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Hyphaene thebaica (Areceaeae) as a Promising Functional Food: Extraction, Analytical Techniques, Bioactivity, Food, and Industrial Applications

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Abstract

Hyphaene thebaica, also known as doum, is a wild plant growing in Egypt, Sudan, and other African countries. It is usually used to prepare nutritive diets, tasty beverages, and other food products. This review aimed to highlight the phytochemical composition of the doum plant using NMR, GC–MS, HPLC, and UPLC/Qtof/MS. The reported active constituents are also described, with flavonoids, phenolic acids, and saponins being the most dominant components. Extraction methods, both conventional and non-conventional, and their existing parameters were summarized. The in vitro and in vivo studies on the extracts and active constituents were also reported. We focused on different applications of doum in functional food products, animal feeding systems, and pharmaceutical applications. Doum is considered a promising dietary and therapeutic candidate to be applied on a wider scale. Proteomic analysis of doum and clinical assessment are still lacking and warrant further investigations in the future.

Keywords *Hyphaene Thebaica* · Arecaceae · Functional Food · Fortified Food · Mannan · Antioxidant · Anti-inflammatory

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Abbreviations

LDL	Low-density lipoprotein
HDL	High-density lipoprotein
GSH	Px-glutathione peroxidase
GST	Glutathione S-transferases
CAT	Catalase
ALT	Alanine transaminase
AST	Aspartate transaminase
TNF	α-Tumor necrosis factor-α
IL-1β	Interleukin 1-β
MDA	Malondialdehyde
AChE	Acetylcholinesterase
SUR1	Sulfonylurea receptor 1

Introduction

Foods that possess additional physiological effects beyond their nutritional functions of providing nutrients are called functional foods. The concept of functional food received scientific attention in the last four decades. Functional food can provide nutraceuticals with the therapeutic value that is capable of protecting against infectious and chronic disease (Rivera et al. 2010; Kumar et al. 2021a). The use of plant-based dietary components or functional foods was promoted globally because of their therapeutic benefits and nutritive properties (Kumar et al. 2021b; Prakash et al. 2021). In the current study, we focused on an interesting nutritive plant, *Hyphaene thebaica* (L.), which is native to Egypt and North Sudan as a potential functional food. This plant is considered sacred by the Egyptian and other African civilizations for its nutritive and therapeutic potential. *H. thebaica* is commonly known as doum and belongs to the Arecaceae family. It is native to the northern half of Africa and grows in Senegal, Mauritania, Tanzania, Sudan, and Arabian Peninsula (Sinai, Yemen, Saudi Arabia, and Palestine). The fruit of *H. thebaica* is edible and oval-shaped. It contains proteins, sugars, fats, calcium, phosphorus, and a high level of iron. Fruits contain a wide array of phytochemical compounds including hydroxy cinnamates, flavonoids, essential oils, and saponins. It is also rich in niacin, amino acids, thiamin and riboflavin (El-Beltagi et al. 2018). It exhibited many industrial applications as a stabilizer, filling powder, the basis of fibers, and nectars, as a flavoring agent. Bioactive compounds of *H. thebaica* demonstrated many biological activities including antimicrobial, anticancer, hyperlipidemia, antioxidant, anti-inflammatory, and antidiabetic effects (Hsu et al. 2006; Abdallah 2021). Recent studies of *H. thebaica* focused on the fruit (doum fruit) because of its medicinal properties and high nutritional value (El-Beltagi et al. 2018; Gibril et al. 2020; Aamer 2016; Salib et al. 2013; Aboshora et al. 2014a). Although in recent years numerous bioactive compounds and plant products were examined for their role as functional foods with potential therapeutic uses and nutritional health benefits, few of them were subjected to thorough clinical investigation of their therapeutic uses and nutritional health benefits. A small number of bioactive compounds and plant foods surpassed the standard requirements by the FDA's significant scientific agreement for the authorization of their health claim (Hasler 2002). Despite the wide use of doum in African countries, there are no detailed reviews on their constituents, biological activities, and potential applications. This study focused on the extraction techniques, food, and industrial applications of *H. thebaica*. We discussed reported studies on the functionality of *H. thebaica* as a potential functional food, research on the bioactivity of the plant extracts, and applications in the food industry. We also provided recommendations for the future production and consumption of doum and its products.

Material and Methods

The review of literature conducted was performed using different search engines including Google Scholar, Scopus, PubMed, and Science Direct. The used keywords were "*H. thebaica*," "Doum," "*H. thebaica* + Arecaceae," "*H. thebaica* + extraction," "*H. thebaica* + formulations," "*H. thebaica* + metabolomics," "*H. thebaica* + GC," "*H. thebaica* + HPLC," and "*H. thebaica* + biological activity." All retrieved reports including reviews and original research manuscripts were considered until 2022, with more focus on studies published within the last 10 years.

Agricultural and Harvesting Aspects (Drying and Storage Effect)

The analysis of bibliographic data related to the collection, drying, and storage of *H. thebaica* revealed interesting results. We found only one study that was carried out by Ewansiha et al. (2021). It presented all aspects of harvesting to the final preparation of the sample including the washing and drying stages. *H. thebaica* grows naturally in the southwestern part of Egypt (Taha et al. 2020) and the Wina region in Cameroon. It is harvested in the period between November 2019 and February 2020 (Kolla et al. 2021). However, crops of this species were reported on the agricultural lands of Jambutu Yola (Ewansiha et al. 2021), and Kayauki of Adamawa and Katsina (Salisu and Saleh 2019) in the respective states in Nigeria. It also grows in the Tayba garden in the city of Elgazira Aba in Sudan (Aboshora et al. 2017), and in the city of Wudil State of Kano in Nigeria (Salihu et al. 2019).

To the best of our knowledge, no bibliographic data mentioned the agricultural practices of this species in the regions mentioned above. The majority of studies on *H. thebaica* focused on the fruit, except for the work reported by Taha et al. (2020) who studied the phytochemical content of the leaves and fruits. Salisu and Saleh (2019) studied the phytochemical content of the stem.

Regarding the collection method, no bibliographic data described this procedure. The plant washing step was optional in most of the studies, only one study cited washing the fruit with 70% ethanol, while another study mentioned washing the fresh stems with distilled water.

