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Original Article

Renin and Angiotensin Converting Enzyme Inhibition of Standardized Bioactive Fractions of *Hyphaene thebaica* L. Mart Growing in Egypt

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ABSTRACT

Introduction: *Hyphaene thebaica* L. fruit is known in Egypt for its antihypertensive activity. However a standardized herbal extract/fraction was never prepared. **Methods:** A biologically guided fractionation was carried out *in-vitro* for the 50% and 70% ethanol extracts of *Hyphaene thebaica* L. fruit using the angiotensin converting enzyme (ACE) inhibition and renin inhibition assays. A validated reversed phase HPLC method was developed for the standardization of the active fractions. **Results:** The ethyl acetate fraction of the 70% extract contained higher percentages of the three bioactive markers chlorogenic acid, quercetin and apigenin (1.940 ± 0.140%, 2.994 ± 0.349% and 0.612 ± 0.0354%, respectively) relative to the ethyl acetate fraction of the 50% extract (1.384 ± 0.157%, 0.342 ± 0.0834% and 0.070 ± 0.00225%, respectively). The butanol fraction of the 70% extract was found to possess the highest antihypertensive activity (93.69 ± 5.695 % renin inhibition activity at 0.5 mg/mL and IC_{50} of 0.001436 +0.00044 mg/mL for ACE inhibition activity). A standard calibration curve for the three compounds was established at a concentration range of 0.1-50 µg/Ml, they showed good linearity with a correlation coefficient (R^2) of (1.00, 1.00 and 0.999; respectively). A high degree of precision (relative standard deviation values <5%) was achieved. The limits of detection for the three compounds were 0.428, 0.368 and 0.849; respectively, while the limits of quantitation were 1.29, 1.11 and 2.57, respectively. **Conclusion:** Current results showed that the butanol fraction of the 70% extract revealed the highest antihypertensive activity through ACE and renin inhibition mechanisms. In addition, recorded observations concerning linearity of the used bioactive markers offer a support for the possible utility of the tested fractions as potent standardized antihypertensive drugs.

Key words: Antihypertensive, Doum, HPLC, Phenolics, Standardization, Validation.

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INTRODUCTION

The dried fruit of *Hyphaene thebaica* L. Mart, a plant of the Palmaceae family, locally known as Doum, is widely used in the Egyptian folk medicine for its anti-hypertensive activity. Also, the tea is well known in Egypt and is widely used as an antihypertensiveagent. Plant extracts are used in the treatment of bilharzias is, haematuria, bleeding, dyslipidemia and as a hematinic agent. The roots are used in treatment of bilharziasis, while the resin of the tree proved to possess diuretic and diaphoretic activities. It is also prescribed for tape-worm and against animal bites.[1] Meanwhile, the aqueous extract showed to stimulate the contractions in rats and it has a lowering blood pressure effect on both normotensive and hypertensive anaesthetized dogs.[2] Several articles discussed the various pharmacological activities of the different parts of the plant extract. However, a standardized extract/fraction of the fruit was never prepared for the treatment of hypertension. Three constituents that have been previously identified in Doum extract were chosen; chlorogenic acid, apigenin and quercetin.[3] These compounds have been previously reported to possess antihypertensive properties.[4,5] Consequently they were selected to be used as bio-active markers for standardization of the doum fruit extracts and fractions. The aim of the present study was to develop a method to identify and quantify chlorogenic acid, apigenin and quercetin content in the prepared extracts and fractions of the fruit to be utilized as a tool for comparison in between them and to conclude if these compounds could be attrib-uted to the antihypertensive effect. Also, to prepare a standardized active extract/fraction through a validated reversed phase high performance liquid chromatography (RP-HPLC) method.

MATERIALS AND METHODS

Plant material

The fruits of *H. thebaica* L. Mart were obtained from local market (Harraz shop for herbal products), Cairo, Egypt. The plant was authenticated by Dr. Treaslabeeb

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(Botanist at Orman botanical garden). A voucher specimen number 0417 was kept at the herbarium of Helwan University.

