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Chemical characterization and phytochemical evaluation of phoenix dactylifera l. Pits from northern Nigeria

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Abstract

The date palm (Phoenix dactylifera) fruits are a popular staple fruit in the Northern part of Africa and the Middle East. The global production of this fruit is nearly 9 million tonnes annually with an increased demand created by the awareness of the therapeutic benefits of this plant. While the fruits have received much research attention over the years, the pits, (seeds) have hugely being considered a waste, and thus used in the formulation of animal feeds for untold centuries. Recently these pits from few studies have demonstrated some pharmacological activities ranging from antioxidant, antidiabetic, anti-bacterial to anti-cancer activities. However most of the studies have been carried out on the varieties mainly found in the Middle East, Egypt, Saudi Arabia and Northern parts of Africa with scanty mention of the variety found in Nigeria. This study investigated the Nigerian variety of dates: by phytochemical analysis and GCMS evaluation. The results of the phytochemical analysis identified alkaloids, flavonoids, phenolic compounds, tannins, lipids, saponins, triterpenoids/steriods carbohydrates, anthraquinone and proteins as the classes of compounds present in the date seed extract (DSE). The GCMS evaluation indicated the following nineteen compounds: Pentane, 3-ethyl-2,2-dimethyl, N-Aminomorpholine, glyoxal imine, 1,4-Cineol, 1,3,8-p-Menthatriene, 3,8,11-Trioxatetracyclo [4.4.1.0 (2,4).0(7,9)] undecane, (1α,2β,4β,6α,7β,9β), Benzene, (2-methyl-1-propenyl)-, Thymine, 2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one, p-Cymen-8-ol, 2-Methyl-6-methylene-octa-1,7-dien-3-ol, 1,4-dihydroxy-p-menth-2-ene, 2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-, (Z)-, 1-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene, (Z,Z)-α-Farnesene , Methyl tetradecanoate, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester and Phenol, 4-(1,1,3,3-tetramethylbutyl as the bioactive constituents in the DSE. This finding is consistent with some of the literature reported compounds from other varieties.

Keywords: GCMS, DSE, DNA

Introduction

Phoenix dactylifera L. popularly known as dates or sometimes referred to as date palm is a sweet fruit widely cultivated in the North of Africa, the Middle East and South of Asia [1,2,3,4]. The plant thrives in tropical and subtropical parts of the world, with over 8.5 tonnes produced annually. P. dactylifera is a flowering plant belonging to the Arecaceae family. Dates is a renowned staple food in the Middle East for thousands of years since 5500-3000 BC with nutritional, ornamental, environmental and economic significance [5]. In Nigeria, the plant is grown in the Northern part of Nigeria from Latitude 10 N downwards in the Sudan Savanna to Sahel regions. This region comprises of Adamawa, Bauchi, Borno, Gombe, Bauchi, Kaduna, Katsina, Kano, Sokoto, Kebbi Jigawa and Yobe States. The three varieties of dates in Nigeria are Red soft type, Tempo 2 and Tempo 3 (Zabia). Dates often attain a height of more than 20 metres and yields red fruits [6]. The ethnopharmacological uses of the plant are vast. These include; as a prophylactic in Morocco, India, Iran, Egypt and India[7], As an antidiabetic and hypertensive medicine in Morocco [8]. Since ancient times in different nations like Morocco, Iraq, India, Algeria, Iran and Egypt [7], pregnant women and people suffering from jaundice historically were placed on dates [9]. The plant is famous for the treatment of malaria and liver diseases in the Arab peninsula[9]. Other notable applications of dates are as anti-aging constituent for women, used as expectorant, diuretic, for gastrointestinal issues, toothaches, demulcent and laxative [7,10,11]. Analysis of dates showed that the fruit contains essential minerals and trace elements, such as selenium, fluorine cobalt, manganese, magnesium, copper, boron and potassium. The major constituent is sugar which makes up over 50% of the fruit, while protein, fiber, minerals and other nutrients make up the remainder [12]. Phytochemical studies documented the presence of anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids in date fruits. Many studies carried out on the fruits demonstrated a number of pharmacological activities such as antioxidant, anticancer, anti-inflammatory, hepatoprotective and nephroprotective activities[13]. A comparison of the glycemic index of three varieties of dates gave the range as 30.5-49.7 [14].

