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Gas chromatography-mass spectrometry analysis and *in vitro* inhibitory effects of *Phoenix dactylifera* L. on key enzymes implicated in hypertension

[Análisis de cromatografía de gases-espectrometría de masas y efectos inhibitorios *in vitro* de *Phoenix dactylifera* L. sobre enzimas clave implicadas en la hipertensión]

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Abstract

Context: *Phoenix dactylifera* (PD) is a medicinal plant reportedly used in folklore for hypertension management. Scientific validation for its use as an antihypertensive agent is scanty.

Aims: To investigate the bioactive compounds present in extract and differential solvent fractions, and the *in vitro* inhibitory effect of PD on the activities of key enzymes associated with hypertension.

Methods: Ethanol and differential solvent (ethyl acetate, butanol and water) fractions of PD were prepared using established methods and were thereafter used for the enzyme inhibition assays, which includes: angiotensin-converting enzyme (ACE), acetylcholinesterase (AChE), phosphodiesterase-5 (PDE-5), adenosine deaminase (ADA) and arginase. Gas chromatography-mass spectrometry was used to evaluate bioactive compounds present in the extracts and fractions.

Results: Preliminary phytochemical evaluations revealed the presence of tannins, flavonoids, betacyanins and phenols in all solvent fractions. Some of the compounds detected in *P. dactylifera* that have been implicated in hypertension management include squalene, lauric acid, palmitic acid, caprate, stearate, vitamin E, β-sitosterol, phytol, linolenic acid, isosorbide, coumarins and taurine. Furthermore, all except the aqueous fraction exerted significantly ($p<0.05$) higher ACE inhibition than the control drug (lisinopril). Also, the ethanol, butanol, and aqueous fractions exerted significantly ($p<0.05$) higher inhibition of PDE-5 than the control drug (sildenafil). However, the aqueous fraction exhibited the highest PDE-5 and AChE inhibition at 86.99 ± 0.10 and 91.81 ± 1.20%, respectively. Also, the aqueous fraction had the highest inhibitory effect on ADA (82.87 ± 4.32%).

Conclusions: These findings, therefore, justify the use of *P. dactylifera* as an antihypertensive agent in the folklore medicine.

Keywords: angiotensin-converting enzyme; arginase; hypertension; *Phoenix dactylifera*; phosphodiesterase-5; phytochemicals.

Resumen

Contexto: *Phoenix dactylifera* (PD) es una planta medicinal utilizada en el folklore para el manejo de la hipertensión. La validación científica para su uso como agente antihipertensivo es escasa.

Objetivos: Investigar los compuestos bioactivos presentes en el extracto y las fracciones de solvente diferencial, y el efecto inhibidor *in vitro* de la PD sobre las actividades de las enzimas clave asociadas con la hipertensión.

Métodos: Las fracciones de etanol y disolvente diferencial (acetato de etilo, butanol y agua) de PD se prepararon utilizando métodos establecidos y posteriormente se usaron para los ensayos de inhibición enzimática que incluyeron: enzima convertidora de angiotensina (ACE), acetilcolinesterasa (AChE), fosfodiesterasa-5 (PDE-5), adenosina desaminasa (ADA) y arginasa. La cromatografía de gases-espectrometría de masas se utilizó para evaluar los compuestos bioactivos presentes en los extractos y fracciones.

Resultados: Las evaluaciones fitoquímicas preliminares revelaron la presencia de taninos, flavonoides, betacianinas y fenoles en todas las fracciones de solventes. Algunos de los compuestos detectados en *P. dactylifera* que han sido implicados en el manejo de la hipertensión incluyen escualeno, ácido láurico, ácido palmítico, caprato, estearato, vitamina E, β-sitosterol, fitol, ácido linolénico, isosorbida, cumarinas y taurina. Además, todos excepto la fracción acuosa ejerció una inhibición de la ECA significativamente mayor ($p<0.05$) que el fármaco de control (lisinopril). Además, las fracciones etanólica, butanólica y acuosa ejercieron una inhibición significativamente mayor ($p<0.05$) sobre PDE-5 que el fármaco control (sildenafil). Sin embargo, la fracción acuosa exhibió la inhibición más alta de PDE-5 y AChE a 86.99 ± 0.10 y 91.81 ± 1.20%, respectivamente. Además, la fracción acuosa tuvo el mayor efecto inhibidor sobre ADA (82.87 ± 4.32%).

Conclusiones: Estos hallazgos, por lo tanto, justifican el uso de *P. dactylifera* como agente antihipertensivo en la medicina popular.

Palabras Clave: arginasa; enzima convertidora de angiotensina; fitoquímicos; fosfodiesterasa-5; hipertensión; *Phoenix dactylifera*.

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INTRODUCTION

Hypertension is a critical health burden globally, and it is reported to significantly impact the risk of stroke, heart attack, blindness, kidney dysfunction, and other life-threatening diseases (WHO, 2019). African and other developing countries are mostly affected by the global increase in the rate of hypertension incidence (WHO, 2013). Some major enzymes associated with the progression of hypertension include angiotensin-converting enzyme, phosphodiesterase-5, cholinesterases, arginase, monoamine oxidase, and adenosine deaminase (Oboh et al., 2018). These enzymes are reportedly elevated in hypertension; thus, it is believed that inhibiting their activities with pharmaceutical drugs or other biomolecules could ameliorate hypertension. For instance, inhibiting angiotensin-converting enzyme (ACE) results in enhanced vasodilation through improved nitric oxide and bradykinin availability (Majumder and Wu, 2015). Nitric oxide and bradykinin are potent vasodilators. Elevated arginase activity leads to reduced nitric oxide, endothelial nitric oxide synthase (eNOS) substrate-L-arginine as well as the cofactor- tetrahydrobiopterin (BH4); leading to elevated reactive oxygen species and inflammation (Majumder and Wu, 2015). The elevated phosphodiesterase-5 activity has been reported to hydrolyze cGMP, thus preventing vasodilatory responses (Oyeleye et al., 2017).

Extracts of plants have been reported to be beneficial to the management of hypertension. This beneficial effect has been attributed to the presence of predominant bioactive compounds present in these plants. For instance, some plants inhibit ACE activity mainly due to the presence of phenolic compounds in their composition (Oboh et al., 2018). The presence of plant phytochemical compounds is determined using various qualitative and quantitative methods of phytochemical analysis. Studying plant bioactive compounds is essential to the discovery of new drugs and scientific validation of folklore medications. It also helps to identify more economical sources of pharmacologically relevant bioactive compounds (Sermakkani and Thangapandian, 2012).