The drying of the plant material is one of the most important steps in the preparation of the extracts. In this step, the plant matrix is dehydrated to eliminate any risk of microbial development thus extending the storage period of the plant material.

Three drying methods were used in the literature including sun drying (Aboshora et al. 2014b), drying in the dark (room temperature) (Aboshora et al. 2017; Bello et al. 2017), and in an oven (45 °C) (Taha et al. 2020) or (50 °C) (Gibril et al., 2020). The plant material was either crushed and directly extracted (Ewansiha et al. 2021; Shehu et al. 2017), or crushed and stored for future use (Bello et al. 2017; Taha et al. 2020; Gibril et al. 2020; Kolla et al. 2021; Aboshora et al. 2014a). The best methods of preserving plant material were either stored in a glass jar or a plastic bag. These methods allowed the samples to be kept hermetically reducing structural changes caused by oxidation phenomena (Méndez and Falqué 2007). Another study published by Kolla et al. (2021) mentioned the use of cardboard boxes for the preservation of *H. thebaica* fruits. A collective list of different culturing and drying methods is shown in Table 1.

Different Extraction Techniques Are Used in the Extraction of *H. thebaica*

Extraction techniques play important role in the preparation of high-quality plant formulations. Modern extraction methods or non-conventional methods are more effective and showed significant advantages over conventional ones. Conventional methods include hot water extraction, reflux extraction, alkali extraction, and maceration extraction. In conventional extraction methods, the extraction yield depends on various parameters including extraction time, pH, number of extraction cycles, and solvent-to-liquid ratio. Conventional extraction methods require a longer extraction time and a large volume of organic solvents (Rasul 2018). Modern extraction methods are also known as green extraction methods or non-conventional methods that were developed to increase efficiency and achieve more yield. These methods include ultrasonic extraction, supercritical fluid extraction, enzyme extraction, and microwave-assisted extraction. These methods require low organic solvent, short extraction time, and offer high selectivity (Kumar et al. 2020; Singh et al. 2020). For the extraction of bioactive compounds from *H. thebaica*, both conventional and non-conventional methods were adopted.

Conventional Methods Used in the Extraction of H. thebaica

Hot Water Extraction

The hot water extraction of 10 g dry fruit powder of *H. thebaica* was carried out under optimized conditions. The extraction time was 30 min with constant stirring and infusion with deionized boiling water (600 mL). The yield of *H. thebaica* obtained under these optimized conditions was 29.3 g (23.9% w/w) (Hsu et al. 2006).

In another study, *H. thebaica* fruit aqueous extraction was carried out using two different methods. In the first method, crushed fruit was soaked in water with the sample-to-solvent ratio of 1:5 w/v, extraction time (4, 8, 12 h) with a temperature of 22 ± 2 °C. In the second method, crushed fruit powder is mixed in boiling water with the sample-to-solvent ratio of 1:5 w/v, extraction time (5, 10, 15 min), and temperature 100 °C. In the first method (12 h), the yield of the total phenolic compounds was 35.98 ± 0.23 mg/100 g and the total flavonoid compounds were 3.60 ± 0.06 mg/100 g which was higher in comparison to the extraction yield of the second method (5 min) with a yield of the total phenolic compounds 23.47 ± 0.25 mg/100 g and flavonoids 3.18 ± 0.03 mg/100 g (Aamer 2016).

Solvent Extraction

In another study, the extraction of *H. thebaica* dry fruits was carried out under optimized conditions with two different solvents including ethanol (70% v/v) and methanol (80% v/v) in a shaking water bath (70 rpm), with an extraction time of 30 min, extraction temperature 70 °C for ethanol extract and 60 °C for methanol extract, and the sample-to-solvent ratio was 1:10. The yields of the phenolic compounds in ethanol and methanol extract were 116.26 ± 0.43 mg/g and 132.51 ± 0.51 mg/g, respectively. The yields of the flavonoid compounds in the ethanol and methanol extracts were 24.04 ± 0.17 mg/g and 41.55 ± 0.17 mg/g obtained under the optimized conditions (Aboshora et al. 2014b).

Maceration Extraction

In a recent study, the maceration of *H. thebaica* fruit powder (100 g) was carried out under optimized conditions,

Table 1 Culture, dry, and storage conditions of *Hyphaene thebaica* according to different regions

Spontaneous or cultivated	Region of collection	Climate	Time of collection	Parts collected	Washing	Drying mode	Drying parameters	Storage Preparation	Storage mode	References
Cultivated	Agricultural land in Jambutu Yola, Adamawa State, Nigeria	Tropical	The rainy season, precisely in May 2019	Fruits	Ethanol (70%)	In the dark	(26 °C)	Grinding		(Ewansiha et al., 2021)
Cultivated	Taybad garden in the City of Elgazira Aba in Sudan	Arid	–	Fruits	–	Sun-dried	–	Grinding	Kept dry at room temperature	(Aboshora et al., 2014a)
Cultivated	Taybad garden in the City of Elgazira Aba in Sudan	Arid	–	Fruits	–	In the dark	(23–25 °C)	Grinding	–	(Aboshora et al., 2017)
Cultivated	Wudil town, Kano state in Nigeria	Tropical	–	Fruits	–	Oven-dried	For 2 weeks	Grinding	In a glass jar	(Bello, et al., 2017)
Spontaneous	Saharan, the south-western part of Egypt	Desert	–	Leaves & fruits	–	Oven-dried	45 °C	Grinding	Stored in closed containers until use	(Taha, et al., 2020)
–	Collected from the local market in the city of Omdurman, Republic of Sudan	Arid	–	Fruits	–	Oven-dried	At 50 °C during 24 h	–	Kept in a plastic bag	(Gibril, et al., 2020)
–	local market (Dokki, Giza, Egypt)	Arid	–	Fruits	–	–	–	–	–	(Farrag et al., 2020)
Spontaneous	Cameroon in Hougno, Wina	Tropical	Collection in November 2019 and February 2020	Fruits	–	–	–	–	Stored in box	(Kolla et al., 20,201)
–	Rimi Market Kano Municipal Town, Kano State, Nigeria	–	February 2018	Fruits	–	–	–	–	–	(Salihu et al., 2019)
Cultivated	Local fields in Kayauki village, along the Daura road, Katsina state, Nigeria	Tropical	–	Stalk	Distilled water	Oven-dried	At 50 °C	–	–	(Salisu and Saleh, 2019)
–	Konduga, Borno State, Nigeria	Tropical	–	Fruits	–	In the dark	–	Grinding	No storage	(Shehu et al., 2017)

extraction time 3 days, extraction temperature 25–30 °C, and used solvent was methanol 80% (v/v). A total of 500 mg/ml experimental yield was obtained using maceration (Abdallah 2021).

