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Original Article

Nutritional/Chemical Constituents and Free Radical Scavenging Potentials of the Aqueous Extract of *Phoenix dactylifera* Fruit

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ABSTRACT

Aims: *Phoenix dactylifera fruit, grown majorly in northern part of Nigeria is acclaimed to possess numerous nutritional and medicinal properties. The study was carried out to investigate the nutritional/chemical constituents and the free radical scavenging potentials of the aqueous extract of Phoenix dactylifera fruit.* ***Methods:*** *The aqueous extract of the fruit was subjected to standard analytical test to assay for the amino acids, phytochemicals and free radical scavenging potentials: Phytochemicals and amino acids screening was carried out via High Performance Liquid Chromatography and Gas Chromatography Mass Spectrometry analyses. The antioxidant effects of the extract on free radicals were determined on radicals 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) , 2, 2-diphenyl-1-picrylhydrazyl , Nitric oxide and Hydroxyl radical.* ***Results:*** *Results revealed the presence of seven secondary metabolites with a high steroid and flavonoid content and low Coumarins, Glycosides, Alkaloids, Saponin, and Terpenoid concentration. The HPLC analysis of the phytochemical showed that it contained fifteen phytocompounds, seventeen amino acids while GCMS analysis reveals eleven fatty acids. For the free radical scavenging activity, it was able to inhibit ABTS, DPPH, NO, and OH^-, in a concentration dependent manner.* ***Conclusions:*** *The results indicate that the fruit possess good nutritional properties and abundant secondary plant metabolites constituents and this might provide evidence which corroborates with the free radical scavenging potentials of the fruit.*

Keywords*: Phoenix dactylifera, Free radical, Aqueous extract, High performance Lipid Chromatography.*

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الأهداف: من المعروف أن فاكهةPhoenix dactylifera ، التي تنمو بشكل رئيسي في الجزء الشمالي من نيجيريا، تمتلك العديد من الخصائص الغذائية والطبية. أجريت هذه الدراسة لمعرفة المكونات الغذائية والكيميائية وإمكانات التخلص من الجذور الحرة للمستخلص المائي لفاكهة Phoenix dactylifera. **الطرق**: تم إخضاع المستخلص المائي للثمرة لاختبار تحليلي قياسي لفحص الأحماض الأمينية والمواد الكيميائية النباتية وإمكانات تنظيف الجذور الحرة: تم إجراء فحص المواد الكيميائية النباتية والأحماض الأمينية عن طريق تحليل كروماتوغرافيا سائلة عالية الأداء وتحليلات مطياف الكتلة كروماتوغرافيا الغاز. تم تحديد التأثيرات المضادة للأكسدة للمستخلص على الجذور الحرة على الجذور 2،2'-أزينو-مكرر (3-إيثيل بنزوثيازولين-6-حمض السلفونيك)، 2، 2-ثنائي فينيل-1-بيكريل هيدرازيل، وأكسيد النيتريك وجذر الهيدروكسيل. **النتائج**: أظهرت النتائج وجود سبعة مستقلبات ثانوية ذات محتوى عالي من الستيرويد والفلافونويد وانخفاض تركيز الكومارينات والجليكوسيدات والقلويدات والسابونين والتربينويد. أظهر تحليل HPLC للمادة الكيميائية النباتية أنها تحتوي على خمسة عشر مركبًا نباتيًا وسبعة عشر حمضًا أمينيًا بينما أظهر تحليل GCMS أحد عشر حمضًا دهنيًا. بالنسبة لنشاط مسح الجذور الحرة، كان قادرًا على تثبيطABTS ، DPPH، NO، و-OH، بطريقة تعتمد على التركيز. **الاستنتاجات**: تشير النتائج إلى أن الثمرة تمتلك خصائص غذائية جيدة ومكونات أيضية نباتية ثانوية وفيرة، وهذا قد يوفر دليلاً يدعم إمكانات الفاكهة في التخلص من الجذور الحرة.

INTRODUCTION

Date palm fruit (*Phoenix dactylifera*), termed simply as date is a specie of the family Arecaceae that is rich in many essential nutrients and polyphenols, and is one of the most consumed fruits in the Middle East and North Africa [1]. Dates is cultivated throughout the Middle East and to an increasing degree in other regions of the world including parts of Central and South America, Europe, India, and the United States. Date palm fruit has been reported to be a good source of high nutritional value food, which is rich in carbohydrates, proteins, dietary fibers, minerals, and vitamin B complex, such as thiamine (B1), riboflavin (B2), niacin (B3), pantothenic (B5), pyridoxine (B6), and folate (B9) [2]. It is also regarded as a good source of antioxidant vitamins A, C, and E, with the anthocyanins, isoquercetrin, quercetin, quercetrin, procyanidins, apigenin, luteolin, and rutin constituting the flavonoid content of date palm fruit [3]. In recent years, a huge interest in the abundant health promoting properties of date fruits had led to many pharmacological studies (*in-vitro* and *in-vivo*) as well as the identification and quantification of different classes of phytochemicals present in this fruit [4].