Fruits of *P. dactylifera* (date fruits) are reportedly used to manage several ailments both in Nigeria and other countries such as Morocco and Iran (Taleb et al., 2016). Biological activities reported for *P. dactylifera* include anti-inflammatory, neuroprotective, hepatoprotective as well as antioxidant effects (Shabani et al., 2013; El Arem et al., 2014; Yasin et al., 2015). To the best of our knowledge, there is a dearth of literature on the antihypertensive activities of *P. dactylifera*. In this study, therefore, the complete GC-MS profile of the fruits of *P. dactylifera* and its *in vitro* inhibitory effects on the activities of key enzymes linked with hypertension were investigated.

MATERIAL AND METHODS

Plant materials

Dry fruits of *Phoenix dactylifera* were purchased from major distributors at a local market (Agege, Lagos State). The fruits together with the plant (obtained from a local farm in Kano, Kano State) were identified and authenticated at Forestry Research Institute of Nigeria (FRIN) Ibadan, Oyo State, and samples were deposited at the institution's herbarium. Dried fruits were cleaned, pits removed, and thereafter milled to a fine powder using an Apex hammer mill. Milled samples were stored in tightly sealed plastic bags.

Solvent extraction and phytochemical screening

A weighed amount of the milled sample (4 kg) was soaked in 25 L of 96% ethanol for 72 h, filtered and extracted 3 times. The combined filtrate was thereafter concentrated at reduced pressure using a rotary evaporator (Adebayo et al., 2010). A weighed amount (200 g) of the ethanol extract was dissolved in 1 L distilled water and subsequently partitioned with organic solvents such as ethyl acetate, butanol and water, with percentage yields of 5.0, 5.0 and 65.0% respectively. The fractions were concentrated separately and thereafter refrigerated (4°C) and used for both *in vitro* and *in*

vivo studies. Phytochemical analysis was done using the methods described by Harborne (1984).

Gas chromatography-mass spectrometry (GC-MS) analysis of various solvent fractions of *P. dactylifera*

Bioactive compounds present in differential solvent fractions of *P. dactylifera* were evaluated using GC-MS according to the method described by Krishnaveni et al. (2014). This was done using GCMS-QP2010SE Shimadzu, Japan instrument. The samples were dissolved in methanol and thereafter introduced into the column by split-less injection system (column and injection temperatures were set at 60 and 250°C, respectively). Ultra-high quality helium was used as carrier gas at a flow rate of 3.22 mL/min, and volume injected was 0.5 µL. The source ionization temperature was set at 230°C. The experiments were conducted on the positive ion mode. Mass spectra scan speed was 1428, and MS detection was completed in 18.9 min. The detection employed the NIST (National Institute of Standards and Technology) -Year 2011 library.

Sample preparation

Aqueous solutions of each solvent fractions and reference drugs were prepared (1 mg/mL) and used for the *in vitro* enzyme assays (Oboh et al., 2018).

Angiotensin-converting enzyme (ACE) inhibitory assay

The required dilution of the extracts (50 µL) and enzyme was incubated at 37°C for a period of 15 mins. The addition of 150 µL of 8.33 mM of the substrate in 125 mM Tris-HCl buffer (pH 8.3) into the reaction mixture initiated the enzymatic reaction. This mix was then incubated for 30 min at 37°C, and thereafter, the reaction was terminated by the addition of 250 µL of 1 M HCl. The solution was extracted using 1.5 mL ethyl acetate and subsequently centrifuged in order to separate the ethyl acetate layer. A portion (1 mL) of the ethyl acetate layer was siphoned into clean a test-tube for evaporation. The resulting residue was suspended in distilled water, and its absorbance was read at 228 nm in a UV/visible spectrophotometer. Percentage inhibition was used to express the inhibitory activity of the extracts (Oboh et al., 2018). Lisinopril (an ACE inhibitor drug), served as a reference compound for this experiment.

Phosphodiesterase-5 (PDE-5) inhibitory assay

A method described by Oboh et al. (2017) was used to determine the inhibitory activity of PDE-5. The reaction mixture contained 20 mM Tris buffer (pH 8.0), 5 mM *p*-nitrophenyl phenylphosphonate (substrate), phosphodiesterase enzyme, and extracts or standard (sildenafil). The mixture was allowed to stand for 10 min at 37°C, thereafter the intensity of p-nitrophenol generated was determined as a change in absorbance at 400 nm after 5 min. The control was carried out in the absence of extracts and the standard. Percentage inhibition was used to express the inhibitory activity of the extracts. Sildenafil (a known inhibitor of PDE-5) served as a reference compound for this experiment.

Acetylcholinesterase (AChE) inhibitory assay

This assay was done in a reaction mixture consisting of 200 µL of acetylcholinesterase, 5,5′-dithio-bis(2-nitrobenzoic) acid solution (DTNB 3.3 mM), extracts and standard drug (prostigmine) and phosphate buffer (pH 8.0). The reaction mixture was thereafter incubated for 20 mins at 25°C. After incubation, acetylthiocholine iodide was added as the substrate, while the activity of the enzyme was measured at 412 nm in a UV/visible spectrophotometer. Results were expressed as percentage inhibition (Oboh et al., 2018). Prostigmine (a standard AChE inhibitor) was used as a reference compound for this experiment.

Adenosine deaminase (ADA) inhibitory assay

The method described by Giusti and Galanti, (1984) was adapted. The assay protocol directly measures ammonia formation in the presence of excess adenosine. To 21 mM of the substrate (adenosine, pH 6.5; incubated at 37°C for 1 h), 25 µL of adenosine deaminase was added. The reaction was terminated by the addition of 106 mM and 167.8 mM sodium nitroprusside and hypo-

chlorite, respectively. Subsequently, 75 µmol of ammonium sulfate served as standard. All experiments were conducted in triplicates, and values were expressed as percentage inhibition.

Arginase inhibitory assay

The reaction mixture contained Tris-HCl buffer (1 mM, pH 9.5, 1 mM $MnCl_2$), 0.1 M arginase solution and the extract. The mixture was made up to 1 mL final volume and was incubated for 10 min at 37°C. The addition of 2.5 mL Ehrlich reagent (2 g *p*-dimethylaminobenzaldehyde in 20 mL of absolute HCl made up to 100 mL with distilled water) terminates the reaction. The absorbance was then measured 20 min later at 450 nm. Mixture without the extracts served as a control, and arginase inhibitory activity was expressed as percentage inhibition (Adefegha et al., 2015).

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, version 21.0 (SPSS Inc., Chicago, IL, USA) via a one-way analysis of variance (ANOVA) followed by DUNCAN multiple range test. The level of significance was considered at $p<0.05$, and all data were represented as the mean ± standard error of the mean (SEM).