In another study, the maceration of *H. thebaica* epicarp (500 g) was carried out. The extraction of epicarp powder was done using the optimized conditions at room temperature in a Soxhlet extractor, acetone was used as the solvent. The yield of *H. thebaica* epicarp powder was 150 g (Salib et al. 2013).

Non-Conventional or Green Methods Used for Extraction of H. thebaica

Ultrasonic Extraction

The extraction of *H. thebaica* dry fruits was performed under optimized conditions with two different solvents including ethanol (70% v/v) and methanol (80% v/v) in an ultrasonic bath (220 V and 50 Hz), extraction time was 30 min, extraction temperature was 70 °C for the ethanol extract, and 60 °C for the methanol extract. The sample-to-solvent ratio was 1:10. The yields of the phenolic compounds in the ethanol and methanol extracts were 123.36 ± 1.48 mg/g and 139.48 ± 1.18 mg/g. The yields of the flavonoid compounds in the ethanol and methanol extracts were 28.62 ± 0.12 mg/g and 47.17 ± 0.17 mg/g, obtained under the optimized conditions (Aboshora et al. 2014b).

In ultrasonic extraction, high yields of phenolic component 139.48 ± 1.18 mg/g and flavonoid component 47.17 ± 0.17 mg/g were reported. Conventional methods were more time-consuming compared to non-conventional methods. A compiled list of the extraction methods of *H. thebaica* is presented in Table 2.

Yield Optimization of Bioactive or Nutritive Compounds

The fruit of *H. thebaica* is consumed widely in some African countries and even its waste has some applications. In a recent study, the seed powder of *H. thebaica* was analyzed showing moisture content of 10.0 wt% (weight%), volatile matter of 85.31 wt%, and ash content of 1.3 wt%. The high-performance liquid chromatography (HPLC) results indicated that 70 wt% of the seed sugar content was mannose. Mannan, a storage polysaccharide having β (1–4) linkage present in the primary cell wall of plants, was extracted from 5 g seed powder of *H. thebaica* under the following optimized conditions including an alkali solution (0.25 N), sample-to-solvent ratio 1:30, extraction time 90 min, and temperature 90 °C. The yield of mannan under optimized conditions was 13.07 wt% suggesting the seed of *H. thebaica* to be a rich and low-cost source for mannan extraction (Gibril et al. 2020).

In another study, the microwave-assisted mercerization of fibers obtained from the stalks of *H. thebaica* was carried out. The obtained fibers were used as low-cost biosorbents to treat wastewater having Pb^{2+} and Cu^{2+} ions. Microwave-assisted mercerization of *H. thebaica* fiber was carried out under the following optimized conditions including extraction time of 20 min, microwave power of 700 W, frequency of 2450 MHz, and solvent sodium hydroxide solution 5% w/v. This pretreatment increased the hydrophilicity of fibers and removed the wax, lignin, and oil (Salisu and Saleh 2019).

Analytical and Detection Methods: GC/MS, LC/MS, and NMR

GC/MS

The essential oil of doum fruit was obtained by hydrodistillation using a modified Karlsruher apparatus. The hydrodistilled oil was then analyzed by GC/MS adopting linear temperature programming (80 °C to 270 °C at 10 °C/min). This resulted in the identification of 57 constituents. Diterpenes represented the predominant class, being amounted to 40.49% of the components, with incensole (17.52%) and incensole acetate (19.81%) as the main components in addition to cembrene A and cembrene C. Monoterpenoids were detected constituting 15.97%, mainly limonene, *β*-pinene, terpinene-4-ol, and sabinene (2.42, 1.98, 1.77 and 0.82%, respectively). Interestingly, the oxygenated compounds reached 66.78% reflecting an economically important value of doum oil. The authors attributed the scent of doum fruits to their richness with volatile diterpenes (Ayoub et al. 2011). In another study, GC–MS analysis was performed to detect the primary metabolites of doum fruits after sample derivatization. This allowed the identification of 26 compounds including mono- and disaccharides and organic and amino acids. Sucrose showed a high abundance accounting for 45–58% of the total ion chromatogram (Farag and Paré 2013).

HPLC and UPLC

Phenolic compounds from the fruit bulbs of *H. thebaica* collected from Sudan were analyzed and quantified for flavonoids and phenolic acids (Salih and Yahia 2015). Sixteen compounds were detected with methoxycinnamic and sinapic acids, catechin, and chlorogenic acid as the major compounds. They amounted to 2219.4, 1367.6, 584.6, and 572 mg/kg DW, respectively. Farag and Paré (2013) analyzed the aqueous and organic extracts of doum via UPLC-PDA-TOF to profile their phenolics and lipid compounds.

Table 2 Conventional and non-conventional methods of *Hyphaene thebaica* extraction