Plants are capable of producing and synthesizing diverse groups of organic compounds which are divided into two major groups: primary and secondary metabolites. Primary metabolite are metabolites that are directly involved in normal growth, development, reproduction, and usually performs a physiological function in the organism (i.e. an intrinsic function) [5]. Secondary metabolites also known as phytochemicals or natural products are metabolic intermediates or products which are not involved in the primary metabolism (photosynthesis, respiration, protein, and lipid metabolism) and seem to have no direct function in growth and development of the producing plants but rather required for interaction of plants with their environment and produced in response to stress [6]. Phytochemicals includes flavonoids, alkaloids, saponins, steroids, phenolics, tannins, terpenes, glycosides etc. Dietary phytochemicals are found in fruits, vegetables, legumes, nuts, herbs, and spices, and they possess anti-inflammatory, anti-bacterial, anticancer and antioxidant properties [7].

Free radicals are molecules with a single, unpaired electron, and are naturally produced in the body through the normal functioning of body systems and various physiochemical conditions, or through exposure to external sources such as pollutants, chemicals, and other environmental agents [8]. Molecules with an unpaired electron are highly unstable and reactive and will seek out another molecule to either give it their extra electron or take one of its electrons, thus creating another free radical. Free radicals thus start chain reactions that can damage a cell and its important constituents, including proteins, lipids and even DNA [9]. This destructive process can lead to overall disruption of the body's delicate balance, known as homeostasis. Over the course of an individual's lifetime free radical damage tends to accumulate, and scientists theorize that this accumulated damage contributes to cellular dysfunction and ultimately to declining health as they age and is thought to be a significant part of the aging process itself. Free radicals cannot be avoided as they are part of the body's normal physiological processes, but their impact on health can be minimized with free radical scavengers. A free radical scavenger also be known as an antioxidant refers to any molecule that is stable enough to donate an electron to a free radical currently on a tear through the body, neutralizing it or reducing its capacity to cause damage in the body [9]. Free radicals and other reactive oxygen species can adversely affect various important classes of biological molecules, such as protein, deoxyribonucleic acid (DNA), and lipids causing oxidative deterioration of biomolecule, which can lead to aging, heart disease, stroke, arteriosclerosis, diabetes, cancer, and inflammation [9]. The protective effects of fruits against chronic diseases are ascribed to their nutrients and bioactive non-nutrients called phytochemicals present in fruits, and as such, fruit have gained increased interest among several researchers due to their potential health benefits such as antioxidant

activity, cholesterol-lowering properties, and cardioprotective activity [2]. Hence, this study investigated the nutritional/chemical constituents and the free radical scavenging potentials of the aqueous extract of *Phoenix dactylifera* fruit.

METHODS

This study was conducted during the year 2023 in laboratories of biochemistry department at the University of Ilorin, Ilorin.

Plant Materials and Authentication

Fresh fruits of *Phoenix dactylifera* were purchased at the Oja Oba market, Ilorin, located in the North central region of Nigeria. The plant was taxonomically identified and authenticated at the herbarium unit of the National Centre for Genetic Research and Biotechnology (NACGRAB) Moor plantation, Apata, Ibadan. An herbarium number; NACGRAB/ARECACEAE/003 was assigned to the *Phoenix dactylifera* sample.

Preparation of Aqueous Extract of the Fruit of Phoenix dactylifera

The purchased date fruits were cleaned, and the edible part of the date was dried at room temperature before grinding it with a grinding machine into fine powder. The sample was extracted by soaking a total of 1000g of the date powder in 4 litres of distilled water for 72 hours. It was sieved and filtered using Wattman filter paper, the filtrate was then concentrated in a water bath at 40℃.

Determination of the Nutritional/Chemical Constituents of the Fruit of Phoenix dactylifera

Secondary Plant Metabolite Analysis

The secondary metabolites in the aqueous extract concentrate of *Phoenix dactylifera* were determined using standard analytical tests for both qualitative and quantitative analysis.

High Performance Liquid Chromatography (HPLC) Analysis of the Secondary Plant metabolite Constituents of the Fruit of Phoenix dactylifera

High performance liquid chromatography (HPLC) analysis was performed using Shimadzu LC-20D dual binary pumps, Shimadzu CTO-10AS column oven, and Shimadzu Prominence SPD-20A UV/Vis detector. The analysis was performed using a C-18 reversed phase column (Phenomenex, Gemini 5 μ, 150 mm length × 4.6 mm internal diameter). The composition of solvents and the gradient elution profile used in this analysis were as described by Kaisoon *et al*., 2011 with slight modifications The mobile phase consisted of acetic acid-acidified deionized water (pH 2.8) as solvent A and acetonitrile as solvent B at a flow rate of 0.8 mL/min. Gradient elution was executed as follows: 0-5 min, 5-9% solvent B; 5-15 min, 9% solvent B; 15-22 min, 9-11% solvent B; 22-38 min, 11-18% solvent B; 38-43 min, 18-23% solvent B; 43-44 min 23-90% solvent B; 44-45 min, 90-80%, solvent B; 45-55 min, 80% solvent B; 55-60 min, and 80-5% solvent B. The column was equilibrated with 5% solvent B for 20 min after each injection of samples. The column temperature was set to 38°C and the injection volume was 20 μL. The wavelengths were set to 280 nm for the detection of HBAs, 320 nm for hydroxycinnamic acids, and 370 nm for flavonoids [10]. Phenolic compound identification and quantification were performed by comparing respective retention times and peak areas with pure standard compounds utilizing the method of external standards to construct calibration curve. The concentrations of standards used for calibration curve ranged from 0.01 mM to 3 mM [10].

High Performance Liquid Chromatography (HPLC) Analysis of the Amino Acids of the Fruit of Phoenix dactylifera

HPLC analysis of the amino acids profile was carried out by HPLC with UV detector method. uBondapack C18 and acetonitrile/water (70:30) were used as column and carrier respectively.