RESULTS

Preliminary phytochemical composition of *P. dactylifera*

Table 1 depicts the phytochemical composition of the crude and solvent fractions of *P. dactylifera.*

GC-MS analysis of ethanol and differential solvent fractions of *P. dactylifera*

The total ion chromatograms (TICs) of the ethanol, ethyl acetate, butanol and aqueous fractions of *P. dactylifera* are presented in Figs. 1A-D, while the compounds identified based on retention time, peak area percentage, and compound names are shown in Tables 2 - 5. More compounds were detected in the ethyl acetate fraction (62 compounds) compared to the other fractions. Based on the ethanol extract had 5-hydroxymethyl - furfural (37.31%) present in it, followed by cis-vaccenic acid (9.14%). Elaidic acid methyl ester had an area percent of 11.28, while cyclopentadecanone, 2-hydroxy- was present at 10.85% in the ethyl acetate fraction. The most abundant compounds in the butanol fraction of *P. dactylifera* include laurostearic acid (17.66%), followed by lauric acid methyl ester (10.21%) and methyl levulinate (8.43%). The most abundant compounds in the aqueous fraction include levulinic acid methyl ester (37.06%), neopentyl glycol (16.05%) and lauric acid (7.95%).

Inhibitory effect of *P. dactylifera* extracts on angiotensin-converting enzyme (ACE) activity

Fig. 2A shows the inhibitory effect of *P. dactylifera* on ACE. From the results obtained, all solvent fractions except the aqueous fruit fraction, exerted significantly ($p<0.05$) higher ACE inhibition (EFPD = 85.24 ± 3.72%; EAPD = 62.48 ± 1.4% and BFPD = 72.70 ± 0.4%) than the standard drug used (lisinopril = 40.22 ± 0.5%).

Inhibitory effect of *P. dactylifera* extracts on phosphodiesterase-5 activity

Results from this evaluation revealed that the aqueous fraction had the highest inhibitory effect (86.99 ± 0.1%) on PDE-5, followed by the ethanol extract (78.13 ± 0.1%). The observed inhibitory effects of the extracts were significantly ($p<0.05$) higher than that of the standard drug Sildenafil (23.06 ± 0.7%), as seen in Fig. 2B.

Inhibitory effect of *P. dactylifera* extracts on acetylcholinesterase activity

The aqueous fraction had significantly ($p<0.05$) higher AChE inhibitory effect (91.81 ± 1.2%) than all other solvent fractions, as shown in Fig. 2C. There was no significant difference ($p<0.05$) between the inhibitory effects of ethyl acetate and butanol fractions on AChE. However, the inhibitory effect of the standard drug (prostigmine, 95.070 ± 0.40%) was significantly higher ($p<0.05$) when compared to all the fractions except for the aqueous fraction.

Table 1. Qualitative phytochemical composition of *P. dactylifera.*

Phytochemical	EFPD	EAPD	BFPD	AFPD
Tannins	+	+	+	+
Saponins	-	-	-	-
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
Anthocyanins	-	-	-	-
Betacyanins	+	+	+	+
Quinones	-	-	-	-
Glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Terpenoids	+	-	-	-
Triterpenoids	-	-	-	-
Phenols	+	+	+	+
Coumarins	-	+	+	-
Steroids	-	-	-	-

EFPD, EAPD, BFPD and AFPD represent ethanol, ethyl-acetate, butanol and aqueous extracts, respectively. While + represents "present" and – represents "absent".

Inhibitory effect of *P. dactylifera* extracts on adenosine deaminase (ADA) activity

The ADA inhibitory activity of *P. dactylifera* is presented in Fig. 2D. Extracts had inhibitory activity ranging from 63.60 ± 10.7 - 82.87 ± 4.3%, with the butanol fraction exerting the least inhibitory effect on the enzyme. However, the inhibitory effect of the fractions on the enzyme was not significantly different ($p<0.05$) from each other.

Inhibitory effect of *P. dactylifera* extracts on arginase activity

Significantly ($p<0.05$) higher inhibitory effect on arginase was recorded for butanol (BFPD = 82.08 ± 1.4%) and aqueous (AFPD = 81.02 ± 0.8%) fractions compared to the other solvent fractions of *P. dactylifera* (Fig. 2E). Also, there was no difference between the arginase inhibitory effect of ethanol and ethyl acetate fractions of the fruit.

DISCUSSION

Hypertension poses a critical public health concern, and despite the presence of various antihypertensive drugs in the market, it is still not under control. This has been attributed partly to its asymptomatic nature as well as complicated interactions of environmental and genetic components (Oboh et al., 2018). In this study, we evaluated the bioactive constituents of *P. dactylifera* using GC-MS and the *in vitro* inhibitory actions of ethanol, ethyl acetate, butanol and aqueous fractions on critical enzymes associated with hypertension including; angiotensin-converting enzyme, phosphodiesterase-5, arginase, acetylcholinesterase and adenosine deaminase.

Table 2. Compounds identified in the ethanol extract of *P. dactylifera*.

Peak No.	Retention time	Area %	Molecular weight	Similarity index (%)	Formula	Compound name	Classification of compound
1	6.707	0.39	110	75	$C_6H_{10}N_2$	1,4,5-Trimethylimidazole	-
2	8.481	2.22	195	71	$C_{12}H_{21}NO$	N-Isobutylbicyclo[2.2.1]heptane-2-carboxamide	-
3	9.489	3.48	144	90	$C_6H_8O_4$	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	-
4	10.276	1.21	144	69	$C_6H_8O_4$	2,3-Dimethylfumaric acid	Carboxylic acids
5	11.233	37.31	126	92	$C_6H_6O_3$	5-Hydroxymethylfurfural	Sugars
6	13.984	7.62	200	90	$C_{12}H_{24}O_2$	Lauric acid	Fatty acid
7	15.383	1.95	162	82	$C_6H_{10}O_5$	3-Deoxy-d-mannoic lactone	Sugar alcohol
8	15.871	2.29	228	95	$C_{14}H_{28}O_2$	Myristic acid	Fatty acid
9	17.160	3.86	270	96	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	Fatty acid methyl ester
10	17.627	6.68	256	95	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
11	18.344	1.51	396	65	$C_{29}H_{48}$	Stigmastan-3,5-diene	Fatty acid
12	18.549	1.25	294	95	$C_{19}H_{34}O_2$	9,12-Octadecenoic acid, methyl ester	Fatty acid
13	18.592	1.08	296	93	$C_{19}H_{36}O_2$	Elaidic acid, methyl ester	Fatty acid methyl ester
14	18.764	0.20	298	89	$C_{19}H_{38}O_2$	Methyl stearate	Fatty acid methyl ester
15	19.100	9.14	282	90	$C_{18}H_{34}O_2$	cis-Vaccenic acid	Fatty acid
16	19.221	1.53	284	88	$C_{18}H_{36}O_2$	Stearic acid	Fatty acid
17	19.530	2.38	492	76	$C_{31}H_{53}ClO_2$	5-Chlorostigmastan-3-yl acetate	Phytosterol
18	20.276	4.27	430	95	$C_{29}H_{50}O_2$	Vitamin E	Terpenoid
19	20.860	0.25	468	77	$C_{32}H_{52}O_2$	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.β,4.α,5.α)-	-
20	21.594	0.36	330	82	$C_{19}H_{38}O_4$	Palmitic acid .β-monoglyceride	Fatty acid
21	22.385	1.43	430	64	$C_{29}H_{50}O_2$	Cholestan-3.β-ol acetate	Steroid
22	22.781	0.59	294	84	$C_{19}H_{34}O_2$	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	-
23	23.092	1.57	452	84	$C_{30}H_{60}O_2$	Myristyl palmitate	Fatty acid
24	23.276	0.76	344	58	$C_{23}H_{36}O_2$	1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris(1,1-dimethylethyl)-3,4-dihydro-	Hydrocarbon
25	23.543	0.48	414	83	$C_{29}H_{50}O$	β-Sitosterol	Phytosterol
26	23.809	0.69	442	74	$C_{30}H_{50}O_2$	Erythrodiol	Terpenoid
27	24.340	0.26	222	63	$C_{15}H_{26}O$	Dihydro-.β-agarofuran	Terpenoid
28	24.768	5.23	246	83	$C_{18}H_{30}$	d-Norandrostane (5.α,14.α)	-