Source and region	Extraction method	Optimized condition (extraction time, sample size, solvent used, other parameters)	Yield/increase in recovery	Reference
Conventional method				
H. thebaica (Cairo, Egypt)	Hot water–assisted extraction	Time: 30 min Temperature: 35–40 °C Solvent used: deionized water	The experimental extraction yield of *H. thebaica* obtained under optimized conditions was 23.9% by weight	(Hsu et al., 2006)
H. thebaica (Aswan desert, Southern Egypt)		Time: 8 h Temperature: 22 °C The solvent used: water Sample to the solvent ratio: 1:5 w/v	The experimental yield of the total phenolic compounds was 34.54 ± 0.18 mg/100 g and the flavonoid compounds yield was 2.44 ± 0.04 mg/100 g	(Aamer, 2016)
H. thebaica (Aswan desert, Southern Egypt)		Time: 10 min Temperature: 100 °C The solvent used: water Sample to the solvent ratio: 1:5 w/v	The experimental yield of the total phenolic compounds was 21.26 ± 0.09 mg/100 g and the flavonoid compounds yield was 2.68 ± 0.04 mg/100 g	(Aamer, 2016)
H. thebaica (Aljazeera Aba City, The Republic of Sudan)	Solvent extraction	Time: 30 min Temperature: 70 °C The solvent used: ethanol (70% v/v) Sample to the solvent ratio: 1:10 w/v	The experimental yield of the phenolic content was 116.26 ± 0.43 mg/g and the flavonoid content yield was 24.04 ± 0.17 mg/g	(Aboshora et al., 2014b)
H. thebaica (Aljazeera Aba City, The Republic of Sudan)		Time: 30 min Temperature: 60 °C The solvent used: methanol (80% v/v) Sample to the solvent ratio: 1:10 w/v	The experimental yield of the phenolic content was 132.51 ± 0.51 mg/g and the flavonoid content yield was 41.55 ± 0.17 mg/g	(Aboshora et al., 2014b)
H. thebaica (Khartoum, Sudan)	Maceration extraction	Time: 3 days Temperature: 25–30 °C The solvent used: Methanol (80% v/v)	A total of 500 mg/ml experimental yield was obtained	(Abdallah, 2021)
H. thebaica (Aswan, Egypt)		Temperature: RT (room temperature) The solvent used: acetone	The extraction yield of *H. thebaica* epicarp powder was 150 g	(Salib et al., 2013)
Non-conventional Method				
H. thebaica (Aljazeera Aba City, The Republic of Sudan)	Ultrasonic extraction	Time: 30 min Temperature: 70 °C Frequency: 50 Hz Power: 220 V The solvent used: ethanol (70% v/v) Sample to the solvent ratio: 1:10 w/v	The obtained experimental yield of the phenolic content was 123.36 ± 1.48 mg/g and the flavonoid content yield was 28.62 ± 0.12 mg/g	(Aboshora et al., 2014b)
H. thebaica (Aljazeera Aba City, The Republic of Sudan)		Time: 30 min Temperature: 60 °C Frequency: 50 Hz Power: 220 V The solvent used: Methanol (80% v/v) Sample to the solvent ratio: 1:10 w/v	The obtained experimental yield of the phenolic content was 139.48 ± 1.18 mg/g and the flavonoid content yield was 47.17 ± 0.17 mg/g	(Aboshora et al., 2014b)

The results revealed the presence of 17 compounds including cinnamates, flavonoids (mainly *O*-glycosides), fatty acids, sphingolipids, unknown stilbene, and isolariciresinol glycoside. Chlorogenic acid and *O*-caffeoyl shikimic acid constituted the major phenolics as represented by 551.4 and 421.1 µg/g fruit dry weight. Aamer (2016) identified 19 phenolic compounds in doum fruit aqueous extracts by RP-HPLC and they also studied the extraction parameters (time and temperature) on the concentration of these compounds. They found 3-hydroxytyrosol, vanillic acid, catechin, and chlorogenic acid as the most enriched metabolites. Their concentration was related to the time of soaking at ambient temperature. Increasing the boiling time resulted in a lower concentration of the phenolics. Recently, Taha et al. (2020) investigated the metabolomics profile of different organs of doum, where 14 compounds were detected against co-injected standards. Interestingly, the fruits exhibited the highest content of chlorogenic acid (0.152 mg/g) being in line with previous reports as a major doum metabolite. Also, apigenin-7-glucoside was detected in doum fruit up to 0.169 mg/g. Apigenin-7-glucoside and rutin were the predominant phenolics detected in doum leaves (5.428 and 2.695 mg/g, respectively). While the male parts were mostly rich in vanillic, rosmarinic, and protocatechuic acids (1.081, 0.544, 0.463 mg/g, respectively).

NMR Analysis

^{1}H-NMR was utilized to identify and further quantify the metabolites of doum fruit. Three classes of compounds were observed in the amino and organic acid regions in addition to the sugar region. Sucrose was detected as the most abundant metabolite constituting 219 mg/g (Farag and Paré 2013). The identification of the previously isolated compounds from doum was confirmed mainly using NMR and these compounds will be discussed in "Isolated Phytochemical Compounds from *H. thebaica* (doum Palm)."

Isolated Phytochemical Compounds from *H. thebaica* (Doum Palm)

An in-depth phytochemical study was carried out on the aqueous ethanolic extract of doum leaves (Eldahshan et al. 2009). It revealed the isolation of quercetin (**1**) and three quercetin glycosides namely, quercetin-3-*O*-β-$_4C^1$-D-glucopyranoside (**2**), quercetin-7-*O*-β-$^4C_1$-D-glucopyranoside (**3**), and 7,3′ dimethoxy quercetin-3-*O*-[6″-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**4**). In addition, apigenin aglycone (**5**) and two *C*-apigenin glycosides, namely, 8-*C*-β-D-glucopyranosyl-5,7,4′-trihydroxyflavone (**6**) and 6-$^4C_1$-β-D-glucopyranosyl-5,7,4'-trihydroxyflavone (**7**), were isolated. Luteolin (**8**), luteolin-7-*O*-β-$^4C_1$-D-glucopyranoside (**9**), tricin (**10**), and tricin-5-*O*-β-$^4C_1$-D-glucopyranoside (**11**) were reported. Kaempferol (**12**) and kaempferol rhamnoglucoside identified as kaempferol-3-*O*-[6″-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**13**) were also obtained from the same extract.

Another study published by Salib et al. (2013) identified flavonoids in the water-soluble fraction of acetone extract from *H. thebaica* epicarp. The authors reported two new isolated flavonoid glycosides namely luteolin 7-*O*-[6″-*O*-α-L-rhamnopyranosyl]-β-D galactopyranoside (**14**) and chrysoeriol 7-*O*-[2″-*O*-β-D-galactopyranosyl]-α-L-arabinofuranoside (**15**). Also, five known flavonoid glycosides were identified including vitexin (**6**), isovitexin (**7**), luteolin 7-*O*-β-D-glucopyranoside (**9**), chrysoeriol 7-*O*-[6″-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**16**), and kaempferol 7, 4′-dimethoxy-3-[6″-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside (**17**). Three aglycones including luteolin (**8**), kaempferol (**12**), and chrysoeriol (**18**) were also obtained. The structures of identified phytochemicals from doum are represented in Fig. 1.