10.00g sample was extracted with acetonitrile and then stabilized with ethyl acetate. The stabilized sample

was introduced into 25ml standard flask and made up to the mark. 5µl of the solution was injected at 2ml/min flow rate [10].

Gas Chromatography Mass Spectrometry (GCMS) Analysis of the Secondary Plant Metabolites Constituents of the Fruit of Phoenix dactylifera Fruit

GC-MS analysis was performed using a 7820A gas chromatograph coupled to a 5975C inert mass spectrometer (with triple axis detector) and an electron-impact source (Agilent Technologies, Santa Clara, CA 95051, USA). 0.5 g of *Phoenix dactylifera fruit extract* was suspended in ethanol to make a concentration of 100 mg/ml (w/v), followed by filtration through Varian Bond Elute C18 solid-phase extraction to remove impurities. The stationary phase of separation of the compounds was carried out on a HP-5 capillary column coated with 5% of phenyl methyl siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness) (Agilent Technologies, Santa Clara, CA 95051, USA). The carrier gas used was GC-grade helium (99.999% purity) at a constant flow rate of 1.573 ml/min, an initial nominal pressure of 1.9514 psi, and at an average velocity of 46 cm/s. One microliter (1 μl) of the samples was injected in the split-less mode at an injection temperature of 260°C. Purge flow was 21.5 ml/min at 0.50 min with a total gas flow rate of 23.355 ml/min; the gas saver mode was switched on. The oven was initially programmed at 60°C (1 min) and then ramped at 4°C/min to 110°C (3 min), followed by temperature program rates of 8°C/min to 260°C (5 min) and 10°C/min to 300°C (12 min). Run time was 56.25 min with a 3 min solvent delay. The mass spectrometer was operated in the electron-impact ionization mode at 70 eV with an iron source temperature of 230°C, quadrupole temperature of 150°C, and transfer line temperature of 280°C. The mass spectrophotometer conditions are solvent delay of 3.00 min, gain factor of 1.00, and resulting EM voltage of 1859, and scanning of possible compounds was from m/z 30 to 550 amu at a 2.62 s/scan rate. Using computer searches on a National Institute Standard and Technology (NIST) 14 Mass Spectral Database and the Mass Spectral Search Program (Version 2.2) and comparing, the spectrum obtained through GC-MS compounds present in the *Phoenix dactylifera fruit* was identified. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library to ascertain its chemical identity [11].

Determination of In vitro Free Radical Scavenging Potential of the Fruit of Phoenix dactylifera

Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

500µl of 0.3mM alcoholic solution of DPPH (Himedia, India) was added to 2.5ml of test samples at varying concentrations (250–1000 µg/ml). The samples were incubated in dark for 30 minutes, and absorbance was measured at 518nm using UV-visible spectrophotometer (Systronics AU-2700, India). Synthetic antioxidant butylated hydroxytoluene (BHT) were used as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage inhibition, using the following formula:

$$\% \text{ Scavenging} = \frac{\text{Abscontrol} - \text{Abssamples}}{\text{Abscontrol}} \times 100$$

Determination of Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the extract was determined as described by Smirnoff and Cumbes (1989) with slight modifications. 2ml of test compounds at 200 to 1000mg/ml, 0.6ml of 8mM ferrous sulfate, 0.5mL of 20mM hydrogen peroxide, and 2ml of 3mM salicylic acid were mixed and incubated at 37°C for 30 minutes. Thereafter, 0.9mL of distilled water was added to each vial, centrifuged at 4472g for 10 minutes and absorbance was read at 510nm. The percentage OH. scavenging activities of the extract was calculated using the following expression:

2.0 $\% \text{ Scavenging} = \frac{\text{ABScontrol} - \text{ABSsamples}}{\text{ABScontrol}} \times 100$

Determination of Nitric Oxide Scavenging Activity

The nitric oxide scavenging activity was determined according to the method of Marcocci, *et al* (1994). 2ml of the test extracts with varying concentrations (250–1000µg/ml) were incubated with 0.5ml of sodium nitroprusside (5mM) for 2 hours at 27°C. Aliquot 1ml of the incubated solution and mixed with 0.6ml of Griess reagent (1.0mL sulfanilic acid reagent [0.33%] in 20% glacial acetic acid at room temperature for 5 minutes with 1ml of naphthyl ethylenediamine dichloride [0.1%]). The absorbance was measured immediately at 550nm, and synthetic antioxidant BHT was used as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage scavenging, using the following formula:

$$\% \text{ Scavenging} = \frac{\text{ABScontrol} - \text{ABSsamples}}{\text{ABScontrol}} \times 100$$

Determination of Hydrogen Peroxide Scavenging Activity

H_2O_2 radical scavenging activity was determined according to the method of Ruch and Duhring (1989). A solution of H_2O_2 (40mM) was prepared in phosphate buffer (50mM, pH 7.4). Briefly, 1ml of test samples of varying concentrations (250–1000µg/ml) were added to the H_2O_2 solution and incubated for 10 minutes. Absorbance was measured at 230nm against blank solution containing phosphate buffer without H_2O_2. Synthetic antioxidant ascorbic acid was used as positive control. The experiments were performed in triplicates, and scavenging activity was expressed as percentage scavenging, using the following formula:

$$\% \text{ Scavenging} = \frac{\text{ABScontrol} - \text{ABSsamples}}{\text{ABScontrol}} \times 100$$