Table 3. Compounds identified in the ethyl acetate fraction of *P. dactylifera*.

Peak No.	Retention time	Area %	Molecular weight	Similarity index %	Formula	Compound name	Classification of compound
1	8.730	0.21	125	91	$C_6H_6O_3$	Levoglucosenone	Sugars
2	9.574	0.10	250	80	$C_8H_{15}AgO_2$	Silver octanoate	Hydrocarbons
3	10.151	0.10	200	74	$C_8H_8O_6$	2,8,4,6-(Epoxyethanediylidenoxy) [1,3]dioxino[5,4-d]-1,3-dioxin, tetrahydro-	-
4	11.141	0.28	168	92	$C_8H_8O_4$	5-Acetoxymethyl-2-furaldehyde	Volatile flavour compound
5	11.252	0.36	150	85	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol	Phenolics
6	11.694	0.10	154	79	$C_8H_{10}O_3$	Syringol	Phenolics
7	11.867	0.31	172	90	$C_{10}H_{20}O_2$	n-Capric acid	Fatty acid
8	11.984	0.17	252	94	$C_{18}H_{36}$	trans-9-Octadecene	Fatty acid
9	12.082	0.06	214	77	$C_{12}H_{22}O_3$	Nonanoic acid, 9-oxo-, 1-methylethyl ester	Carboxylic acid methyl ester
10	12.241	0.05	242	89	$C_{16}H_{34}O$	n-Cetyl alcohol	Fatty alcohol
11	12.821	0.14	194	70	$C_{13}H_{22}O$	Cyclopentanone, 2-(2-octenyl)-	-
12	12.949	0.12	168	86	$C_{12}H_{24}$	Cyclododecane	Hydrocarbon
13	13.150	0.04	268	88	$C_{19}H_{40}$	n-Nonadecane	Hydrocarbon
14	13.366	2.38	206	97	$C_{14}H_{22}O$	2,4-Di-tert-butylphenol	Phenolics
15	13.425	0.27	214	89	$C_{13}H_{26}O_2$	Lauric acid, methyl ester	Fatty acid methyl ester
16	13.968	0.92	200	87	$C_{12}H_{24}O_2$	Laurostearic acid	Fatty acid
17	14.111	0.96	280	94	$C_{20}H_{40}$	9-Eicosene, (E)-	-
18	14.203	0.63	222	90	$C_{12}H_{14}O_4$	Diethyl Phthalate	Aromatic carboxylic acid
19	14.267	0.17	210	84	$C_{14}H_{26}O$	Methyl 7,9-tridecadienyl ether	-
20	14.605	0.16	242	77	$C_{16}H_{34}O$	2-Hexyl-1-decanol	Alcohol
21	15.139	0.58	268	93	$C_{19}H_{40}$	n-Nonadecane	Hydrocarbon
22	15.380	0.99	256	88	$C_{16}H_{32}O_2$	Methyl 12-methyltetradecanoate	Hydrocarbon
23	15.414	0.22	222	90	$C_{12}H_8Cl_2$	3,5-Dichlorobiphenyl	-
24	16.009	1.40	266	93	$C_{19}H_{38}$	1-Nonadecene	Hydrocarbon
25	16.100	0.50	242	91	$C_{16}H_{34}O$	2-Hexyldecanol	Alcohol
26	16.295	0.33	312	81	$C_{20}H_{40}O_2$	Methyl 17-methyloctadecanoate	-
27	16.334	2.05	178	94	$C_{14}H_{10}$	Phenanthrene	Hydrocarbon
28	16.719	0.72	212	84	$C_{11}H_{16}O_4$	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	-
29	16.997	1.82	256	64	$C_{12}H_7Cl_3$	2,4,4'-Trichlorobiphenyl	-
30	17.190	4.60	270	96	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	Fatty acid methyl ester
31	17.705	4.77	256	93	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
32	17.748	1.91	284	90	$C_{18}H_{36}O_2$	Ethyl palmitate	Fatty acid

Table 3. Compounds identified in the ethyl acetate fraction of *P. dactylifera* (continued…).

