<sub>2</sub>O<sub>2</sub> assay, wherein All solutions were prepared freshly.1.0mL of the reaction mixture contained 100µL of 28mM of 2-deoxy-2-ribose ( dissolved in phosphate buffer,pH 7.4), 500µL solution of various concentrations of the extract (10µL,20µL,30µL,40µL,50µL) 200µL of 200µM FeCl<sub>3</sub> and 1.04mM EDTA (1:1 v/v),100µL H<sub>2</sub>O<sub>2</sub>(1.0mM) and 100µL ascorbic acid(1.0mM).After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution. Vitamin E was used as a positive control.

## RESULTS

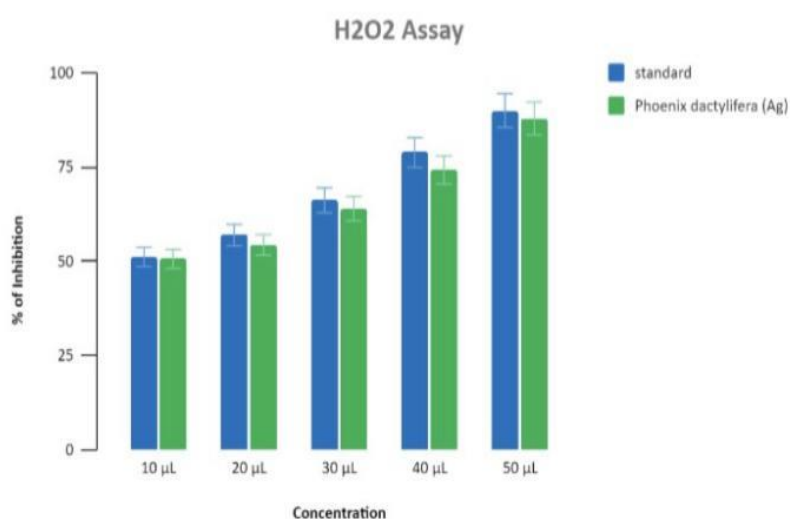
The graphs represent the evaluation of our extract with the standards that they're being compared to in different tests. For BSA and EA assay ( for anti- inflammatory activity) the standard taken for both is Diclofenac sodium, while for H<sub>2</sub>O<sub>2</sub> assay (for anti oxidant activity) is vitamin E and for DPPH assay (for anti oxidant property) is ascorbic acid. Our extract is more or less equal to the standard in all the four graphs hence proving the fact that it has a good anti inflammatory and anti oxidant property which can be used in the form of drugs in many applications.



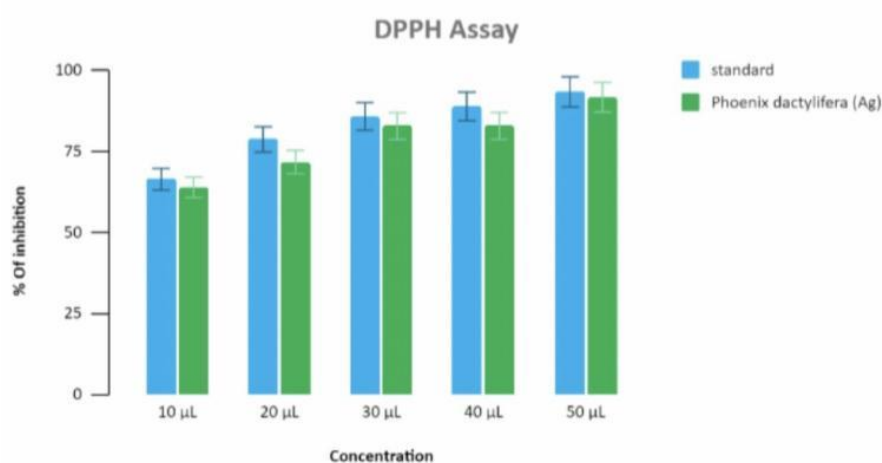
**FIGURE 1:** this graph (Bradford's assay) shows that our extract was found out to be more or less equal to the standard used.