Preparation of extracts and fractions

Two kg of the air-dried powdered fruit of *Hyphaene thebaica* L. were extracted by percolation with 5 liters of 50% ethanol in water on cold for 3 days and then filtered. This process was repeated three times till exhaustion. The alcoholic extract was then concentrated under pressure to give a dark brownish viscous residue (50% ethanol extract residue= 192 g equivalent to 9.6% of the total plant material). For the comparative study, this procedure was repeated using another two kg of the powdered fruit but this time using 70% ethanol in water for extraction (70% ethanol extract residue= 240 g equivalent to 12% of the total plant material). The dried alcoholic extracts were separately digested with one liter of distilled water, transferred into a separating funnel and successfully partitioned using n-hexane (H), chloroform (C), ethyl acetate (E) and n-butanol (B) saturated with water. The fractions of the 50% extract were given symbols H1, C1, E1 and B1, respectively. While the fractions of the 70% extract were given symbols H2, C2, E2 and B2 respectively. The yield of each fraction in g is shown in Table 1.

Another 0.125 kg of the air-dried powdered fruit of *Hyphaene thebaica* L. were extracted by percolation with 500 mL cold water for 3 days and then filtered. This process was repeated three times till exhaustion (Cold water extract residue = 9.417 g equivalent to 7.5% of the total plant material). This procedure was repeated using another 0.125 Kg of the same powdered fruit but using boiled water for extraction (Hot water extract residue= 17.30 g equivalent to 13.84% of the total plant material).

Chemicals

The angiotensin converting enzyme (ACE) extracted from rabbit lung, Hippuryl-histidyl-leucine (HHL) substrate, captropril, NaOH, borate saline buffer (boric acid, NaCl), TRIS HCL, aliskiren, renin enzyme and renin substrate were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Round bottom 96-well clear and black micro plates were obtained from BD International (Mississauga, ON, Canada) and Corning Incorporated (Edison, NY, USA), respectively. All Solvents used in this work were of analytical grade and were purchased from ADWIC (Cairo, Egypt). They include, absolute ethyl alcohol (99%), n-hexane, chloroform, ethyl acetate, n-butanol. Chlorogenic acid, quercetin and apigenin standards were purchased from Alpha Aeser (Ward Hill, Massachusetts, U.S.). Solvents used in analysis were of HPLC MS grade, Methanol D4 and Acetonitrile were provided from Sigma Aldrich (St. Louis, MO., U.S.A.). Orthophosphoric acid was purchased from Merck (Darmstadt, Germany). The acidic aqueous solution used for HPLC analysis was filtered through Agilent Ecno 0.45 µm polytetrafluoroethylene (PTFE) membrane filter and degassed in an ultrasonic bath before use.

RP-HPLC analysis of the fractions and aqueous extracts of the fruit

An HPLC method was developed for the comparative study of the different fractions of the 50% and 70% alcoholic extracts. The same method was applied for the analysis of the hot and cold water extracts in order to reach the best solvent for extraction of the active compounds. An Agilent technologies 1100 series HPLC was used, equipped with an Agilent 1200 series G1322A quaternary pump and a degasser, a G1314A variable wavelength detector and an Agilent Chemstation software, Santa Clara, California, United States software. Chlorogenic acid and quercetin (1 mg/mL 30% methanol in orthophosphoric acid) and apigenin (1 mg/mL 30% absolute ethyl alcohol in orthophosphoric acid) were injected into a lichrosphere 100 RP-18 5 µm, 250 mm × 4.6 mm column (Merck, Germany) maintained at a temperature of 25°C. The mobile phase used was 0.15% orthophosphoric acid in water (solvent A) and acetonitrile (solvent B). A continuous gradient elution was carried out at a flow rate 1.0 mL/min (0-33 min 10-70% B in A). The injection volume was 20 µL and detection was made at 325 nm. The same RP-HPLC method was used for the analysis of the chloroform, ethyl acetate, n-butanol fractions, hot water and cold water extracts (1 mg/mL 30% methanol in orthophosphoric acid, each).

Table 1: The yield of different fractions of the 50% and 70% ethanol extracts expressed in g.

Sample number	Weight in grams
H1	0.034
H2	2.97
C1	0.35
C2	0.47
E1	2.35
E2	1.70
B1	8.00
B2	12.76

Standardization using RP-HPLC

Sample preparation

For comparative HPLC analysis of the fractions and extracts, 1mg of each fraction and extract was dissolved in 1 mL of 30% methanol in orthoposphoric acid (0.15%). Prepared fractions and extracts were filtered through a PTFE 0.45 µm syringe filter and eluted on YMC prepacked column. All samples were injected three times and an average concentration for each compound was determined.

