Evaluation of The Antioxidant Properties of Abid Rahim Date Palm (*Phoenix Dactylifera* L.) fruit

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**ABSTRACT**

Free radicals are implicated as a cause and consequence of diverse health pathologies including neuro-degenerative diseases, cardiovascular ailments, diabetes mellitus, cancer, nephropathies, inflammatory disorders, auto-immune diseases, idiosyncratic reactions etc. There is however a renewed interest in the study of plants for novel antioxidants. The present study evaluated the antioxidant properties of the ethanol extract of Date palm (*Phoenix dactylifera* L.) fruit using 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay and also conducted phyto-chemical analysis using standard protocols. The crude extract produced a reduced antioxidant effect compared to ascorbic acid. Specifically, at high test concentrations (0.50 and 0.25 mg/ml), the mean antioxidant activity of the extract was 65.7% and 55.2% respectively relative to 79.0% and 76.8% with ascorbic acid at the same concentration. The extract also induced an abysmally low antioxidant activity of less than 32% below 0.25 mg/ml. Phyto-chemical analysis revealed that the extract contained flavonoids, alkaloids, steroids, terpenoids and cardiac glycosides. *Phoenix dactylifera* L. fruit could be a potential source for isolation of potent antioxidant principles.

**Keywords:** Free radicals, Antioxidant, *Phoenix dactylifera*, Health implications
**INTRODUCTION**

*Phoenix dactylifera* L. commonly referred to as Date or Date palm is a flowering plant species in the palm family *Arecaceae*. The species name *dactylifera* "date bearing" comes from the Greek words *daktylos*, which means "date" (also "finger") [1] and *fero*, which means "I bear", [2]. Date fruits are important stable food in the diet of population living in the arid and semi-arid regions of North Africa, Middle East and South-Asian countries [3]. Dates support the diet and ceremonial festivities of consumers since it is widely consumed during the Muslims holy months of Ramadan, in funerals and to welcome guests [4].

Beside fresh consumption, the fruit is processed into a wide variety of value-added products such as dry dates, date paste, date syrup, date juice, fibre concentrate, date flour, date jam, date butter, date-based fruit bar, date chutney, date relish and date pickles. Date oil and date coffee are some of the by-products from date seed [5].

The chemical intermediates in between oxygen ($O_2$) and water ($H_2O$) in the body are called the superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH$) and are largely within control limits in the respiratory chain. However, small quantities of these ions and molecules sometimes escape cellular regulation, enabling them to elicit other reactions elsewhere in the body tissues.

These are referred to as oxidants. Reactive oxygen species (ROS) comprises other chemicals, other chemicals such as free radicals and combinations of oxygen with nitrogen and chlorine. Apart from their formation in the mitochondria, ROS can also come from a variety of other sources, both endogenous (inflammation, exercise, ischemia reperfusion, xanthine oxidase and arachidonate pathways, transition metals, peroxisomes, etc) and exogenous, such as smoking, environmental pollutants, radiation, ultraviolet light and certain chemical exposure.

This study was aimed at evaluating the antioxidant properties and phytochemical constituents of Date palm (*Phoenix dactylifera* L.) fruit using standard assay procedures.

This fruit is found to contain carbohydrates (44-88%), fats (0.2-0.4%), fiber (6.4-11.5%), minerals, vitamins and an interestingly higher concentration of protein (2.3-5.6%) compared with other major cultivated fruits such as apples, oranges, bananas and grapes that contain only 0.3%, 0.7%, 1.0% and 1.0% of protein respectively [6].

Phytochemicals are naturally produced, non-nutritive and bioactive compounds which are synthesized by plants for protection against external stresses and attack by pathogenic microorganisms. Phytochemicals are reported to have various biological effects, such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial and anti-inflammatory.

**MATERIALS AND METHODS**

**Study Area**

Gwagwalada is one of the six Local Government Area Councils of the Federal Capital Territory (Abuja), Nigeria. The main town also bearing Gwagwalada, has an area of 1,043 km² with a population of 157,770 in the 2006 census. It is located at an elevation of 210 meters above sea level.

**Collection And Preparation of The Plant Material**

*Abid Rahim* date (*Phoenix dactylifera* L.) fruit were collected at Gwagwalada market and identified by a botanist in the Biological Science Herbarium, University of Abuja. The fruit was washed with sterile distilled water to remove sand and other debris and oven dried at 40 °C for one week. The dried fruit was ground into powder using a laboratory mortar and pestle.