Recent In Vitro and In Vivo Studies on *H. thebaica* (Doum Palm)

Different extracts of *H. thebaica* were evaluated in a panel of in vitro and in vivo assays as illustrated in Fig. 2 and Tables 3 and 4.

In Vitro Studies

Antioxidant Activity

The hot water extract of *H. thebaica* fruits acted as a potent source of antioxidants. It showed hydrogen-donating activity of 2.85 mmol ascorbic acid equivalent, Fe^{2+}-chelating activity of 1.78 mmol ethylenediamine tetra acetic acid equivalent, hydroxyl radical–scavenging activity of 192 mmol gallic acid equivalent, inhibition of substrate site-specific hydroxyl radical formation of 3.36 mmol gallic acid equivalent, superoxide radical–scavenging activity of 1.78 mmol gallic acid equivalent, and reduction of the power of 3.93 mmol ascorbic acid equivalent (Hsu et al. 2006). Faten (2009) reported the antioxidant activity of the *H. thebaica* fruits to extract using the DPPH assay. It showed an IC_{50} value of 1000 µg/mL and showed 80% inhibition at a concentration of 1500 µg/mL as compared to the quercetin standard which showed 69% inhibition at a concentration of 1000 µg/mL.

Regarding the 70% ethanol extract of the leaves, it showed inhibition of the reactive oxygen species (ROS) attack on salicylic acid with an IC_{50} value of 1602 µg/mL using xanthine/hypoxanthine oxidase assay. The antioxidant potential

would be attributed to the major phenolic compounds, namely, gallic acid, quercetin glucoside, kaempferol rhamnoglucoside, and dimethyoxyquercetin rhamnoglucoside identified by HPLC-ESI analysis (Eldahshan et al. 2009).

The methanolic extract of *H. thebaica* showed 64.55% scavenging effects towards DPPH at a concentration of 150 µg/mL as compared with the standards butylated hydroxytoluene (BHA) and butylated hydroxyanisole (BHT), which sowed inhibitory activity at 70.45 and 67.55 µg/mL, respectively (Mohamed et al. 2010).

The methanol extract of the bark showed 90.7% inhibition of the free radical–scavenging activity using the DPPH assay at a concentration of 100 µg/mL. And the recorded IC_{50} value was 44 ± 1.5 µg/mL (Fayad et al. 2015).

Atito et al. (2019) reported a significant difference in the DPPH-scavenging activity of the 80% methanol extract of the endocarp, mesocarp, and coat samples of *H. thebaica* fruits where the IC_{50} values were 53.76, > 500, and 137.89 µg mL, respectively. The results revealed that endocarp showed the most potent radical–scavenging activity. Another study tested the DPPH-scavenging activity of the aqueous *H. thebaica* extract and the IC_{50} was 771 µg/mL (Abd-ELmageed et al. 2019).

A recent study revealed that the 80% methanol extract of the leaves and male parts of doum palm showed more potent DPPH-scavenging activity with IC_{50} values of 62.6 and 66.2 µg/mL, respectively, as compared with the fruits with an IC_{50} value of 76.8 µg/mL. The antioxidant activity could be correlated with their total antioxidant capacity (TAC). The male parts showed the highest TAC (308.8 mg ascorbic acid equivalent/g extract), and the leaves and fruits showed lower values (123.8 and 127.9 mg ascorbic acid equivalent/g extract, respectively) (Taha et al. 2020).

Anti-inflammatory Activity

The anti-inflammatory activity of the chloroform extract of *H. thebaica* seeds was evaluated using atropinized rat fundus strip by induced inflammation with kidney homogenate. The results revealed that the chloroform extract at a dose of 5 ng/mL showed significant inhibition of kidney muscle stimulation by 60% as compared with the reference drug, indomethacin (5 µg/mL), which showed complete inhibition of the kidney muscle stimulation (Eltayeb et al. 2009).

Also, the 80% methanol extract of *H. thebaica* fruits showed anti-inflammatory potential through cyclooxygenase-1 enzyme inhibition. The extract demonstrated inhibition of COX-1 with an IC_{50} value of 20.9 ± 3.2 mg/mL in comparison with the reference COX-1 inhibitor (SC-560) that showed an IC_{50} value of 50 ± 6.7 µM. Its anti-inflammatory activity was likely to be mediated by flavonoid conjugates, oxygenated fatty acids, and sphingolipids found in fruits (Farag and Paré 2013).

Antimicrobial Activity

The methanolic and aqueous extracts of *H. thebaica* showed potent inhibitory effects against Gram-positive (*S. aureus* & *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* & *S. typhi*). However, slight inhibition was observed against *L. monocytogenes*, and no inhibitory effect was observed against *E. coli*. The methanolic extract of *H. thebaica* showed more potent antifungal (*A. niger*) and anti-yeast (*C. albicans*) activities than the aqueous extracts (Mohamed et al. 2010).

The aqueous extract obtained from the pericarp showed MIC values of 25 mg/mL against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi*. The MIC value for *E. coli* and *Shigella dysenteriae* was 50 mg/mL. It also showed (minimum bactericidal activity) MBC values of 50 mg/mL (Auwal et al. 2013).

The *n*-hexane extract of the fruits showed antibacterial activity with a diameter zone of inhibition (DZI) ranging from 15.10 ± 0.51 to 2.0 ± 0.55 mm towards *K. pneumoniae*, 10.20 ± 0.57 to 2.00 ± 0.35 mm towards *P. aeruginosa*, and 8.00 ± 0.35 to 1.00 ± 0.55 mm towards *S. typhi*, while the DZI for the aqueous extract was 7.10 ± 0.23 to 2.0 ± 0.35 mm towards *K. pneumoniae*, 6.20 ± 0.31 to 2.00 ± 0.35 mm towards *S. typhi*, and 5.42 ± 0.55 to 2.05 ± 0.75 mm towards *P. aeruginosa* using agar well diffusion method. The MIC and MBC values of the extracts were 100 mg/mL and 200 mg/mL, respectively (Ewansiha et al. 2021). The aqueous extract of fruits showed high antimicrobial activity towards *S. aureus* with the highest DZI of 20.33 mm followed by *E. coli* with a DZI of 16.00 mm (Abd-ELmageed et al. 2019). Atito et al. (2019) reported that the 80% methanol extract of *H. thebaica* endocarp showed the most potent activity against two strains of bacteria *S. pneumonia* and *B. subtilis*.