Construction of the standard calibration curve

Stock solutions of 1 mg of quercetin and chlorogenic acid standards were dissolved in 1 mL methanol, while apigenin standard was dissolved in 1mL of absolute ethyl alcohol. All standard solutions were stored away from light at low temperature (- 4°C). Serial dilutions of each standard were prepared from the stock solution having final concentrations of (0.10, 0.50, 1.00, 5.00, 10.0, 50.0 µg/mL).The prepared dilutions of the three compounds (chlorogenic acid, quercetin and apigenin) were injected in triplicates.

Validation of the RP-HPLC method

Linearity was determined by injecting six different concentrations of the three standard solutions (0.10 – 50.0 µg/ml). Accuracy was assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range. In this study, three concentration levels of chlorogenic acid, quercetin and apigenin, three replicates for each concentration were measured for accuracy determination. To determine the intra- and inter-day precision of the method, the three standards were assayed at two different concentrations (1.00 and 10.0 µg/ml) on 1 day in six replicates and on 3 separate days in triplicates. Limits of quantitation (LOQ) and limits of detection (LOD) were determined based on the standard deviation of the response (σ) and the slope of the calibration curve (S) following the International Conference on Harmonization Guidelines (ICH)(Food and Drug Administration 1996) LOQ = 10 (σ/S), LOD = 3.3 (σ/S).[6]

Screening for in-vitro antihypertensive activity

The 70% and 50% ethanol extracts of the fruit of *H. thebaica* were assessed for their antihypertensive activity using two different assays; ACE inhibition assay and renin inhibition assay. In addition, the chloroform, ethyl acetate and n-butanol fractions of the 50% and 70% ethanol extracts along with the pure compounds were tested.

ACE inhibition assay

The ACE inhibition activity of the pure compounds, plant extracts and their fractions was tested according to the method described by Balasuriya, N. and Rupasinghe, H.P.V. with some modifications.[7] The enzyme reaction was carried out using 2mU ACE, 0.80mM HHL, 0.1 mM sodium borate buffer (PH 8.3) and different concentrations of tested samples. Reaction mixtures were incubated at 37°C using a shaker stove (Model HP50, Appolo Instrumentation for molecular biology, CA, USA) for 1 hr. The reaction was then stopped using 0.5 M NaOH. The evaluation of the ACE inhibition potential of the test compounds was dependent on His-Leu formation by the cleavage of HHL in the presence of ACE. The formation of His-Leuwas then measured by the fluorescence method, using the FLUOstar OPTIMA plate reader (BMG Labtech Inc., Offenberg, Germany) at excitation and emission wavelengths of 360 nm and 500 nm, respectively. The mean fluorescence estimations of samples were obtained. The % inhibition of enzyme was expressed with respect to the control used. The equation is as follows:

$$\% \text{ Inhibition} = 1 - \frac{\text{Fluorescence}_{\text{sample}} - \text{Fluorescence}_{\text{blank}}}{\text{Fluorescence}_{\text{control}} - \text{Fluorescence}_{\text{blank}}} \times 100$$

The results were expressed as the percentage inhibition of ACE inhibition and IC_{50} values were calculated by linear interpolation as shown in Tables 3 and 4.

Renin inhibition assay

The renin inhibitory potential of the pure compounds, *H. thebaica* extracts and their fractions were tested according to the method described by Bhullar, K.S and Rupasinghe, H.P.V. with few modifications.[8] NaCl (100 mM, 0.58 g) were first dissolved in 100 mL distilled water then 0.6 g of 50 mMTris-HCl was dissolved portion wise in NaCl solution. A 50 µL renin enzyme was then dissolved in 950 µL buffer (PH 8.0). The aliquot was then stored at -80°C for further analysis. Renin substrate (Arg-Glu (EDANS)-Ile-His-Pro-PheHis-Leu-Val-Ile-His-Thr-Lys (Dabcyl)-Arg) was diluted with dimethyl sulfoxide (DMSO) to achieve a concentration of 500 µM. The assay was performed using 96-well black polypropylene plates. The background wells were formed by adding a 10 µL sample to 150 µL assay buffer, 10 µL*DMSO* (solvent control) and 20µL substrate. The control contained 20 µL of substrate, 150 µL of assay buffer, 10 µL of DMSO and 10 µL renin enzyme. The tested samples were analyzed using 20 µL renin substrate, 150 µL assay buffer, 10 µL renin enzyme and 10 µL of the test compound at the desired concentrations. The reaction mixture was incubated at 37°C for 45 min and the fluorescence signal produced from the cleavage of fluorophore-EDANS to peptide-EDANS by renin was measured at the excitation wavelength of 340 nm and the emission wavelength of 490 nm using the FLUOstar OPTIMA plate reader (BMG LabtechInc., Offenburg, Germany). The renin inhibition was expressed at percentage inhibition using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Fluorescence}_{\text{control}} - \text{Fluorescence}_{\text{test compound}}}{\text{Fluorescence}_{\text{control}}} \times 100$$