Determination of 2, 2′ -azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization

$ABTS^{\bullet+}$ decolorization was measured as described by Pellegrini *et al* (1999) with minor modifications. $ABTS^{\bullet+}$ solution was prepared by mixing aqueous ABTS (7mM) solution with 2.45mM potassium persulfate (1:1 *v/v*) and incubating in darkness at room temperature for 16 hours. The working solution was then obtained by diluting $ABTS^{\bullet+}$ solution in methanol to an absorbance of 0.70 ± 0.05 at 734nm. In each well of a 96 well-plate, 25µL of TDB sample was added to 200µL of the working solution. After a slight shake, the plate was covered by an aluminum foil and kept at room temperature for 30 minutes. Subsequently, the absorbance was recorded by a Multiskan™ Microplate Spectrophotometer (Thermo Fisher Scientific, Osaka, Japan). The ABTS radical decolorizing activity was calculated by the following formula:

$$\text{ABTS radical decolorizing activity (\%)} = \frac{1 - \text{Asample}}{\text{Acontrol}} \times 100$$

Statistical Analysis

Each data represents the mean of three replicates ± SEM. All results were statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test (DMRT) [16]. Differences between group means were considered significant at $p \leq 0.05$.

RESULTS

Nutritional/Chemical Constituents of the Fruit of *Phoenix dactylifera*

Qualitative and Quantitative Analysis of the Secondary Plant Metabolite Constituents of the Fruit Phoenix dactylifera

The qualitative secondary plant metabolite screening of the fruit of *Phoenix dactylifera* revealed the presence of seven secondary metabolites which are alkaloids, saponin, terpenoids, coumarins, cardiac glycosides, flavonoids, and steroids, and the quantitative analysis of the detected secondary metabolites shows that the fruit of *Phoenix dactylifera* has a relatively high steroid content (52.58 ± 0.08) and flavonoid content (25.77 ± 0.13) with low Coumarins, Glycosides, Alkaloids, Saponin, and Terpenoid concentration (Table 1).

High Performance Liquid Chromatography (HPLC) Analysis of the Phytochemical Constituents of the Fruit of Phoenix dactylifera

The HPLC analysis of the phytochemical constituents of the fruit of Phoenix dactylifera showed that it contained Fifteen (15) compounds namely, chlorogenic

acid, p-hydroxybenzoate, gallic acid, p-coumaric acid, catechin, protocatecuhic acid, cinammic acid, betanin, rutin, betanidin, ferulic acid, tricin, vanillic acid and indicaxanthin (Figure 1). Chlorogenic acid was the first to be eluted with the least retention time of 1.266 minutes while indicaxanthin was the last to be eluted with the highest retention time 19.416 minutes, and Betanin has the highest peak area of 9634.9820 cm2 while catechin has the lowest peak area of 90.5950 cm2 (Table 2).

*High Performance Liquid Chromatography (HPLC) Analysis of the Amino Acid Constituents of the Fruit of Phoenix dactylifer*a

The HPLC analysis of the amino acid constituents of the fruit of Phoenix dactylifera showed that it contained Seventeen (17) amino acids namely, glycine, alanine, serine, proline, threonine, cysteine, isoleucine, leucine, aspartic acid, lysine, glutamic acid, histidine, methionine, phenylalanine, arginine, tyrosine, and tryptophan (Figure 2). Glycine was the first amino acid to be eluted first with the least retention of 3.700 minutes while tryptophan was the last to be eluted with the retention time of 23.083 minutes, histidine had the highest peak area of 3212.6195 cm2 while serine has the lowest peak area of 76.4455 cm2 (Table 3).

Gas Chromatography Mass Spectrometry (GCMS) Analysis of Secondary Plant Metabolite Constituents of the Aqueous Extract of the Fruit of Phoenix dactylifera

The gas chromatography-mass spectrometry analysis chromatogram of the chemical constituent of fruit of Phoenix dactylifera showed that the extract contains decanoic acid, dodecanoic acid, methyl tetradecanoate, cis-13-Octadecenoic acid, cis-Vaccenic acid, Ethanol, 2-(tetradecyloxy)- 1-Heneicosyl formate 1-Hexacosene, trans-13-Octadecenoic acid, 1,19-Eicosadienee, Pentadecanoic acid, and 22-Tricosenoic acid (Figure 3).

Table 1: Secondary plant metabolite constituents of aqueous extract of the fruit of Phoenix dactylifera

Secondary plant metabolites	Concentration (mg/100g)
Flavonoids	25.77 ± 0.13
Glycosides	0.85 ± 0.00
Alkaloids	0.62 ± 0.02
Saponin	0.99 ± 0.00
Terpenoids	0.57 ± 0.04
Coumarins	5.29 ± 0.06
Steroids	52.58 ± 0.08
Tannins	Nd
Phenolics	Nd
Triterpenes	Nd
Anthocyanin	Nd
Phlobatanin	Nd

Values are means of three determinations ± SEM. Nd- Not detected.