**Extraction Of Plant Material**

The powdered *Abid Rahim* date (*Phoenix
Two (2 ml) of extract was dissolved in 2 ml of Chloroform and 3 ml of concentrated tetraoxosulphate (vi) acid was added to form a lower layer. A reddish brown colour at the interface indicated the presence of terpenoids [12].

**Test For Steroids**
Two (2 ml) of extract was added to 2 ml of acetic anhydride in a test tube. One millilitre (1 ml) of concentrated tetraoxosulphate (vi) acid was added down the side of the tube. A Blue-green colour indicated the presence of steroids [12].

**Test For Terpenoids**
Two (2 ml) of extract was dissolved in 2 ml of Chloroform and 3 ml of concentrated tetraoxosulphate (vi) acid was added to form a lower layer. A reddish brown colour at the interface indicated the presence of terpenoids [12].

**Phytochemical Analysis of *Phoenix dactylifera* L. Fruit Extract**

The extract was subjected to standard phytochemical qualitative screening for secondary metabolites as described by [8][12].

**Test For Saponins (frothing Test)**
Two (2 ml) of extract was dissolved in 10 ml of distilled water and then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. A honey comb-like froth formed for more than 30 minutes indicated the presence of saponins. To confirm the presence of saponins, hemolysis test was used. Two (2 ml) of extract was added to blood coated agar, hemolysis of the red blood cell in the blood agar indicated the presence of saponins [12].

**Test For Alkaloids**
To about 5 ml of the sample, a small amount of magnesium chips and few drops of conc. HCl were added down the side of the tube, a reddish colouration was observed which indicated the presence of flavonoids. To the extract (about 5 ml) a small quantity of zinc chips and drops of conc. HCl was inserted down the side of the test tube. A reddish colouration was observed which also indicated the presence of flavonoids [12].

**Test For Tannins**
Ferric chloride test. Two (2 ml) of the extract was dissolved in 10 ml of distilled water, and then filtered. Two drops of Ferric chloride solution were added to the filtrate. Formation of a blue-black precipitate indicated hydrolysable tannins and green precipitate indicated the presence of condensed tannins [12].

**Test For Free Anthraquinones Derivatives**
Test for free anthraquinones (Borntrager's Test) Ten (10 ml) of benzene was mixed with 2 ml of extract and filtered. Five (5 ml) of 10% ammonia solution was added to the filtrate and stirred. The production of a pink-red or violet colour indicated the presence of free anthraquinones [12].

**Test For Cardiac Glycosides (kella-killiani Test)**
Two (2 ml) of extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 45°, then 1 ml of concentrated tetraoxosulphate (vi) acid was added down the side of the tube, a reddish colouration was observed which also indicated the presence of flavonoids.
was added down the side. A brown-coloured ring at the interface indicated cardiac glycosides.

**Determination of Antioxidant Properties**

DPPH scavenging activity. Antioxidant activity was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. A portion of 1.950 ml of DPPH solution (6 x10^{-5} M) diluted in methanol was incubated with 50 µL of the extract in varying concentrations (0.03-0.50 mg/ml) in a cuvette. The absorbance at 517 nm was taken after 30 min of incubation in the dark at room temperature. The experiment was done in triplicates. The radical scavenging activity was expressed as EC_{50}, the effective concentration, which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50% [12]. Ascorbic acid (vitamin C) was used as the reference standard. A volume (0.5 ml) methanol plus 1 ml of the extract was used as the blank while 0.5 ml of 6 x10^{-5} M DPPH solution plus 1 ml of methanol was used as the negative control.

**Statistical Data Analysis**

Data obtained were subjected to One-way analysis of variance (ANOVA) and Duncan multiple range post hoc test, differences at p<0.05 were considered significant. Values were expressed as mean percentage ± standard error of mean (s.e.m.).