The results were expressed as the percentage inhibition of renin and were shown in Tables 5 and 6.

RESULTS

By comparing the RP-HPLC-UV chromatograms of *H. thebaica* L. fractions and extracts, it was observed that the fractions of the 70% ethanol extract of the fruit have relatively higher peak intensities of the three compounds rather than the fractions of the 50% extract. So, the percentages of chlorogenic acid, apigenin and quercetinwere found to be higher in the fractions of the 70% ethanol extract than the fractions of the 50% ethanol extract. In addition, ethyl acetate fraction of the 70% ethanol extract was found to contain the highest percentages of the three compounds; chlorogenic acid, quercetin and apigenin; respectively (1.384, 0.342 and 0.070%; respectively) relative to that of the other tested fractions. Also it was observed that the hot water extract contained higher percentages of the three compounds (0.350, 1.125, and 0.840%; respectively) than that of the cold water extract (0.165, 0.087, and 0.755%; respectively) and this supports the traditional use of Doum fruit as a decoction for hypertension. Results are illustrated below in Table 2 and Figure 1.

Table 2: Results of RP-HPLC-UV analysis of the average content of the three compounds in each fraction in addition to hot and cold water extracts.

Sample	Chlorogenic acid content mg%	Quercetin content mg%	Apigenin content mg%
C1	0.0214±0.041	0.254±0.157	0.149±0.101
C2	0.0507±0.075	0.273±0.147	0.742±0.334
E1	1.384 ± 0.157	0.342 ± 0.083	0.07 ± 0.0022
E2	1.940±0.140	2.994±0.349	0.612±0.0354
B1	0.528±0.045		0.173±0.0580
B2	1.314±0.193	0.0265±0.0032	0.1005±0.112
Hot water extract	0.350±0.092	1.125±0.451	0.840±0.360
Cold water extract	0.165±0.047	0.087±0.0135	0.755±0.121

*Results are expressed as mean ± SD of at least three independent experiments.

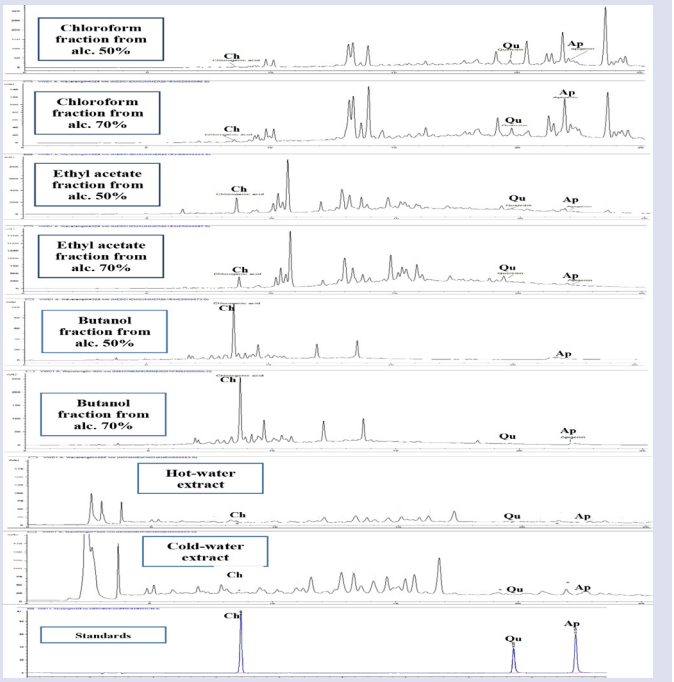

Figure 1: Reversed phase RP-HPLC-UV chromatograms of the fractions and extracts tested showing the three compounds chlorogenic acid at R_t= 8.5, quercetin at R_t= 19.4 and apigenin at R_t=21.8 compared with the chromatogram of the three pure standards.
Ch – Chlorogenic acid
Qu – Quercetin
Ap – Apigenin
*The retention times of the compounds in the fractions and extracts were confirmed by spiking.