Table 2: High Performance Liquid Chromatography Analysis of the Phytochemicals in Phoenix dactylifera fruit

Component	Retention (minutes)	Area (cm^2)	Height (cm)
Chlorogenic acid	1.266	1194.7065	33.746
p-Hydroxybenoate	2.750	2721.8470	26.244
Caffeic acid	4.450	838.0950	13.860
Gallic acid	5.466	380.3530	9.212
p-Coumaric acid	6.483	263.6720	7.639
Catechin	7.333	90.5950	5.949
Protocatechuic	7.950	108.6790	5.407
Cinnamic acid	9.316	96.1330	8.542
Betanin	11.050	9634.9820	162.322
Rutin	12.166	3699.0520	65.488
Betanidin	13.700	3338.5190	43.598
Ferulic acid	16.250	128.2480	6.453
Tricin	17.616	648.3270	8.243
Vanillic acid	18.900	97.0590	6.343
Indicaxanthin	19.416	102.4520	6.488

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Table 3: High Performance Liquid Chromatography Analysis of the Amino acid profile of the Fruit of Phoenix dactylifera

Component	Retention (minutes)	Area (cm²)	Height (cm)
Glycine	3.700	2621.3140	52.516
Alanine	5.883	767.6190	13.163
Serine	7.233	76.4455	4.743
Proline	7.966	927.8450	`7.463
Threonine	9.950	98.4310	5.792
Cysteine	10.500	201.4200	5.531
Isoleucine	11.300	122.8860	5.370
Leucine	11.850	180.4900	5.643
Aspartic acid	13.466	109.3930	5.279
Lysine	13.833	103.8980	5.313
Glutamic acid	15.500	1077.9995	16.944
Histidine	17.233	3212.6195	40.018
Methionine	19.166	130.6225	9.142
Phenylalanine	19.950	101.3825	7.007
Arginine	20.500	197.3760	6.236
Tyrosine	21.416	191.0525	5.608
Tryptophan	23.083	105.4955	4.361

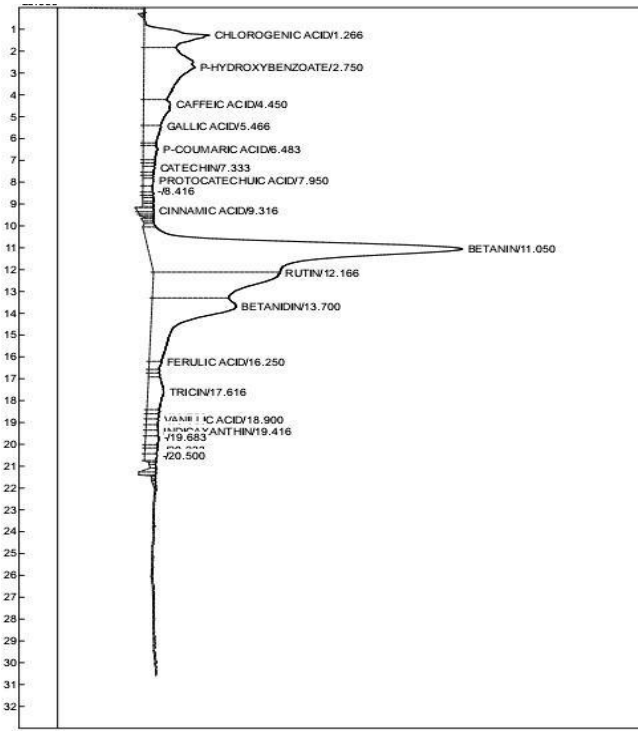

Figure 1: High Performance Liquid Chromatography Analysis of the Phytochemical Constituents of the Fruit of Phoenix dactylifera

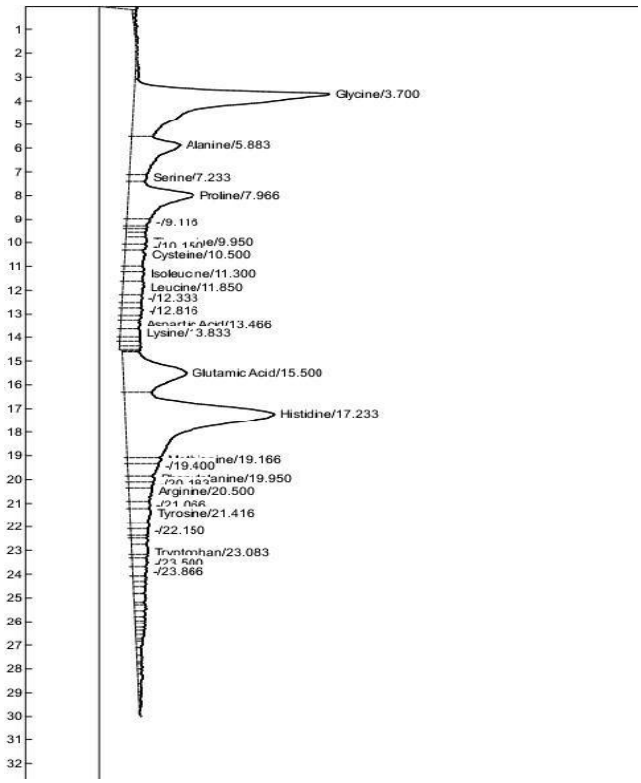

Figure 2: High Performance Liquid Chromatography Analysis of the Amino acid Constituents of the Fruit of Phoenix dactylifera