**RESULTS**

**Phytochemical Analysis of the ethanol extract of Phoenix dactylifera L. fruit**

Phyto-chemical analysis revealed the presence of flavonoids, alkaloids, steroids, terpenoids and cardiac glycosides but no saponin, tannins, or anthraquinones in ethanol extract of *P. dactylifera* L. fruit (Table 1).

| Table 1: Phytochemical Constituents of *Phoenix dactylifera* L fruit extract |
|------------------|-------------------------|
| Constituents     | Ethanol extract of *P. dactylifera* L, fruit |
| Flavonoids       | +                       |
| Saponins         | -                       |
| Tannins          | -                       |
| Alkaloids        | +                       |
| Steroids         | +                       |
| Terpenoids       | +                       |
| Anthraquinones   | -                       |
| Cardiac glycosides | +                     |

Key: + Presence      - Absence
Determination of Antioxidant properties of *P. dactylifera* L, fruit extract

All the test concentrations (0.03-0.50 mg/ml) of vitamin C, the reference antioxidant exerted a consistently high antioxidant activity above 75% in DPPH spectrophotometric assay. The crude extract of *Phoenix dactylifera* fruit demonstrated appreciable but reduced antioxidant effect with mean radical scavenging activity of 65.7% and 55.2% at the higher test concentrations of 0.50 and 0.25 mg/ml relative to 79.0% and 76.8% with ascorbic acid respectively at the same concentration. The extract however induced antioxidant activity of less than 32% at concentrations below 0.25 mg/ml (Table 2).

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Ascorbic acid</th>
<th><em>P. dactylifera</em> L. fruit extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>79.03 ± 1.02</td>
<td>65.70 ± 2.30*</td>
</tr>
<tr>
<td>0.25</td>
<td>76.82 ± 1.92</td>
<td>55.20 ± 1.10*</td>
</tr>
<tr>
<td>0.13</td>
<td>79.40 ± 1.30</td>
<td>31.30 ± 2.20**</td>
</tr>
<tr>
<td>0.06</td>
<td>79.07 ± 0.94</td>
<td>17.90 ± 1.70**</td>
</tr>
<tr>
<td>0.03</td>
<td>81.24 ± 0.86</td>
<td>31.70 ± 3.50**</td>
</tr>
</tbody>
</table>

*,** Significantly lower at p<0.05 and p<0.01 respectively compared to ascorbic acid.

**DISCUSSION**

The phytochemical analysis revealed that ethanol extract of *P. dactylifera* L. fruits contained flavonoids, alkaloids, steroids, terpenoids and cardiac glycosides whereas anthraquinones, tannins, and saponins were absent. Earlier quantitative phytochemical studies reported that *P. dactylifera* L. fruits contain alkaloids (1.59 g/100 g), anthraquinones (0.17g/100g), flavonoids (3.36g/100g), tannins (0.69g/100g), saponins (1.37 x 10^{-3} g/100g) and terpenoids (1.97 x 10^{-3} g/100g) [9][11]. Masmoudi-Allouche et al. [10] also reported the presence of sterols and triterpenes from 16 different extracts of *P. dactylifera* L. fruits. The fruit was found to contain carbohydrates (44-88%), fats (0.2-0.4%), fiber (6.4-11.5%), minerals, vitamins and an interestingly higher concentration of protein (2.3 - 5.6%) compared with other major cultivated fruits such as apples, oranges, bananas and grapes that contain only 0.3%, 0.7%, 1.0% and 1.0% of protein respectively [6].

Phytochemicals are naturally produced, non-nutritive bioactive compounds which are synthesized by plants for protection against external stresses and attack by pathogenic microorganisms. Some are reported to have various biological and medicinal effects, such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial and anti-inflammatory properties. (Provide a citation here). Bioactive principles in plants exist as phenolic acids, alkaloids, flavonoids, carotenoids, resins, glycosides, glycoproteins, tannins, polysaccharides, sterols etc.

The extract displayed a high radical scavenging ability at test concentrations of 0.25 and 0.50
mg/ml; the antioxidant activity was not appreciable (below 32%) at concentrations below 0.25 mg/ml. The antioxidant potency of the extract was however lower comparatively to that of ascorbic acid at the same concentration. The reason for this may not be far-fetched because ascorbic acid is a reference antioxidant while the extract is crude and a complex mixture of different compounds. The antioxidant activity of the components of the extract may increase following isolation of the active principle.

Antioxidants consists of vitamins, polyphenols, flavonoids (Rêka and Varga, 2002), minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules called free radicals. Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol) and selenium are valuable antioxidants

Conclusion
The findings from the study suggest that ethanol extract Phoenixdactylifera L. fruits collected from Gwagwalada Area Council of the FCT, Nigeria has potent antioxidant activity and various phyto-constituents that could be further investigated and exploited to complement therapeutic interventions in diverse health challenges.

Recommendation
It is therefore recommended that Phoenix dactylifera L. fruits be subjected to further studies for isolation of the antioxidant principles present.

REFERNCES