Taha et al. (2020) reported the antimicrobial activity of the 80% methanol extract (500 µg) of doum palm's different parts using the disc diffusion assay. The results revealed that the leaf extract exhibited the highest impact followed by fruit and male part extracts. While only the fruit extract showed antifungal activity towards *Candida albicans* with an inhibition zone of 9.0 ± 0.0 mm. The presence of secondary metabolites such as anthocyanins, saponins, phenolics, flavonoids, and tannins, which are well-known antibacterial agents against a wide range of Gram-positive and negative bacteria, could explain the potential antimicrobial activity of doum palm in different parts (Taha et al. 2020). The inhibition of proteases and/or inactivation of microbial adhesions may contribute to the mechanism of polyphenols toxicity towards microbes (Cowan 1999).

The antimicrobial activity of the 80% methanol extract of the fruit pulp at 500 mg/mL was evaluated using the agar-well diffusion technique. The results revealed a good antimicrobial activity against *Staphylococcus aureus*

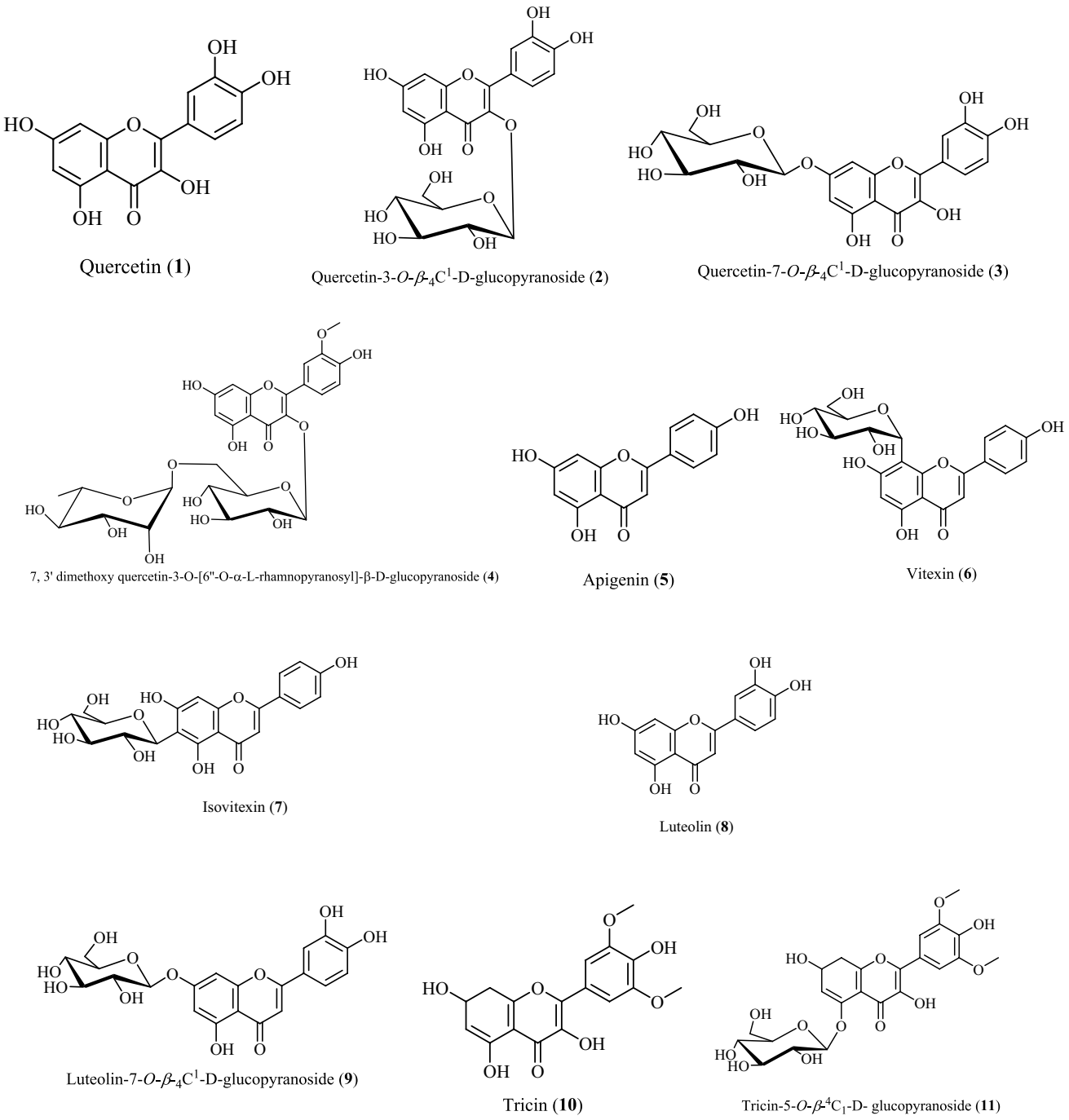
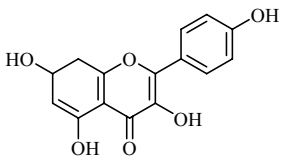


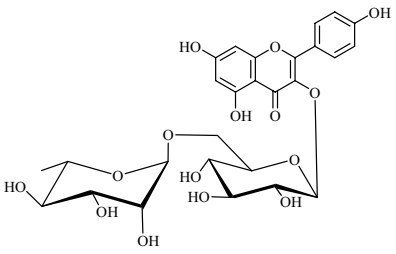
Fig. 1 Chemical structures of flavonoids isolated from *Hyphaene thebaica*

and *Pseudomonas aeruginosa* with inhibition zones of 16.0 ± 1.0 mm and 18.5 ± 0.5 mm, respectively. On the other hand, weak antibacterial activity was recorded with *Bacillus cereus* and *Escherichia coli* with inhibition zones of 9.0 ± 0.0 mm and 7.5 ± 0.5 mm, respectively. The minimum inhibitory concentrations (MIC) of the methanolic extract of the fruit were 62.5 mg/mL and 125 mg/mL for *S. aureus* and *P. aeruginosa,* respectively (Abdallah 2021).