Table 3: The % inhibition of ACE and IC_{50} calculated for 70% extract and its fractions.

Sample	% ACE inhibition at different concentrations mg/mL				
	20	2	0.2	0.02	IC_{50}
50%Extract	98.624+0.413	80.275+1.898	75.229+1.949	67.431+1.374	0.00925 + 0.00178
70%Extract	99.541+0.800	96.789+1.040	83.945+1.074	74.771+1.989	0.00217 +0.00049
C1	98.165+1.567	63.303+0.960	57.798+0.729	34.862+0.499	0.128 + 0.0538
C2	95.413+1.414	90.826+1.795	28.440+0.653	26.606+1.115	0.6721 +0.0872
E1	94.495+2.452	57.798+1.493	23.853+1.833	17.889+3.530	0.7204+ 0.0614
E2	98.165+1.423	73.395+2.888	71.559+1.107	8.257+0.670	0.03163 +0.00624
B1	83.028+2.121	84.404+0.896	37.156+3.600	10.755+4.007	0.2731 + 0.0551
B2	91.743+1.714	89.908+2.480	79.817+2.045	70.642+2.560	0.001436 +0.0004

*Results are expressed as mean ± SD of at least three independent experiments.

Table 4: The% ACE inhibition and IC_{50} calculated for the standard compounds.

Concentration mg/ml	Quercetin	Apigenin	Chlorogenic acid
20	95.878+1.023	99.433+0.401	78.578+ 3.529
2	79.568+5.500	81.454+1.511	58.867+1.045
0.2	38.755+1.286	64.568+2.341	42.867+0.722
0.02	15.865+1.081	35.865+2.028	27.979+1.075
IC_{50}	0.604 + 0.11336	0.07 + 0.0134	0.637 + 0.096

*Results are expressed as mean ± SD of at least three independent experiments.

Both the 50% and 70% ethanol extracts and their fractions of the fruit of *Hyphaene thebaica* L. were chosen for the *in vitro* assays as they contained relatively high content of three bioactive markers to be tested for their antihypertensive activity. They showed a significant antihypertensive activity through both mechanisms; ACE and renin mechanisms with varying potencies. The 70% extract was found to possess relatively higher ACE inhibition activity (IC_{50}=0.002173 mg/mL) than that of the 50% extract (IC_{50}=0.009257 mg/mL). Also by comparing all the fractions, the butanol fraction of the 70% extract proved to have the highest activity (IC_{50}=0.001436 mg/mL), followed by 70% ethyl acetate fraction (IC_{50}=0.03163 mg/mL). Also, it was shown that the apigenin standard possesses the highest ACE inhibition activity (IC_{50}= 0.07 mg/mL), while the other two compounds quercetin and chlorogenic acid proved to have almost the same potency (IC_{50}= 0.604 mg/mL and 0.637 mg/mL, respectively). Results are illustrated below in Tables 3 and 4. Captopril was used as reference standard with an IC_{50}=0.0000464 ± 0.0000038 mg/mL.

By comparing 50% extract to the 70% extract of the *H. thebaica* fruit, it was observed that there was no significant difference between the rennin inhibition activity of the 50% extract (% inhibition=82.84%) and that of the 70% extract (% inhibition=75.18%) at the same concentration (0.5 mg/ml). Also by comparing all the fractions, the B2 proved to have the highest activity (% inhibition=93.69%). In addition by comparing the renin inhibition activity of the pure compounds, it was shown that the apigenin possesses the highest activity (% inhibition= 83.82%) at the same concentration 87.5µg/ml. Aliskiren was used as a reference molecule for each work with a % inhibition of 91.79 %. Results are shown below in Tables 5 and 6.