**FIGURE 2:** demonstrates Egg albumin assay and again our extract is almost equivalent to the standard used



**FIGURE 3:** shows the hydrogen peroxide assay and proves to be almost very close to the values of the standard



**FIGURE 4:** the 2,2 diphenyl-1-picryl-hydrazyl-hydrate assay too depicts that our extract was very close to the values of the standard

## DISCUSSION

After evaluating the results on SPSS version 23 it was evident that date seeds show a good potential to be used as anti-inflammatory and anti-oxidant drugs. The findings of this research are consistent to that of Khaled.S Allemailen et al- Ajwa dates (Phoenix dactylifera) mediated synthesis of silver nano particles and their anti- bacterial, anti biofilm and cytotoxic potential, 2022 applied sci. 12,4537

A study by Al-Shwyyeh HA implies that The evaluated research represent just a small sample of the many studies on the health benefits of date palm fruits. According to those studies, date palm fruits are a secure, all-natural alternative and complementary therapy that is on par with synthetic medications in treating a wide range of illness conditions. The value should extend beyond that and be used to create new goods as food additives. (Al-Shwyyeh 2019)

The research by Abdoh Yousef at al described in this thesis identified the nutritional makeup of 19 different dates that come from four ecologically varied regions of Saudi Arabia. In all seven of the selected cultivars, the amounts of phenolics, anthocyanins, and antioxidant capacity all dropped as the plants grew. There were significant relationships between this antioxidant capacity and the amounts of total phenolic and anthocyanin, indicating that these are the primary sources of dates' nutritional value (Abdoh 2018)

In a study by Abdul Hamid et al Phoenix dactylifera seeds, namely the Algerian date variety of Deglet, were tested for their impact on the metabolome of LPS-IFN-induced RAW 264.7 cells using a nuclear magnetic resonance (NMR)-based metabolomics approach. The gathered information overall supported the potential use of Deglet seeds as a functional food with anti-inflammatory characteristics (Abdul-Hamid et al. 2019)

In a study by Hajer Taleb et al stated that prior research has revealed and documented P. dactylifera's use in the treatment of inflammatory diseases. A rich source of polyphenols, anthocyanins, sterols, and carotenoids is the date fruit as well as date fruit byproducts like date syrup. Date fruit has antibacterial, anti-inflammatory, and anti-angiogenic properties, according to in vitro research. Date fruit and date syrup both offer positive health effects that can be ascribed to the existence of natural bioactive

chemicals, according to growing interest in identifying the many health advantages of dates utilising in vitro and in vivo studies (Taleb et al. 2016). Henceforth, this article too is consistent with our findings.

A study by Heba. A Sahyon on Antimicrobial, anticancer and antioxidant activities of nano-heart of Phoenix dactylifera tree extract loaded chitosan nanoparticles, proved high vitality of the plant as an anti-microbial, anti-cancer and anti-oxidant drug in biomedical field. Rats treated with HP-ChNPs against doxorubicin oxidative damage showed improved heart and renal antioxidant enzymes as well as biochemical markers in this in vivo research (Sahyon et al. 2023)

A study by Reem. A Alajmi on Anti-Toxoplasma activity of silver nanoparticles green synthesized with Phoenix dactylifera and Ziziphus spina-christi extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice proved the high anti inflammatory effects of the plant. However, liver homogenate's antioxidant enzyme activity was markedly increased after nanoparticle treatment, which considerably reduced hepatic LPO and NO concentrations and proinflammatory cytokines (Alajmi et al. 2019)

Most of the studies discussed are consistent with our findings, making these findings an eligible study to be conducted forward for commercial use and for the welfare of our health.

## CONCLUSION

In conclusion the present study demonstrates that silver nanoparticles synthesized using Phoenix dactylifera possess potent anti oxidant and anti inflammatory activities which comparable with the references synthetic antioxidant and anti inflammation can be replaced by these synthetic compounds. Since this is a preliminary study, it is hereby recommended that further research must be conducted to isolate, purify and standardize the active anti-inflammatory and antioxidant constituents of P. dactylifera seed for use in herbal concoctions for topical use only or in combination with other herbs. Additionally, further work should be conducted to determine the best solvent for its extraction and the possible toxicity of the active constituents of the P dactylifera seeds in human subjects.

## CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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