Peak No.	Retention time	Area %	Molecular weight	Similarity index %	Formula	Compound name	Classification of compound
33	17.848	3.98	320	59	$C_{20}H_{32}O_3$	Butyric acid, 2-(2,4-di-tert-pentylphenoxy)-	Carboxylic acid
34	17.928	1.48	506	79	$C_{28}H_{58}O_7$	d-Mannitol, 1-O-(22-hydroxydocosyl)-	Alcohol
35	17.991	0.39	296	88	$C_{19}H_{36}O_2$	Methyl dihydrochaulmoograte	Carboxylic acid
36	18.433	1.02	310	78	$C_{20}H_{38}O_2$	Butyl 9-hexadecenoate	Fatty acid ester
37	18.601	6.92	294	93	$C_{19}H_{34}O_2$	Methyl octadeca-9,12-dienoate	Fatty acid methyl ester
38	18.647	11.28	296	93	$C_{19}H_{36}O_2$	Elaidic acid, methyl ester	Fatty acid methyl ester
39	18.732	0.49	312	89	$C_{20}H_{40}O_2$	n-Octadecyl ethanoate	Fatty acid ester
40	18.786	2.56	298	94	$C_{19}H_{38}O_2$	Methyl stearate	Fatty acid methyl ester
41	19.063	4.76	280	91	$C_{18}H_{32}O_2$	cis,cis-Linoleic acid	Fatty acid
42	19.109	10.85	240	88	$C_{15}H_{28}O_2$	Cyclopentadecanone, 2-hydroxy-	-
43	19.241	4.20	284	85	$C_{18}H_{36}O_2$	Stearic acid	Fatty acid
44	19.282	1.02	282	84	$C_{18}H_{34}O_2$	Ethyl 9-hexadecenoate	Fatty acid ester
45	19.452	9.52	368	97	$C_{24}H_{48}O_2$	Docosyl acetate	Fatty acid ester
46	19.710	2.37	304	64	$C_{20}H_{32}O_2$	13Alpha-delta(8)-dihydroabietic acid	-
47	20.055	2.72	210	77	$C_{14}H_{26}O$	13-Tetradecenal	-
48	20.180	0.25	312	88	$C_{20}H_{40}O_2$	n-Octadecyl ethanoate	Fatty acid ester
49	20.689	1.56	354	93	$C_{24}H_{50}O$	Lignoceryl alcohol	Alcohol
50	20.783	0.14	286	66	$C_{20}H_{30}O$	Cyclopenta[a,d]cycloocten-5-one, 1,2,3,3a,4,5,6,8,9,9a,10,10a-dodecahydro-7-(1-methylethyl)-1,9a-dimethyl-4-methylene	-
51	21.521	0.84	396	88	$C_{26}H_{52}O_2$	Tetracosyl acetate	-
52	21.677	0.85	330	90	$C_{24}H_{38}O_4$	Phthalic acid, bis(2-ethylhexyl) ester	Aromatic carboxylic acid
53	21.995	0.82	396	89	$C_{27}H_{56}O$	Heptacosanol	Fatty alcohol
54	22.226	0.27	368	85	$C_{24}H_{48}O_2$	Methyl 21-methyldocosanoate	-
55	22.552	0.18	224	72	$C_{13}H_{20}O_3$	3-(1-Hydroxy-2-isopropyl-5-methylcyclohexyl)-2-propynoic acid	-
56	22.637	0.47	490	80	$C_{35}H_{70}$	17-Pentatriacontene	-
57	22.884	0.84	286	81	$C_{17}H_{34}O_3$	Methyl 16-hydroxy-hexadecanoate	-
58	23.039	0.52	196	77	$C_{13}H_{24}O$	(2E)-2-Tridecenal	Flavour compound
59	23.324	0.92	490	82	$C_{35}H_{70}$	17-Pentatriacontene	-
60	23.807	0.43	618	67	$C_{20}H_{23}F_{17}O_2$	Heptadecafluorononanoic acid, undecyl ester	-
61	23.971	0.53	618	64	$C_{20}H_{23}F_{17}O_2$	Heptadecafluorononanoic acid, undecyl ester	-
62	24.775	0.34	380	62	$C_{23}H_{44}O_2Si$	cis-11,14-Eicosadienoic acid, trimethylsilyl ester	-

Table 4. Compounds identified in the butanol fraction of *P. dactylifera*.

Peak No.	Retention time	Area %	Molecular weight	Similarity index %	Formula	Compound name	Classification of compound
1	6.280	4.87	126	76	$C_2H_6O_4S$	Dimethyl sulphate	-
2	6.502	2.19	124	75	$C_4H_9ClO_2$	2-Methoxyethoxymethyl chloride	-
3	7.246	8.43	130	90	$C_6H_{10}O_3$	Methyl levulinate	Carboxylic acid methyl ester
4	8.081	3.65	144	96	$C_7H_{12}O_3$	Ethyl laevulinate	Keto acid ester
5	8.523	1.79	128	69	$C_7H_{12}O_2$	Z-3-Methyl-2-hexenoic acid	Fatty acid
6	8.961	1.25	162	88	$C_6H_{10}O_5$	Malic acid, dimethyl ester	Carboxylic acid ester
7	9.055	1.56	118	79	$C_5H_{10}O_3$	Propanoic acid, 3-methoxy-, methyl ester	-
8	9.321	0.63	140	78	$C_8H_{12}O_2$	Hepta-2,4-dienoic acid, methyl ester	-
9	9.764	0.75	174	90	$C_8H_{14}O_4$	Methyl levulinate ethylene ketal	-
10	10.639	0.24	188	89	$C_9H_{16}O_4$	1,3-Dioxolane-2-propanoic acid, 2-methyl-, ethyl ester	-
11	11.227	1.56	186	91	$C_{11}H_{22}O_2$	Capric acid methyl ester	Fatty acid ester
12	12.025	0.84	368	80	$C_{24}H_{48}O_2$	Docosanoic acid, ethyl ester	Fatty acid ester
13	13.546	10.21	214	95	$C_{13}H_{26}O_2$	Lauric acid, methyl ester	Fatty acid methyl ester
14	13.608	0.24	204	68	$C_9H_{16}O_5$	1,3:2,5-Dimethylene-4-methyl-d-rhamnitol	Sugar alcohol
15	14.174	17.66	200	97	$C_{12}H_{24}O_2$	Laurostearic acid	Fatty acid
16	15.079	1.49	256	76	$C_{14}H_{28}N_2O_2$	Hexanoic acid, (2-hexanoylaminoethyl)-amide	-
17	15.472	6.30	242	97	$C_{15}H_{30}O_2$	Myristic acid, methyl ester	Fatty acid methyl ester
18	15.798	2.33	146	77	$C_6H_{10}O_4$	Dianhydromannitol	Sugar alcohol
19	15.961	2.71	284	87	$C_{18}H_{36}O_2$	Ethyl palmitate	Fatty acid ester
20	16.043	3.89	216	80	$C_{12}H_{24}O_3$	2-Methoxyethyl nonanoate	-
21	16.138	0.82	318	87	$C_{18}H_{35}ClO_2$	2-Chloroethyl palmitate	-
22	16.342	0.53	328	83	$C_{20}H_{40}O_3$	Stearic acid, 2-hydroxyethyl ester	Fatty acid
23	16.461	2.32	270	97	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	Fatty acid
24	17.230	5.14	256	96	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
25	17.679	4.28	284	94	$C_{18}H_{36}O_2$	Palmitic acid, ethyl ester	Fatty acid
26	17.738	1.53	282	78	$C_{19}H_{38}O$	Methyl heptadecyl ketone	-
27	17.818	0.45	284	88	$C_{18}H_{36}O_2$	Methyl 14-methylhexadecanoate	Fatty acid methyl ester
28	17.990	0.17	290	88	$C_{16}H_{31}ClO_2$	2-Chloroethyl myristate	-

Table 4. Compounds identified in the butanol fraction of *P. dactylifera* (continued…).