Date seeds though considered a waste are used in animal feed. The seed oil has been employed in cosmetics and dermatological applications, owing to its high content of fatty acids such as lauric acid and oleic acid. The oils are a potential source of oxalic acid. The anti-diabetic activities of the fruits have been documented [15]. Recently our research team reported the antidiabetic activities of the date pits (seeds) on the Nigerian variety [16]. The date seeds have been shown to inhibit genotoxicity and ameliorate DNA damage by N-nitroso-N-methylurea [17]. The seeds are used as additives to coffee beans. The browning substrates; caffeic acid glycoside 3-O-caffeoylshikimic acid (also known as dactylifric acid) and its isomers, have been identified in dates [18]. The oral toxicity of date seeds has been investigated, with no fatality recorded at a dose of 3000 mg/kg, in the animals[19]. The need for GCMS analysis of dates pits cannot be over-emphasized as the pits merits further research judging from the recent reported pharmacological activities. Furthermore, there is a paucity of data on the analysis of date pits sourced from Nigeria.

Materials and methods

Plant materials: Dried fruits of P. dactylifera were obtained from the Northern part of Nigeria where the fruits are grown in the month of March. The fleshy parts were taken off and the seeds collected. The seeds were air-dried under shade before being milled into fine particles.

Reagents

Methanol, Dichloromethane, Chloroform, Diethyl Ether, Acetic Anhydride, Glacial acetic acid, Sodium picrate, 2% 3,5-Dinitrobenzoic acid, Picric acid, Iodine solution, Dimethylsulfoxide, (JHD company, Guangdong. GuanghuaSci-Tech. Co. Ltd. China), Hydrochloric acid, Million's reagent, Benedict's solution, Wagner's reagent, Sodium Hydroxide, Ferric chloride solution, Saturated lead acetate solution, Dragendorff's reagent, Kedde reagent, Ammonia solution, 7.5% Potassium Hydroxide, Fehling's solution A and B (Sigma Aldrich Chemicals, St Louis, USA), Distilled water, Deionized water (Pharmaceutical Chemistry Lab, University of Port Harcourt).

Extraction of plant materials

About 500g of the pulverized P. dactylifera seeds were extracted according to the American National Cancer Institute (NCI) method of extraction [20]. The pulverized plant material was macerated in a 1:1 mixture of dichloromethane and methanol for 24 h to obtain the extracts. The procedure was repeated thrice. The ratio of plant material to solvent used was 1:5. The residue was further macerated in methanol for another 24 h to yield the methanol extract, which was combined with the dichloromethane/methanol extract to yield the total organic extract, which was henceforth referred to as date seed extract (DSE). The obtained dry extracts were further dried in a desiccator to remove any trace of solvent. Phytochemical Screening Phytochemical tests were carried out on the plants' extract by the method described by Trease and Evans [21].

GC-MS Analysis of the crude extracts

The gas chromatography mass spectrometry (GC-MS) analysis of the DSE was quantitatively determined using an Agilent 7890B GC system coupled with an Agilent 5977A MSD with a Zebron-5MS column (ZB-5MS 30 m × 0.25 mm × 0.025µm) (5%-phenylmethylpolysiloxane). The GC-grade helium served as the carrier gas at a constant flow rate of 2 mL/min. The DSE was dissolved with ethanol and filtered before use. The column temperature was maintained at 60°C and gradually increased at 10°C per minute until a final temperature of 300°C was reached. The time taken for the GC-MS analysis was 30 min. The compounds were identified based on computer matching of the mass spectra with the NIST 11 MS library (National Institute of Standards and Technology library).

Results

The results of the phytochemical screening are presented in Table 1.