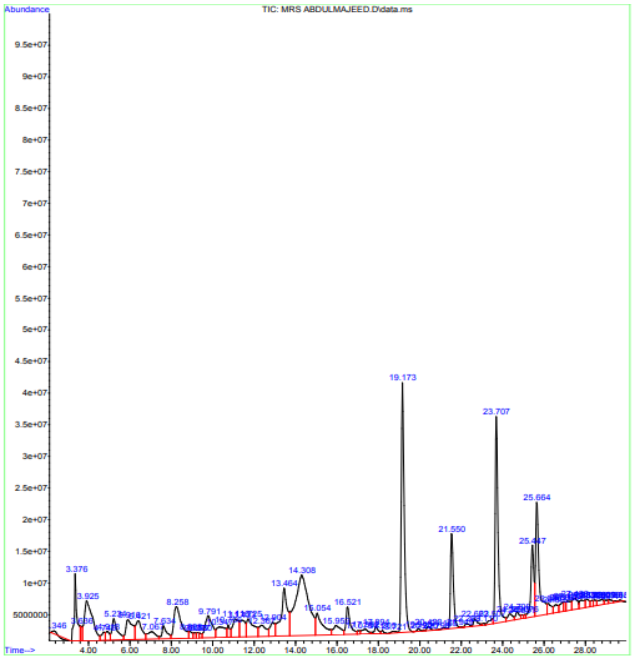

Figure 3: Gas chromatography mass spectrometry analysis of chemical constituent of the fruit of Phoenix dactylifera

In vitro Free Radical Scavenging Potentials of the Aqueous Extract of the Fruit of Phoenix dactylifera

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the aqueous extract of the fruit of Phoenix dactylifera

The result of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the aqueous extract of the fruit of *Phoenix dactylifera* showed an increase in the inhibition of DPPH as the concentration of the aqueous extract of the fruit of *Phoenix dactylifera* increases but was significantly lower ($p < 0.05$) when compared with the control (butylated hydroxytoluene) at the same concentration (Figure 4). The result revealed that DPPH, which is a stable free radical was scavenged by the aqueous extract of the fruit of *Phoenix dactylifera* in a dose dependent manner.

Hydroxyl radical (OH^-) scavenging activity of the aqueous extract of the fruit of Phoenix dactylifera

The result of the Hydroxyl radical (OH^-) scavenging activity of the aqueous extract of the fruit of *Phoenix dactylifera* showed an increase in the inhibition of hydroxy radical as the concentration of the aqueous extract of the fruit of *Phoenix dactylifera* increases, with a significant difference ($p< 0.05$) in the inhibition of hydroxy radical by the aqueous extract of the fruit of *Phoenix dactylifera* at higher concentrations, but was significantly lower ($p < 0.05$) when compared with the control (butylated hydroxytoluene) at the same concentration (Figure 5). This result revealed that the hydroxyl radical (OH^-) scavenging activity of the aqueous extract of fruit of *Phoenix dactylifera* increased in a concentration dependent manner.

Nitric Oxide scavenging activity of the aqueous extract of the fruit of Phoenix dactylifera

The result of the Nitric Oxide scavenging activity of the aqueous extract of the fruit of *Phoenix dactylifera* showed an increase in the inhibition of Nitric oxide as the concentration of the aqueous extract of the fruit of *Phoenix dactylifera* increases, but was significantly lower ($p < 0.05$) when compared with the control (butylated hydroxytoluene) at the same concentration (Figure 6), indicating that the Nitric oxide scavenging activity of the aqueous extract of fruit of *Phoenix dactylifera* increased in a concentration dependent manner.

2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity of the aqueous extract of the fruit of Phoenix dactylifera

The result of the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activity of the aqueous extract of the fruit of *Phoenix dactylifera* showed an increase in the inhibition of ABTS as the concentration of the aqueous extract of the fruit of *Phoenix dactylifera* increases, with a significant difference ($p< 0.05$) in the inhibition of ABTS by the aqueous extract of the fruit of *Phoenix dactylifera* at higher concentrations, but was significantly lower ($p < 0.05$) when compared with the control (butylated hydroxytoluene) at the same concentration (Figure 7). This result revealed that the ABTS radical scavenging activity of the aqueous extract of fruit of *Phoenix dactylifera* increased in a concentration dependent manner.

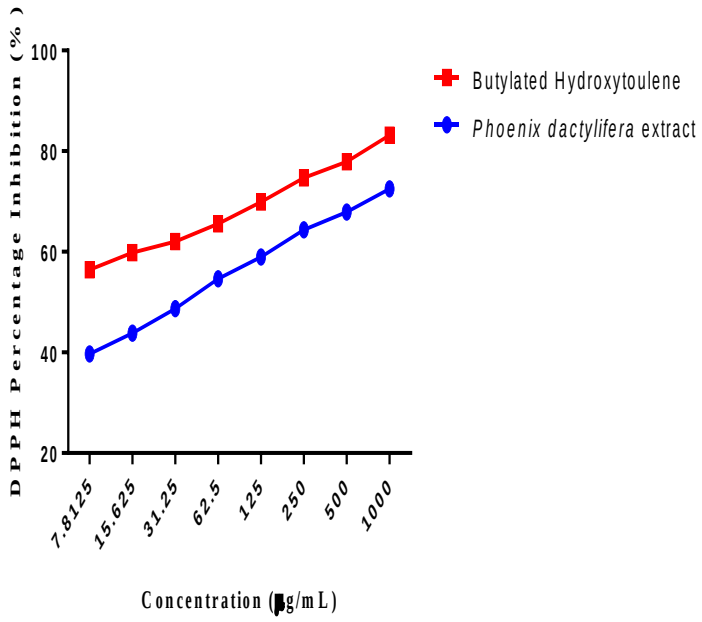

Figure 4: 2,2-diphenyl-1-picrylhydrazyl (DPPH) percentage inhibition of the aqueous extract of the fruit of Phoenix dactylifera. Values are means of three determinations± SEM