Peak No.	Retention time	Area %	Molecular weight	Similarity index %	Formula	Compound name	Classification of compound
29	18.036	0.24	296	94	$C_{19}H_{36}O_2$	Elaidic acid, methyl ester	Fatty acid
30	18.598	0.88	296	95	$C_{19}H_{36}O_2$	Elaidic acid, methyl ester	Fatty acid
31	18.650	1.26	298	97	$C_{19}H_{38}O_2$	Methyl stearate	Fatty acid
32	18.798	0.50	280	83	$C_{18}H_{32}O_2$	cis,cis-Linoleic acid	Fatty acid
33	18.993	0.20	284	85	$C_{18}H_{36}O_2$	Stearic acid	Fatty acid
34	19.159	0.86	312	92	$C_{20}H_{40}O_2$	Stearic acid, ethyl ester	Fatty acid
35	19.274	0.72	282	75	$C_{19}H_{38}O$	Methyl heptadecyl ketone	-
36	19.358	0.43	318	91	$C_{18}H_{35}ClO_2$	2-Chloroethyl palmitate	Fatty acid ester
37	19.584	0.22	430	93	$C_{29}H_{50}O_2$	Vitamin E	Terpenoids
38	20.170	0.97	382	88	$C_{25}H_{50}O_2$	Methyl lignocerate	Fatty acid ester
39	22.882	0.24	270	73	$C_{15}H_{26}O_4$	Ethylene brassylate	-
40	24.111	0.51	246	82	$C_{18}H_{30}$	d-Norandrostane (5.α,14.α)	-
41	24.705	1.31	228	96	$C_{14}H_{28}O_2$	Myristic acid	Fatty acid

Preliminary phytochemical evaluations of *P. dactylifera* revealed the presence of tannins, flavonoids, betacyanins, and phenols in all solvent fractions studied. Al-Orf et al. (2012) reported the presence of flavonoids, phenols, carotenoids as well as anthocyanins in date fruits. A good number of studies have documented that phenol-containing plants confer health benefits including prevention of atherosclerosis, cancer, coronary heart disease, cardiovascular disease as well as neurological disorders (Al-Farsi et al., 2005; Alahyane et al., 2019). Biological activities reported for phenolic compounds include antioxidant, free radical scavenging, antimicrobial, anti-inflammatory, as well as anticarcinogenic effects (Baliga et al., 2011; Al-Orf et al., 2012). Thus, it could be inferred that the presence of phenols in *P. dactylifera*, could mitigate some of the risk factors associated with hypertension.

GC-MS analysis revealed the presence of 28, 62, 41 and 30 compounds in the ethanol, ethyl acetate, butanol and aqueous fractions, respectively. Based on the area percentage, the ethanol extract had 5-hydroxymethylfurfural (37.31%) present in it, followed by cis-vaccenic acid (9.14%). Elaidic acid methyl ester had an area percent of 11.28, while cyclopentadecanone, 2-hydroxy- was present at 10.85% in the ethyl acetate fraction. The most abundant compounds in the butanol fraction *P. dactylifera* include laurostearic acid (17.66%), followed by lauric acid methyl ester (10.21%) and methyl levulinate (8.43%). The most abundant compounds in the aqueous fraction include levulinic acid methyl ester (37.06%), neopentyl glycol (16.05%) and lauric acid (7.95%). Some of the compounds detected in *P. dactylifera*, which have been implicated in hypertension management, include squalene, lauric acid, palmitic acid, caprate, stearate, vitamin E, β-sitosterol, phytol, linolenic acid, isosorbide, coumarins and taurine. These compounds have been reported to reduce blood pressure, cholesterol and triglyceride levels, and they possess cardio-protective properties (Rallidis et al., 2003; Martirosyan et al., 2007; Jeon et al., 2009; Alves et al., 2017). Isosorbide is used to manage coronary artery disease, thereby opening narrowed blood vessels for the free flow of blood (MedlinePlus, 2019).

Table 5. Compounds identified in the aqueous fraction of *P. dactylifera*.

Peak No.	Retention time	Area %	Molecular weight	Similarity index %	Formula	Compound name	Classification of compound
1	6.207	0.47	178	61	$C_{10}H_{10}O_3$	Benzaldehyde, 4-(1,3-dioxolan-2-yl)-	-
2	6.507	1.05	120	81	$C_5H_{12}O_3$	Diethylene glycol monomethyl ether	-
3	6.596	1.45	312	55	$C_{14}H_{20}N_2O_4S$	Ethanone, 1-[4-[4-(2-hydroxyethyl)-1-piperazinylsulfonyl]phenyl]-	-
4	7.116	37.06	130	91	$C_6H_{10}O_3$	Levulinic acid, methyl ester	Keto acid
5	7.967	3.84	114	94	$C_7H_{12}O_3$	Levulinic acid, ethyl ester	Keto acid
6	8.808	16.05	104	81	$C_5H_{12}O_2$	Neopentyl glycol	-
7	9.064	1.15	132	78	$C_5H_8O_4$	Succinic acid, monomethyl ester	Fatty acid ester
8	9.268	2.35	140	78	$C_8H_{12}O_2$	Hepta-2,4-dienoic acid, methyl ester	-
9	9.714	0.70	174	94	$C_8H_{14}O_4$	Methyl levulinate ethylene ketal	-
10	10.162	0.68	164	85	$C_7H_{16}O_4$	Methoxytriethylene glycol	-
11	11.216	1.54	146	82	$C_6H_{10}O_4$	Isosorbide	Organic nitrate
12	13.416	1.51	214	94	$C_{13}H_{26}O_2$	Lauric acid, methyl ester	Fatty acid
13	13.979	7.95	200	96	$C_{12}H_{24}O_2$	Lauric acid	Fatty acid
14	15.020	1.57	146	74	$C_5H_{10}N_2O_3$	Dimethylol ethylene urea	-
15	15.382	0.61	242	95	$C_{15}H_{30}O_2$	Myristic acid, methyl ester	Fatty acid methyl ester
16	15.699	2.49	146	77	$C_6H_{10}O_4$	Dianhydromannitol	Sugar alcohol
17	15.816	3.33	228	95	$C_{14}H_{28}O_2$	Myristic acid	Fatty acid
18	16.408	1.89	272	77	$C_{16}H_{32}O_3$	Myristic acid, 2-hydroxyethyl ester	Alcohol
19	17.145	0.65	270	93	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	Fatty acid methyl ester
20	17.527	2.06	256	93	$C_{16}H_{32}O_2$	Palmitic acid	Fatty acid
21	18.611	0.29	268	80	$C_{17}H_{32}O_2$	7-Hexadecenoic acid, methyl ester, (Z)-	Fatty acid methyl ester
22	18.753	0.29	296	85	$C_{19}H_{36}O_2$	Methyl dihydrochaulmoograte	-
23	18.976	1.63	212	78	$C_{11}H_{16}O_4$	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	-
24	19.453	0.54	488	73	$C_{30}H_{52}O_3Si$	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3.beta.,5Z,7E)-	-
25	20.154	0.93	430	74	$C_{29}H_{50}O_2$	Vitamin E	Terpenoids
26	21.128	2.88	314	67	$C_{19}H_{38}O_3$	Methyl 2-hydroxy-16-methyl-heptadecanoate	Fatty acid methyl ester
27	21.786	0.69	454	62	$C_{31}H_{50}O_2$	Stigmasterol acetate	Sterols
28	23.369	2.13	212	68	$C_{13}H_{24}O_2$	2-Tridecenoic acid, (E)-	Fatty acid
29	23.717	1.79	370	66	$C_{16}H_{34}O_7S$	d-Mannitol, 1-decylsulfonyl-	Sugar alcohol
30	24.636	0.44	322	66	$C_{21}H_{38}O_2$	1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	-