Table 1: Phytochemical results

Test	Prescence
1. Carbohydrate	
a. Molisch test	-
b. Fehlings test	+
2. Anthraquinone	
a. Free (Bomtrager's test)	+
3. Triterpenoids/Steriods	
a. Liebermann-Buchard Test	+
b. Salkwoski's Test	+
4. Phenolics test	

a.	$FeCl_3$ test	+
5.	Tannin test	
a.	Phlobatannins test	+
6.	Flavonoids	
a.	Shinoda Reduction Test	+
b.	$AlCl_3$ test	-
7.	Alkaloids	
a.	**Dragendorff's** (orange colour)	+
b.	Mayer's test (cream)	-
c.	Hager's test (yellow ppt)	+
8.	Saponin	
a.	Frothing Test	+
b.	Emulsion Test	+
9.	Proteins	
a.	Million's Test	+
b.	Picric acid test	+
10.	Fats and Oils	
a.	Oil Stain test	+

Key: +=present, -=absent

The results presented in Table 1 showed that the DSE contained the important classes of phytoconstituents notably alkaloids, flavonoids, phenolic compounds, tannins, fats & oils, saponins, triterpenoids/steriods carbohydrates, anthraquinone and proteins. It has been reported that the seed is rich in unsaturated fatty acids, which were non-toxic [22]. Other studies showed that the seed of P.dactylifera constitutes 5-14% of the total weight of the fruit. The proximate analysis of the seed as documented noted that the carbohydrate content was 20.95%, with the protein content being 2.63% while the fat content was 8.55% [23].The same researchers documented the flavonoid content as 45.28+0.32 mg/100 g, while the phenolic content which was lesser was determined as 28.22+0.43 g/100 g.

The GCMS chemical characterization of the DSE was carried out and the results presented in Table 2. The interpretation of GC-MS mass-spectra was based on the NIST library of the equipment. The individual spectrum were matched with that of the library and the following parameter; molecular weight, structure, retention time and fragmentation patterns compared. Nineteen bioactive compounds were ascertained from the spectral match.

Table 2: GC-MS analysis of P. dactylifera seeds

Peak	RT	Area %	MW	MF	Name of Compound	Documented Bioactivity
1	2.017	4.61	128	C9H20	Pentane, 3-ethyl-2,2-dimethyl	
2	2.183	0.94	142	C6H10N2O2	N-Aminomorpholine, glyoxal imine	
3	4.958	0.74	154	C10H18O	1,4-Cineol	Anxiolytic effects [24]
4	5.016	7.73	134	C10H14	1,3,8-p-Menthatriene	Antimicrobial [25,26]
5	5.187	5.65	154	C8H10O3	3,8,11-Trioxatetracyclo[4.4.1.0(2,4). 0(7,9)]undecane,	

					(1α,2β,4β,6α,7β,9β)	
6	6.006	0.54	132	C10H12	Benzene, (2-methyl-1-propenyl)-	
7	6.206	0.42	126	C5H6N2O2	Thymine	
8	6.675	1.29	126	C7H10O2	2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one	Antimicrobial [27]
9	7.430	1.25	150	C10H14O	p-Cymen-8-ol	Antioxidant and Antifungal activities [28]
10	8.317	1.13	152	C10H16O	2-Methyl-6-methylene-octa-1,7-dien-3-ol	
11	8.695	1.11	170	C10H18O2	1,4-dihydroxy-p-menth-2-ene	
12	8.958	0.01	150	C10H14O	2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-, (Z)-	Antioxidant [29]
13	9.118	1.11	150	C10H14O	1-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene	
14	9.370	0.55	204	C15H24	(Z,Z)-α-Farnesene	Anticancer [30]
15	14.068	1.60	242	C15H30O2	Methyl tetradecanoate	
16	16.162	9.38	270	C17H34O2	Hexadecanoic acid, methyl ester (methyl palmitate)	Antifungal,Antioxidant, Antimicrobial, hypocholesterolemic, nematicidal, pesticidal, antiandrogenic flavour, haemolytic, 5-Alpha reductase inhibitor [31]
17	17.821	9.51	294	C19H34O2	9,12-Octadecadienoic acid, methyl ester (oleic acid derivative)	Antibacterial and Antifungal [32]
18	17.867	22.09	296	C19H36O2	9-Octadecenoic acid (Z)-, methyl ester	Antioxidant activity, Anticarcinogenic,dermatitig

					(oleic acid derivative)	enic and flavouring [31,33]
19	33.379	10.81	206	C14H22O	Phenol, 4-(1,1,3,3-tetramethylbutyl)-	Antibacterial [34]

Discussion

The phytochemical analysis results presented in Table 1, showed that ten important classes of compounds are contained in DSE. This observation is in consonance with the results obtained from the phytochemical studies of date seeds from India. In the referenced study the researchers documented the presence of flavonoids, tannins, saponins, phenolic compounds, alkaloids, sterols, triterpenes and anthraquinone glycosides [35].