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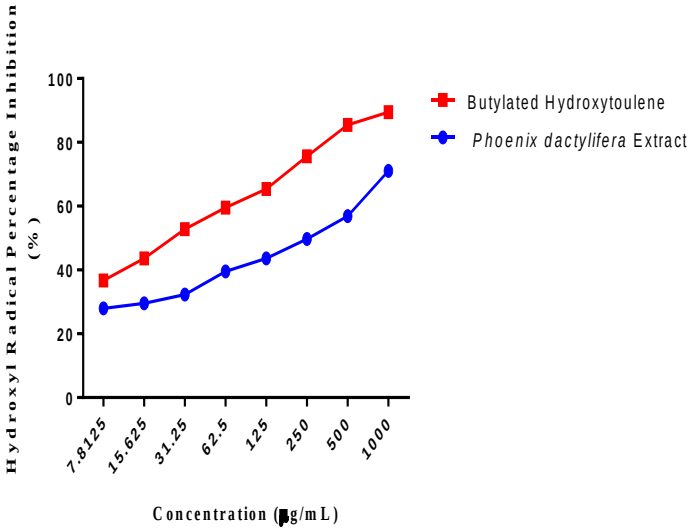

Figure 5: Hydroxyl radical (OH-) percentage inhibition of the aqueous extract of the fruit of Phoenix dactylifera Values are means of three determinations ± SEM

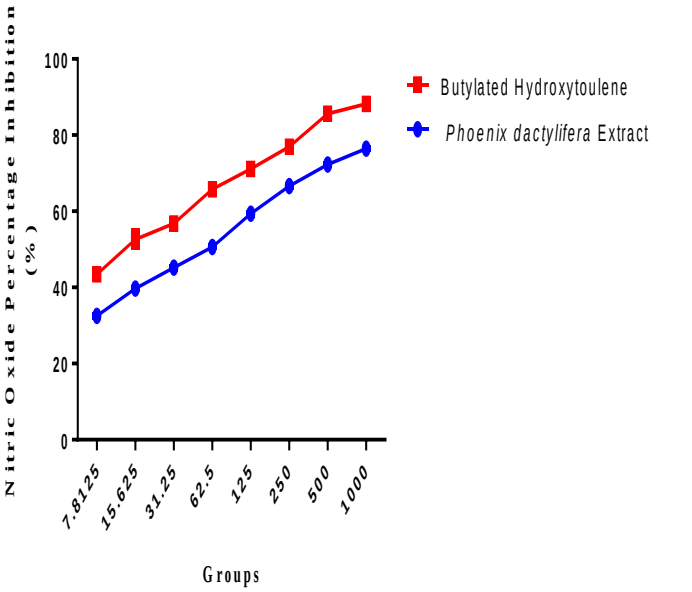

Figure 6: Nitric oxide (NO) percentage inhibition of the aqueous extract of the fruit of Phoenix Dactylifera. Values are means of three determinations ± SEM

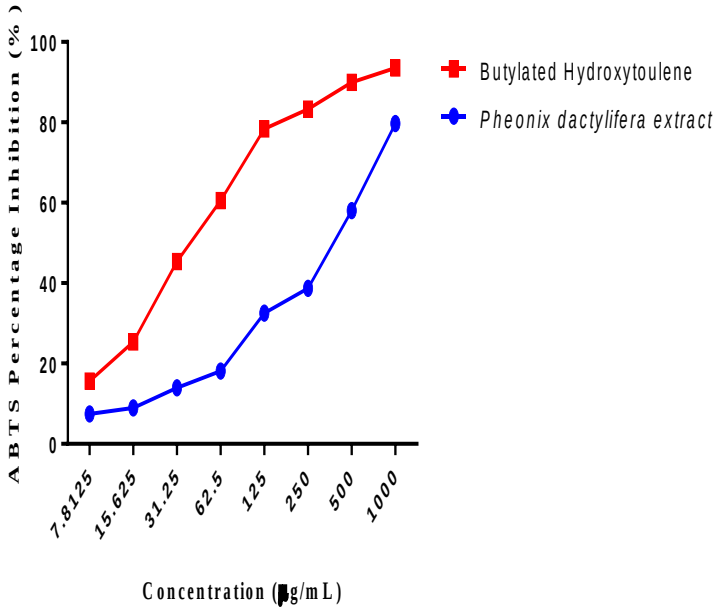

Figure 7: 2, 2′ -azinobis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) percentage inhibition of the aqueous extract of the fruit of Phoenix dactylifera Values are means of three determinations ± SEM

DISSCUSION

Nutritional/Chemical Constituents of the Fruit of *Phoenix dactylifera* Secondary Plant Metabolite Constituents

The protective and health promoting effect of secondary plants metabolites also referred to as phytochemicals have been investigated extensively and have been reported to impact physiologically beneficial effects in humans, both for disease treatment and prevention [17]. *Phoenix dactylifera* fruit has been reported to contain rich phytochemical constituents with various health promoting effects such as antidiabetic, anti-inflammatory, antimicrobial, antioxidant, cytotoxic and aphrodisiac effects [18; 19]. The phytochemicals identified in this study are similar with those from the research by Eze-Steven *et al.* (2021) which reported that *Phoenix dactylifera* contains tanins, alkaloids, flavonoids, terpenoids, glycosides, steroids and phenols. Flavonoids present in plants possess diverse health benefits, which includes antioxidant and free radical scavenging activities, and reduction in the progression of certain chronic diseases [21]. This

suggests that flavonoids in *Phoenix dactylifera* fruits can act as a free radical scavenger due to their hydroxyl groups and exhibit modulatory effects on a variety of metabolic and signaling enzymes, thus preventing chronic diseases. Studies have also revealed that steroids possess antioxidants properties *in vitro* which can be explored in the scavenging of free radicals involved in the manifestation of cardiotoxicity [22]. Alkaloids are important therapeutic plant secondary metabolites and have been studied for their potential anti-hyperglycemic and cardioprotective effect [23]. Saponins possess the potential to lower cholesterol levels due to their anti-hypercholesterolemic effect and thus, it could be deduced that the aqueous extract of the fruit of *Phoenix dactylifera* could lower cholesterol levels in humans [24].