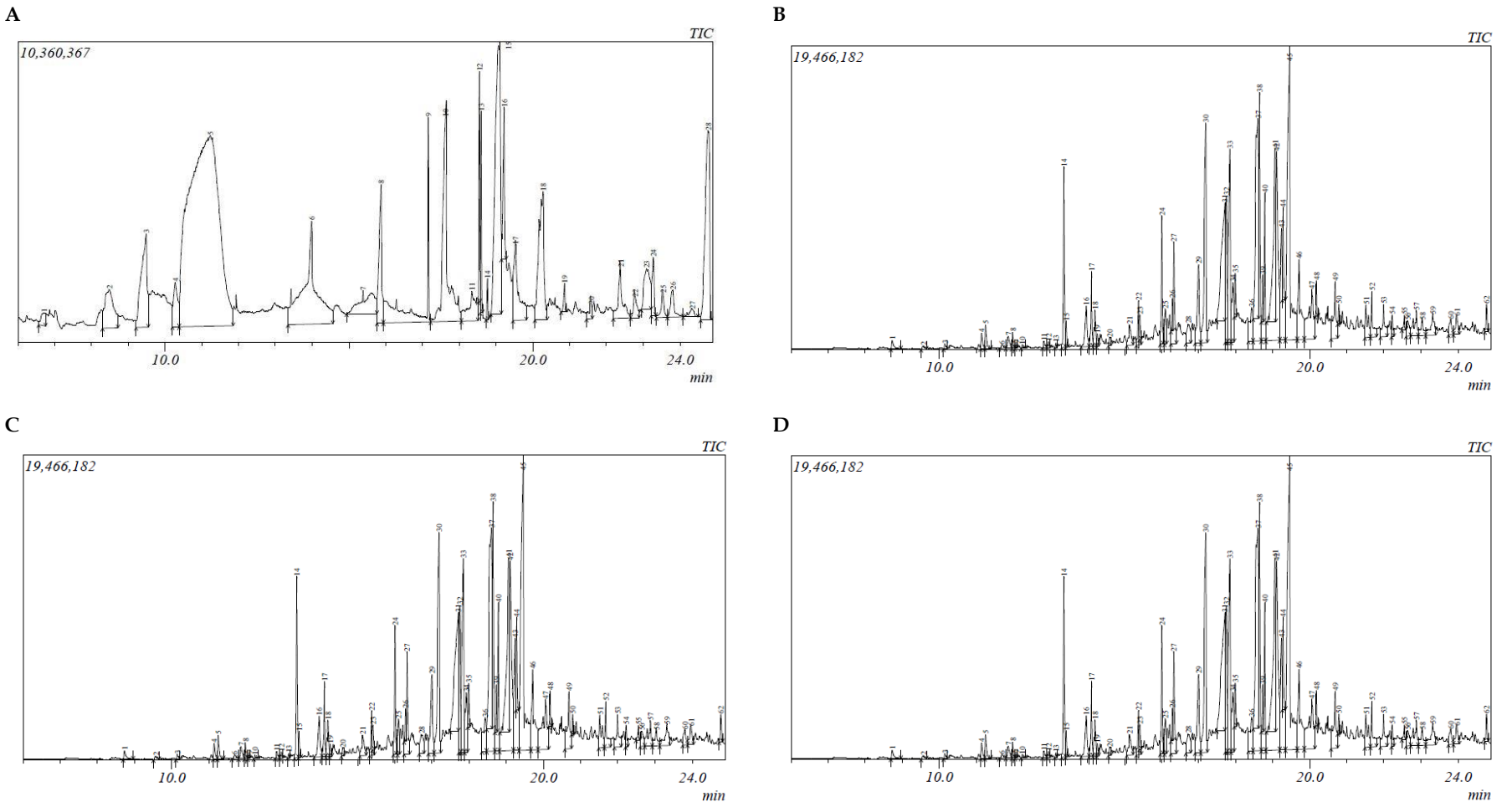

Figure 1. Total Ion Chromatogram of **(A)** ethanol fruit extract of *P. dactylifera*, **(B)** ethyl acetate fraction, **(C)** butanol fraction, and **(D)** aqueous fraction.