The results of the GC-MS analysis presented in table 2 revealed that the DSE contained numerous bioactive compounds; Pentane, 3-ethyl-2,2-dimethyl, N-Aminomorpholine, glyoxal imine, 1,4-Cineol, 1,3,8-p-Menthatriene, 3,8,11-Trioxatetracyclo[4.4.1.0(2,4).0(7,9)]undecane, (1α,2β,4β,6α,7β,9β), Benzene, (2-methyl-1-propenyl)-, Thymine, 2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one, p-Cymen-8-ol, 2-Methyl-6-methylene-octa-1,7-dien-3-ol, 1,4-dihydroxy-p-menth-2-ene, 2-Cyclopenten-1-one, 2-(2-butenyl)-3-methyl-, (Z)-, 1-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene, (Z,Z)-α-Farnesene , Methyl tetradecanoate, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester and Phenol, 4-(1,1,3,3-tetramethylbutyl)- as observed from the documented bioactivities reported in literature. An isomer of 11-Oxaspiro[2.5]oct-5-ene, 8,8-dimethyl-4-methylene , which is -Oxaspiro[2.5]octane,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)- has been reported in the GC-MS evaluation of Aspilia africana; tagged the 'hemorrhage plant' which is synonymous with a plethora of bioactivities such as wound healing properties, antimicrobial, anti-inflammatory, anti-fertility activity, haemostatic and anti-ulcer activity [36]. Similarly, Methyl tetradecanoate was reported in the GC-MS of Cyperus iria L. a plant used traditionally in India as a diuretic, for fever, rheumatism, amenorrhoea and regulation of the menstruation [37]. A comparison with the GC-MS analysis from an earlier study reported the presence of Pentane, 3-ethyl-2,4-dimethyl- with a peak area % of 2.14% as against 4.6% in this present study [38].

Date seed oil contains numerous fatty acids, as observed from the GC-MS analysis. The three main classes of fatty acids; i. saturated fatty acids such as palmitic, lauric and myristic acids; ii. Monosaturated fatty acids which includes oleic and palmitoleic and iii. Polyunsaturated fatty acids made up of linoleic and linolenic have been identified in date seed oil [12,39,40]. Some of the studies on date seed oil determined oleic acid to be the most abundant unsaturated fatty acid in the seed oil. The presence of Capric, linoleic, Myristoleic, linolenic, myristic, palmitoleic and stearic acids were also documented [41]. In this present study, two oleic acid derivatives were identified from the GC-MS analysis; 9,12-Octadecadienoic acid, methyl ester (9.51%) and 9-Octadecenoic acid (Z)-, methyl ester (22.09%). These two fatty acids were the most abundant unsaturated fatty acids present in the DSE. Hexadecanoic acid, methyl ester (9.38%), another highly bioactive fatty acid was found in high amounts in the DSE. In comparison with olive oil, the degree of unsaturation of date seeds oils is lesser than that of most olive oils [12]. Oils rich in oleic acid contents

possess high stability and nutritional benefits which enhances the protective effects against cardiovascular diseases, antioxidant potentials and anticholesterol activities [18]. The presence of Phenol, 4-(1,1,3,3-tetramethylbutyl)- (10.8%) and 1,3,8-p-Menthatriene (7.73%), with potent antibacterial activities suggest that the date pits would possess good antibacterial properties.

Conclusion

The phytochemical and GC-MS analysis of date seed extract, (DSE) of the Nigerian variety has been carried out. The study showed that the variety is rich in biologically-important classes of compounds such as alkaloids, flavonoids, phenolic compounds, tannins, Lipids, saponins, triterpenoids/steriods carbohydrates, anthraquinone and proteins. Nineteen compounds were identified from the GC-MS analysis. The high abundance of unsaturated fatty acids underscores the antioxidant potentials of the date pits. Also the presence of three highly antibacterial compounds heightens the possibilities of the pits exhibiting good antimicrobial activities. The date pits deserves further studies to explore yet-undiscovered bioactivities.

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