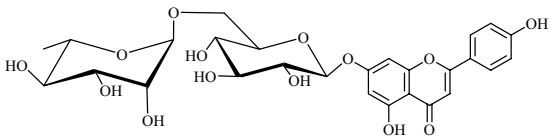
Another report revealed that the 80% methanol extract of *H. thebaica* fruits showed potent antibacterial activity towards two antibiotic-resistant bacteria, namely, Gram-positive ß-lactamase bacteria (*Staphylococcus aureus*) and one Gram-negative multidrug-resistant bacteria (*Proteus mirabilis*). The MIC values were 31.25 and 250.00 mg/mL, respectively, and MBC values were 31.25 and 125.00 mg/mL, respectively (Abdallah 2021).



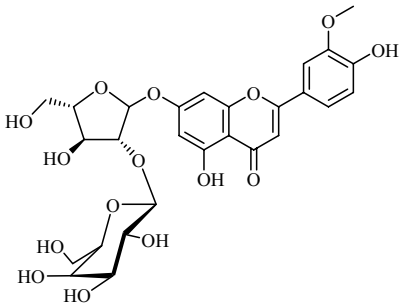
Kaempferol (**12**)



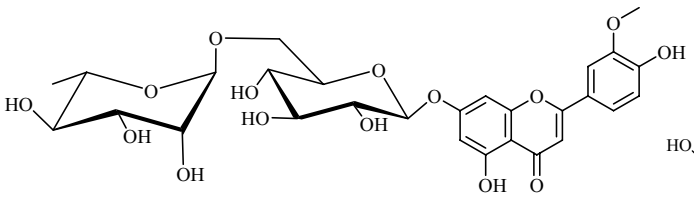
kaempferol-3-O-[6"-*O*-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside (**13**)



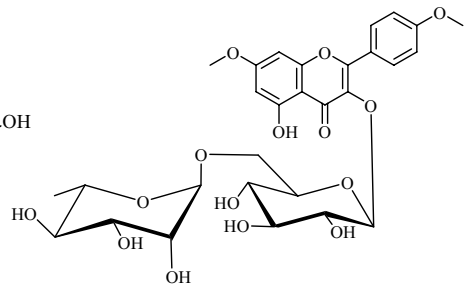
luteolin 7-O-[6"-*O*-*α*-L-rhamnopyranosyl]-*β*-D galactopyranoside (**14**)



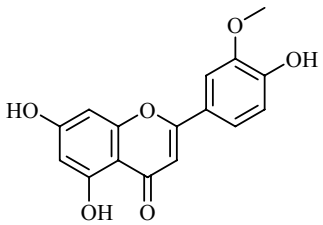
chrysoeriol 7-O-[2"-O-β-D-galactopyranosyl]-β-L-arabinofuranoside (**15**)



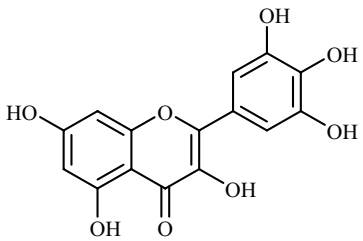
chrysoeriol 7-O-[6"-O-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside (**16**)



kaempferol 7, 4'-dimethoxy-3-*O*-[6"-*O*-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside (**17**)



chrysoeriol (**18**)



Myricetin **(19)**

Fig. 1 (continued)

Cytotoxic Activity

Faten (2009) reported the cytotoxic activities of the *H. thebaica* fruit extract. The extract showed antiproliferative activity against acute myeloid leukemia (AML) cells with an IC_{50} value of 3 µg/mL.

Fayad et al. (2015) reported that the methanol extract of the bark showed cytotoxicity against A549 (lung carcinoma cell line) and MCF-7 (breast cancer cell line) with IC_{50} values of 32 ± 0.9 and 38 ± 1.2 µg/mL, respectively, and at a concentration of 100 µg/mL, it showed inhibition by 87% and 89%, respectively (Fayad et al. 2015).

The antiproliferative activity of the 80% methanol extract of the leaves, male parts, and fruits was evaluated using the sulforhodamine B (SRB) colorimetric assay towards human hepatocellular carcinoma (HepG-2) and lung carcinoma (A549)

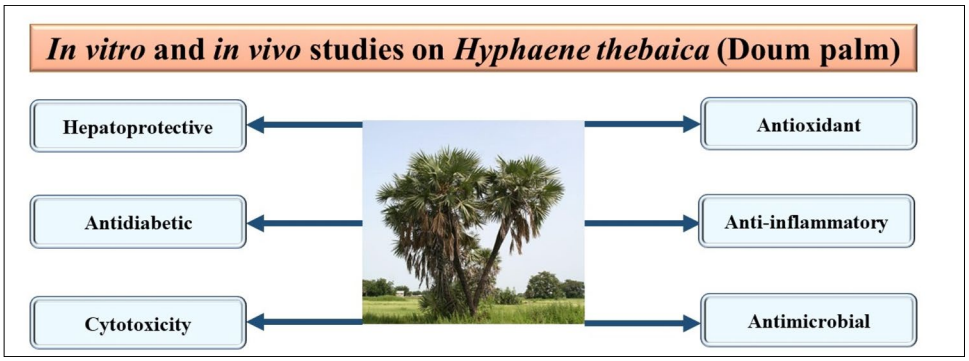


Fig. 2 The schematic diagram for the recent in vitro and in vivo studies on *Hyphaene thebaica*

cell lines. The leaves, male parts, and fruits showed cytotoxic activity against HepG-2 cells with IC_{50} values of 3.08, 1.14, and 3.07 µg/mL, respectively. While their cytotoxic activity against A549 cells was more potent with IC_{50} values of 2.07, 1.15, and 2.76 µg/mL, respectively. The results indicated that the male part extract exhibited the most potent antiproliferative activity against HepG2 and A549 cell lines (Taha et al. 2020).

Antidiabetic Activity

In the digestive system, α-amylase is a key enzyme that catalyzes the hydrolysis of starch into simple monosaccharides, which are further degraded by α-glucosidases to produce glucose for intestinal absorption, which in turn, increases blood glucose levels (Van de Laar et al. 2005; Zhu et al. 2020). Inhibiting the function of these enzymes can decrease blood glucose post-prandial levels since only monosaccharides can be absorbed through intestinal mucosa thus decreasing the demand for insulin, and consequently reducing hyperglycemia in patients with type-2 diabetes (Teng and Chen 2017).