Linear regression analysis of the three compounds; chlorogenic acid, quercetin and apigenin were established by plotting the mean peak area versus concentration and are represented in Figure 2. A standard calibration curve for the three compounds was established at concentration

Table 5: The% renin inhibition activity for 50% and 70% extracts and their fractions.

Sample name	% Inhibition at 0.5 mg/ml
Extract 50%	82.84+3.201
Extract 70%	75.18+1.509
C1	83.19+0.842
C2	86.95+1.931
E1	62.36+1.671
E2	64.18+6.915
B1	68.06+8.545
B2	93.69+5.695
Hot water extract	74.98+9.191
Cold water extract	60.19+4.629

*Results are expressed as mean ± SD of at least three independent experiments.

Table 6: The % renin inhibition activity for the standard compounds.

Sample name	% Inhibition at 87.5 µg/ml
Quercetin	77.85+0.842
Apigenin	83.82+0.867
Chlorogenic acid	1.944+0.524
Aliskiren	91.79±2.375

*Results are expressed as mean ± SD of at least three independent experiments.

range of 0.10-50.0µg/Ml, it showed good linearity with a correlation coefficient (R^2) of (1.00, 1.00 and 0.999, respectively). The chromatogram used to construct the calibration curves for the three compounds was shown in Figure 1. A high degree of precision (relative standard deviation values <5%) was achieved. Precision of the method through intra- and inter-day runs are given in Table 7. The LOD for the three compounds were 0.428, 0.368 and 0.849; respectively, while the LOQ for the three compounds were 1.29, 1.11 and 2.57; respectively. Recovery tests were carried out to further investigate the accuracy of the method and the results are shown in Table 8.

DISCUSSION

Quercetin and apigenin were qualitatively identified in the leaves of *H. thebaica* using (UV, ^{1}H, ^{13}C NMR and ESI/MS).[9] Also, the phytochemical analysis of the potent water extract of the fruits showed the presence of quercetin in the fruits of *H. thebaica* using ^{1}H and ^{13}C NMR methods.[10] Besides the MS/MS profiling done on the fruits of *Hyphaene thebaica* L.

Table 7: Results for the precision of the three standard compounds.

Compound	Intraday % RSD	Interday % RSD
Chlorogenic acid	2.17 % at conc. 1 ug/mL	1.50 % at conc. 1 ug/mL
	2.99 % at conc. 10 ug/mL	1.63 % at conc. 10 ug/mL
Quercetin	3.87 % at conc. 1 ug/mL	3.58 % at conc. 1 ug/mL
	3.80% at conc. 10ug/mL	1.97 % at conc. 10 ug/mL
Apigenin	3.53 % at conc. 1 ug/mL	4.40 % at conc. 1 ug/mL
	1.45 % at conc. 10ug/mL	2.76 % at conc. 10ug/mL

Table 8: Results for the recovery% and % RSD for the three standard compounds.

Compound	Recovery	% RSD
Chlorogenic acid	102.00	0.472
Quercetin	100.95	0.650
Apigenin	94.525	2.434

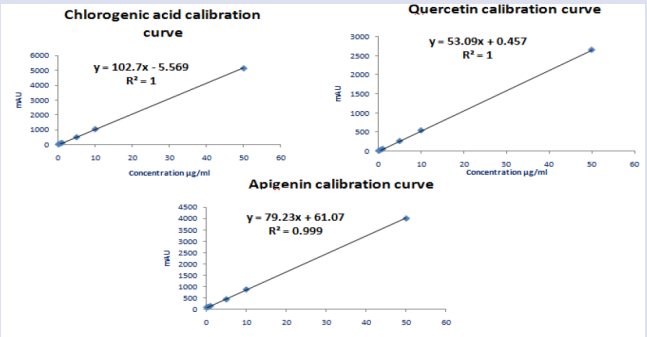

Figure 2: Standard calibration curves for the three compounds using RP-HPLC-UV.