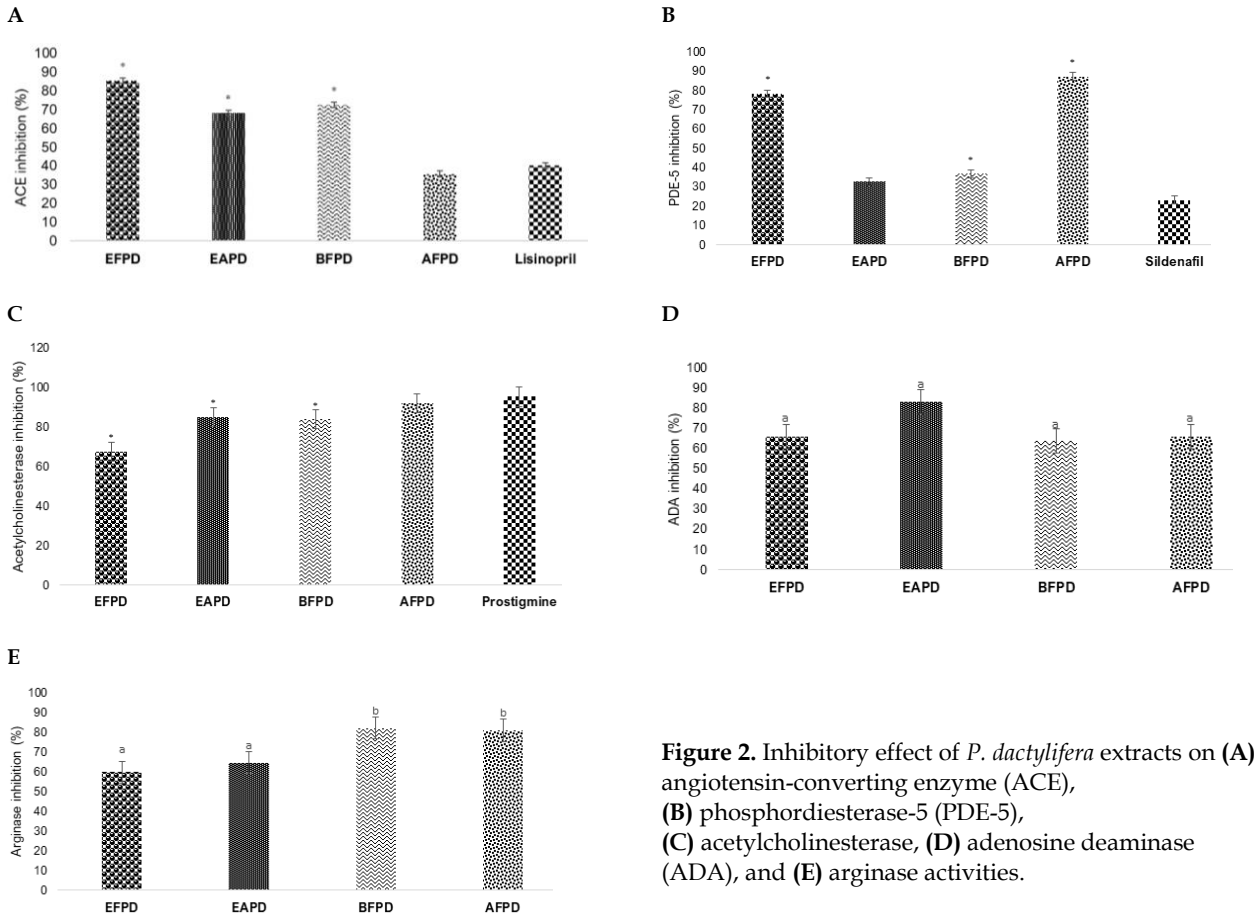

Figure 2. Inhibitory effect of *P. dactylifera* extracts on **(A)** angiotensin-converting enzyme (ACE), **(B)** phosphordiesterase-5 (PDE-5), **(C)** acetylcholinesterase, **(D)** adenosine deaminase (ADA), and **(E)** arginase activities.

Values are presented as mean ± standard error of mean (n = 3). Values marked with * are significantly different ($p<0.05$) from the standard drugs used (lisinopril, sildenafil, prostigmine). Values marked with different superscript alphabet are significantly different ($p<0.05$) from each other. EFPD, EAPD, BFPD and AFPD represent ethanol, ethyl acetate, butanol and aqueous fruit extracts of *P. dactylifera,* respectively.

From our study, the extracts of *P. dactylifera* exerted significant inhibitory activity on ACE, which is significantly higher ($p<0.05$) when compared with the control drug- lisinopril. ACE has been implicated in hypertension, where it activates the renin-angiotensin system (RAS). Elevated activity of the RAS has been shown to aggravate hypertension (Majumder and Wu, 2015). ACE converts angiotensin I to the vasoconstrictive angiotensin II, which in pathological states thwarts the production of nitric oxide for vasodilatory purposes. Extracts of plants have been reported to inhibit ACE activity mainly due to the presence of phenolic compounds in their composition. Phenolic compounds have been reported to elicit strong antioxidant and other protective effects both *in vitro* and *in vivo* (Laranjinha, 2001; Cos et al., 2002). Also, Oboh et al. (2018), reported the ACE-inhibitory effects of caffeine and caffeic acid (phenolic compounds) *in vitro*. The presence of some phenolic compounds in date fruits may thus be responsible for the observed ACE inhibitory effects.

This study also revealed that extract of *P. dactylifera* inhibited the activity of phosphodiesterase-5 (PDE-5). This enzyme hydrolyzes cGMP to GMP, thus counteracting the nitric oxide (NO)-mediated role of cGMP in vasodilation (Kukreja et al., 2004). Thus, inhibiting the activity of the enzyme could be beneficial to hypertension management. Results from our study revealed that the ethanol and aqueous fractions of *P. dactylifera* had the highest inhibitory effects on PDE-5 compared with its

standard inhibitor – sildenafil. This finding is in agreement with a similar study by Boswell-Smith et al. (2006) and Oboh et al. (2018), who reported that caffeine (a phenolic compound of plant origin) inhibited PDE-5.

Similarly, all extracts of *P. dactylifera* inhibited adenosine deaminase (ADA) activity, with the ethyl acetate fraction having the highest inhibitory effect on the enzyme when compared to the other fractions. ADA catalyzes the irreversible deamination of adenosine to inosine, which is subsequently broken down to hypoxanthine, xanthine, and uric acid for elimination by the kidneys. Adenosine is a purine nucleoside formed from adenosine triphosphate (ATP) hydrolysis, and it produces certain physiologic effects such as vascular smooth muscle relaxation (Klabunde, 2012). Acetylcholinesterase (AChE) primarily catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetate. The regulatory role of AChE in hypertension has been established. For instance, Lataro et al. (2015) reported that the inhibition of AChE mitigates the development of hypertension in a hypertensive rat model. Our results show that all extracts of *P. dactylifera* possessed an inhibitory effect on AChE, with the aqueous fraction having the highest inhibitory effect (91.81%). However, the AChE inhibitory effect of a standard AChE inhibitor drug (prostigmine), was significantly higher (95.07%) than all extract fractions of *P. dactylifera*. Our finding is in agreement with previous works of Uddin et al. (2016) and Oboh et al. (2017), who reported that alkaloids and phenolic compounds inhibited acetylcholinesterase *in vitro*. Inhibiting AChE activity enhances the bioavailability of acetylcholine for its vasodilatory role.

Arginase catalyzes the hydrolysis of L-arginine to L-ornithine and urea. Its elevated activity has been implicated in the pathogenesis of hypertension. Reports show that elevated activity of arginase diminishes the availability of L-arginine needed for the production of nitric oxide by nitric oxide synthase (Cadwell et al., 2015). Thus, inhibiting the activity of arginase has been proposed as a crucial approach to hypertension management. The observed inhibitory effect of the extract of *P. dactylifera* on arginase activity could be a possible mechanism of the use of these plants in folkloric management of hypertension. Oboh et al. (2018) reported that caffeine inhibits arginase activity and enhances nitric oxide availability.

CONCLUSIONS

The study showed that extracts of *P. dactylifera* exhibited significant inhibitory effects on key enzymes (angiotensin-converting enzyme, acetylcholinesterase, phosphodiesterase-5, adenosine deaminase and arginase) implicated in hypertension. Inhibition of these enzymes could possibly be the mechanisms through which this plant exerts its antihypertensive effects in ethnomedicinal claims. *P. dactylifera* could, therefore, serve as an alternative therapy for hypertension management and help to curtail the adverse effects reportedly associated with antihypertensive drugs. Further studies on the individual bioactive compounds of the fruit in relation to these key enzymes will help discover novel antihypertensive candidate drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Obode OC	Adebayo AH	Li C
Concepts or ideas	x	x	
Design	x	x	x
Definition of intellectual content	x	x	x
Literature search	x		
Experimental studies	x		
Data acquisition	x		
Data analysis	x		
Statistical analysis	x		
Manuscript preparation	x	x	
Manuscript editing	x	x	x
Manuscript review	x	x	x

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