Amino Acid Profile

Amino acids are one of the central chemicals needed by the body to function well, they are the building blocks of proteins and serve as intermediates in protein metabolism [25]. HPLC analysis of the aqueous extract of the fruit of *Phoenix dactylifera* revealed the presence of 17 amino acids and these amino acids are useful nutrients that are well absorbed and utilized by cells to synthesize various proteins. Davies *et al*. (1997) reported that the administration of some amino acids are capable of attenuating oxygen free radical induced damage and the degradation of proteins. Also, some amino acids especially the sulfur containing compounds such as cysteine and methionine can react with free radicals and result in reduced oxidative degradation thereby attenuating the free radical generated during the cell damage [26].

Free Radical Scavenging Potential

Free radicals and other reactive oxygen species can adversely affect various important classes of biological molecules, such as protein, deoxyribonucleic acid (DNA), and lipids causing oxidative deterioration of these biomolecules, which can lead to aging, heart disease, stroke, arteriosclerosis, diabetes, cancer, and inflammation [9]. Numerous studies have conclusively shown that aqueous extract of fruit of *Phoenix dactylifera* act as a potent antioxidant, anti-inflammatory, and antitumoral agent [9]. Radical scavenging antioxidants are significant in protecting cells from the injury of free radicals, thus have enormous significance in the prevention and therapeutics of diseases [9].

2, 2′ -azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) Scavenging Activity

The ABTS radical scavenging activity of the aqueous extract of fruit of *Phoenix dactylifera* was concentration dependent. The increasing scavenging activity of the aqueous extract on ABTS radical with increasing concentration could be attributed to the presence of antioxidants, especially phenols such as flavonoids [27]. This is because flavonoids, especially those having hydroxyl groups, are potent hydrogen donators and consequently can neutralize free radical easily [28]. This result is in agreement with the study by Biglari *et al.* (2008) where a strong correlation between the antioxidant activity and the total flavonoids of palm dates were investigated.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity

DPPH antioxidant assay is based on the ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical, which accept an electron or hydrogen atom and decolorize in the presence of antioxidants [30]. DPPH, a stable free radical was scavenged by the aqueous extract of the fruit of *Phoenix dactylifera* in a dose dependent manner. The results were in line with that of [31] who reported that date fruits exhibited potent DPPH scavenging capacities. This suggests that scavenging of DPPH radical by hydrogen and /or electron donation by *Phoenix dactylifera* extract might prevent reactive radical species from reaching biomolecules such as lipoproteins, poly unsaturated fatty acids (PUFA), deoxyribonucleic acid (DNA), amino acids and proteins [32]. This antioxidant activity could be due to the high content of the phytochemical compounds present in the fruit [9].

Nitric Oxide Scavenging Activity

Nitric oxide radical is an unstable radical that could be involved in chain reaction to produce its oxidation products, which is detrimental to human health and can result in the development of some diseases [33]. The increase in the inhibition of Nitric Oxide by the aqueous extract of fruit of *Phoenix dactylifera* as its concentration increased could be indicative of the hydrogen donating ability of the antioxidants present in *Phoenix dactylifera* aqueous extract, such as phenolic and polyphenolic compounds. As a result, the aqueous extract could be a potent novel therapeutic agent for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation products (nitrite and peroxynitrite) [33]. Futhermore, the aqueous extract of *Phoenix dactylifera might* be involved in competition with oxygen to react with nitric oxide and thus inhibit generation of the mentioned anions, consequently terminating the radical chain reaction that are detrimental to human health [34].

Hydroxyl Radical (OH-) Scavenging Activity

The Hydroxyl radical (OH-) is an extremely reactive free radical formed in biological systems, that has the capacity to break DNA strands, which contributes to carcinogenesis, mutagenesis, and cytotoxicity [33]. In addition, this radical species is thought to be one of the quick initiators of lipid peroxidation process by abstracting hydrogen atoms from unsaturated fatty acids [35]. The increase in the hydroxyl radical (OH-) scavenging activity of the aqueous extract of fruit of *Phoenix dactylifera* in a concentration dependent manner is in agreement with the study carried out by Anjum (2015) where *in vitro* studies exposed that the aqueous extract of date fruit is a powerful scavenger of hydroxyl radicals to restrain protein oxidation and iron-induced lipid peroxidation in the rat brain homogenate in a concentration dependent manner.

CONCLUSION

From the result obtained from this study, it can be deduced that the aqueous extract of the fruit of *Phoenix dactylifera* possess nutritional and antioxidant properties which is evident from its free radical scavenging activities against the free radicals used and also from the presence of some phytochemicals such as flavonoids, saponin and alkaloids known to possess antioxidant potentials.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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