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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 datylifera 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 datylifera L. fruit could be a potential source for isolation of potent antioxidant principles.

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

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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 populationliving 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) andwater (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 moleculessometimes 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 andcombinations of oxygen with nitrogen and chlorine.Apart from their formation in the mitochondria, ROS can alsocome 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, ultraviolent light and certan chemical exposure

This study was aimed at evaluating the antioxidant properties and phytochemical constiuents 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 pathogenicmicroorganisms. Phytochemicals are reported to have variousbiological 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 atGwagwalada market and identified by a botanist in theBiological Science Herbarium, University of Abuja. The fruitwas washed with sterile distilled water to remove sand andother debris and oven dried at 40 °C for one week. The driedfruit was grounded into powder using a laboratory mortar andpestle.

Extraction Of Plant Material

The powdered *Abid Rahim* date *(Pheonix*

dactylifera.) fruit was extracted using ethanol following procedure described by[7]. Two hundred (200g) of *Abid Rahim* date fruit(*Pheonixdactylifera L.*) powder was soaked in 1000 ml of ethanol at room temperature. The mixture was allowed to stand for 24 hours, shaken at intervals of 3hours after which it was filtered using a fine mesh cloth. The solvent was evaporated to dryness at a temperature of 40^0C using water bath.

Phytochemical Analysis of *Phoenix dactylifera* L Fruit Extract

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

Test For Saponins (frothing Test)

Two (2ml) of extract was dissolved in 10ml of distilled waterand then shaken vigorously for 30 seconds and allowed tostand for 30 minutes. A honey comb-like froth formed formore than 30 minutes indicated the presence of saponins. To confirmthe presence of saponins, hemolysis test was used. Two (2ml)of extract was added to blood coated agar, hemolysis of thered blood cell in the blood agar indicated the presence of saponins[12].

Test For Steroids

Two (2 ml) of acetic anhydride was added to 2 ml of extract ina test tube. One millilitre (1ml) 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 (2ml) of extract was dissolved in 2ml of Chloroform and3ml of concentrated tetraoxosulphate (vi) acid was added to form a lowerlayer. A reddish brown colour at the interface indicated thepresence of (terpenoids) [12].

Test For Flavonoids

To about 5 ml of the sample, a small amount of magnesiumchips and few drops of conc. HCl were added down the sideof the tube, a reddish colouration was observed whichindicated the presence of flavonoids.To the extract (about 5 ml) a small quantity of zinc chips anddrops 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 (2ml) of the extract was dissolved in 10 ml of distilledwater, and then filtered. Two drops of Ferric chloride solutionwas added to the filtrate. Formation of a blue-black precipitate Indicatedhydrolysable tannins and green precipitate indicatedthe presence of condensed (tannin) [12].

Test For Alkaloids

Dragendroff's Test. Two(2ml) of the extract was dissolved in 0.2ml of dilutehydrochloric acid in a test tube, then 1ml of dragendroff reagent.

Orange brown precipitate was formed which indicatedthe presence of alkaloids[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 tothe filtrate and stirred. The production of a pink-red or violetcolour indicated the presence of free anthraquinones[12].

Test For Cardiac Glycosides (kella-killiani Test)

Two (2ml) of extract was dissolved in glacial acetic acidcontaining traces of ferric chloride. The test tube was held atan angle of 45^oC, then 1ml of concentrated tetraoxosulphate vi acid

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-picryl-hydrazyl (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 *Phoenixdactylifera 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 2: Antioxidant activity of *P. dactylifera* L. fruit extract with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) photometric assay

Concentration (mg/ml)	Percentage antioxidant activity ± s.e.	
	Ascorbic acid	*P. dactylifera* L. fruit extract
0.50	79.03 ± 1.02	65.70 ± 2.30*
0.25	76.82 ± 1.92	55.20 ± 1.10*
0.13	79.40 ± 1.30	31.30 ± 2.20**
0.06	79.07 ± 0.94	17.90 ± 1.70**
0.03	81.24 ± 0.86	31.70 ± 3.50**

*,** 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 anthroquinones, tannins, and saponins were absent. Earlier quantitative phytochemical studies reported that *P. dactylifera*L. fruits contain alkaloids (1.59g/100g), anthraxquinones (0.17g/100g), flavonoids (3.36g/100g), tannins (0.69g/100g), saponins (1.37×10^{-3} g/100g) and terpenoids (1.97×10^{-3} g/100g) [9][11]. Masmoudi-Allouche*et al.* [10] also reported the presence of sterols and triterpens from 16 different extracts of *P. dactylifera L.* fruits. The fruitwasfound 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 only0.3%, 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. Someare reported to have various biological and medicinal effects, such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial and anti-inflammatoryproperties.(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 (Réka 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.

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