was able to reveal the presence of quercetin in the 80% methanolic extract.[3] Also, regarding chlorogenic acid, the presence of this compound was determined using UPLC-PDA-TO (ultra performance-photo-diodearray-timeofflight) in the 80% methanolic extract of the fruit.[11] Nevertheless, an HPLC-UV comparative quantitative assay on the fruits of *H. thebaica* regarding their content of the three compounds of the ethanolic extracts and the different fractions of the fruits was never performed before. The described analysis method of chlorogenic acid, quercetin and apigenin in Doum fractions is examined for linearity, precision, and accuracy. Results demonstrate that the method showed good linearity with a high degree of precision (relative standard deviation values <5% was achieved). It also showed that the method is suitable for its intended use, where a simple, accurate, sensitive and reproducible HPLC method has been developed for the determination of the three detected compounds. Previous results showed that Doum proved to cause a significant decrease in systolic and diastolic blood pressure towards normal values when given to hypertensive patients.[1] Those results were consistent with other previous results which suggested that the hypotensive action of Doum in rabbits may be due to ganglion blockade.[2] However it was suggested that further work should be done to determine whether it is the flavonoids content that is responsible for the protective effects of Doum or due to other mechanisms.[1] In the current study, results showed that all doum fractions possess an antihypertensive activity through ACE inhibition and renin inhibition mechanisms with varying potencies (butanol fraction of 70% extract was shown to be the most active fraction through both mechanisms). Concerning total extracts, 50% ethanol extract and 70 % ethanol extract recorded highest potency as an antihypertensive drug through renin inhibition and ACE inhibition mechanisms; respectively. Also, when testing the activity of the pure flavonoids apigenin and quercetin, they proved to have an anti-hypertensive effect through both an ACE inhibition mechanism and renin inhibition mechanism. While when testing the phenolic compound chlorogenic acid, it proved to possess only ACE inhibition activity with minimal or low renin inhibitory mechanism. Those findings showed that the antihypertensive activity of the fractions and extracts could be attributed to their phenolic content. Consequently, the potency of the butanol fraction of the 70% extract as an antihypertensive drug through both mechanisms ACE and renin activity could be attributed to the presence of apigenin and quercetin, those compounds were proved to be the most active compounds through both mechanisms. Also chlorogenic acid could be responsible for the potency through the ACE inhibition mechanism. So those tested compounds may synergistically along with other non-tested polar compounds owe for the potency of the butanol fraction of the 70% extract. Therefore, the three compounds were chosen as the pharmacological markers owing to the antihypertensive activity of the plant and the butanol fraction of the 70% extract was standardized to contain not less than 1.314 mg % $\pm$ 0.193635 chlorogenic acid, 0.0265 mg % $\pm$ 0.003182 quercetin and not less than 0.1005 mg % $\pm$ 0.112308 apigenin.

CONCLUSION

So based on the results of this study, it could be concluded that *Hyphaene thebaica* L. is a very promising fruit with a potent antihypertensive activity. However formulating the butanol fraction of the 70% extract into a pharmaceutical dosage form will require further toxicological studies and clinical trials.

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ABBREVIATIONS

ACE: Angiotensin converting enzyme; **HPLC:** High performance liquid chromatography; **RPHPLC:** Reversed phase high performance liquid chromatography; **HHL:** Hippuryl-histidyl-leucine; **PTFE:** Polytetrafluoroethylene; **LOQ:** Limit of Quantitation; **LOD:** Limit of detection; **ICH:** International conference on harmonization; **RP-HPLC-UV:** Reversed phase high performance liquid chromatography ultraviolet; **Ch:** chlorogenic acid; **Qu:** Quercetin; **Ap:** Apigenin; **SD:** standard deviation; **RSD:** Relative standard deviation; **NMR:** Nuclear magnetic resonance; **ESI:** Electron spray ionization; **Ms:** Mass spectroscopy; **UPLC-PDA-TO:** Ultra performance-photodiode array-time off light; **H:** Hexane; **E:** Ethyl acetate; **C:** Chloroform; **B:** Butanol.

CONFLICT OF INTEREST

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome. As corresponding author, I confirm that the

manuscript has been read and approved for submission by all the named authors.

SUMMARY

Hyphaene thebaica L., with common name doum palm, is an African palm. Local people use it as a medicinal plant for treatment of several diseases as diabetes, hypertension, dislipidemia and other diseases. This study was carried out to investigate the preliminary steps needed to develop an antihypertensive drug from the fruits of this plant. Therefore the study included preparing an extract from the fruits, comparing between the 50% and 70% ethanolic fractions of the fruit regarding the content of their polyphenolic compounds, screening for the antihypertensive activity and standardizing the active fraction.

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