Shady et al. (2021) reported that the aqueous extracts of *H. thebaica* fruits are rich in flavonoids including myricetin (**19**), luteolin (**8**), and apigenin (**5**). The in vitro insulin secretion assay revealed that the major bioactive flavonoids (**19**, **8**, **5**) were able to promote insulin release by human pancreatic cells by 20.9 ± 1.3, 13.74 ± 1.8, and 11.33 ± 1.1 ng/mL, respectively.

The 80% methanol extract of the endocarp, mesocarp, and coat samples of *H. thebaica* fruits showed different *α*-amylase inhibitory activity with IC_{50} values of 87.06, 81.20, and 81.83 µg/mL, respectively. Where the mesocarp showed the most potent activity followed by the coat and endocarp (Atito et al. 2019). Also, a recent report by Khallaf et al. (2022) investigated various fractions obtained by different solvents from the total ethanolic extract of *H. thebaica* for α-glucosidase inhibitory activity, a key enzyme in carbohydrate metabolism. They found that the dichloromethane fraction exerted potent inhibition with IC_{50} = 52.40 µg/ml. The subsequent fractions from the dichloromethane showed powerful inhibition as compared with acarbose (IC_{50} 3.79–5.13 µg/ml versus 2.33 µg/ml). However, in another study, El-Manawaty and Gohar (2018) tested the inhibitory effect of methanol extract of *H. thebaica* flowers on the enzymatic activity of α-glucosidase. They showed that *H. thebaica* extract exhibited very low inhibitory activity with 2% inhibition on α-glucosidase at the tested concentration of 25 ppm (El-Manawaty and Gohar 2018). Thus, further investigations were carried out to demonstrate the potential of the antidiabetic effect of *H. thebaica* using in vivo models (Table 3).

In Vivo *Studies*

Antidiabetic Activity

The phytochemical investigation of the water-soluble fraction of *H. thebaica* fruits showed its richness in flavonoids. One of the isolated flavonoids identified as a new natural flavonoid was chrysoeriol 7-*O*-*β*-D galactopyranosy-(1 → 2)-*α*-L-arabinofuranoside (**15**). Both water-soluble fractions of *H. thebaica* and compound (**15**) at a dose of 20 mg/Kg b.wt. showed antidiabetic activity in alloxan-induced diabetic rats. The results revealed an improvement in glucose, insulin tolerance, and kidney function as well as a significant reduction in blood glycosylated hemoglobin levels. Also, compound (**15**) significantly reduced AST and ALT levels in the liver (Salib et al. 2013).

An in vivo study of the STZ-induced diabetic model showed that the aqueous extract of the fruits at a dose of 1 g/Kg b.wt. increased blood glucose level. And improved the levels of different biomarkers including serum total lipids, cholesterol, triglycerides, LDL, and HDL. Also, the activities of serum enzymes ASAT, ALAT, ALP, GGT, and LDH were ameliorated (Tohamy et al. 2013).

AbdEl-Moniem et al. (2015) reported the recovery ability of the fruit extract on streptozotocin (STZ)-induced diabetic nephropathy at a dose of 150 mg/Kg b.wt. The biochemical results revealed that the levels of blood glucose, urea, and

Table 3 In vitro studies of *Hyphaene thebaica*

Type of activity	Part used/extract	Used assay	Potency/efficiency	Ref
Antioxidant activity	Fruit hot water extract	Hydrogen donating activity	2.85 mmol ascorbic acid equivalent	(Hsu et al., 2006)
		Fe^{2+} chelating activity	1.78 mmol ethylenediamine tetra acetic acid equivalent	
		Hydroxyl radical-scavenging activity	192 mmol gallic acid equivalent	
		Inhibition of substrate site-specific hydroxyl radical formation	3.36 mmol gallic acid equivalent	
		Superoxide radical-scavenging activity	3.36 mmol gallic acid equivalent	
		Reducing power	3.93 mmol ascorbic acid equivalent	
	Fruit water extract	DPPH assay	$IC_{50} = 1000$ µg/mL	(Faten 2009)
	70% ethanol extract from the leaves	Xanthine/hypoxanthine oxidase assay	$IC_{50} = 1602$ µg/mL	(Eldahshan et al., 2009)
	Fruit methanolic extract	DPPH assay	64.55% scavenging effects towards DPPH at a concentration of 150 µg/mL	(Mohamed et al., 2010)
	Bark methanol extract	DPPH assay	IC_{50} value was 44 ± 1.5 µg/mL	(Fayad et al., 2015)
	80% methanol extract of the endocarp, mesocarp, and coat samples	DPPH assay	$IC_{50} = 53.76, > 500$ and 137.89 µg mL, respectively	(Atito et al. 2019)
	Fruit aqueous extract	DPPH assay	$IC_{50} = 771$ µg/mL	(Abd-ELmageed et al., 2019)
	80% methanol extract from leaves and male parts	DPPH assay	$IC_{50} = 62.6$ and 66.2 µg/mL, respectively	(Taha et al., 2020)
Anti-inflammatory activity	Chloroform extract of seeds	Atropinized rat fundus strip by induced inflammation with kidney homogenate	A dose of 5 ng/mL showed significant inhibition of kidney muscle stimulation by 60% as compared to Indomethacin	(Eltayeb et al., 2009)
	Fruits with 80% methanol extract	Cyclooxygenase-1 enzyme inhibition	$IC_{50} = 20.9 \pm 3.2$ mg/ mL	(Farag and Paré, 2013)
Antimicrobial activity	Fruit methanolic and aqueous extracts	Disc assay procedure (40 µg/mL extract)	Both extracts showed potent inhibitory effects against *S. aureus, B. subtilis, P. aeruginosa* & *S. typhi* The methanolic extract showed more potent antifungal (*A. niger*) and anti-yeast (*C. albicans*) activities than the aqueous extracts	(Mohamed et al., 2010)

Table 3 (continued)

Type of activity	Part used/extract	Used assay	Potency/efficiency	Ref
Antimicrobial activity	The pericarp aqueous extract	MIC assay by the dilution method	MIC = 25 mg/mL for *S. aureus, Strep. pyogenes* & *Salmonella typhi*/ MIC = 50 mg/mL for *E. coli* & *Shigella dysenteriae* MBC = 50 mg/mL	(Auwal et al., 2013)
	Fruit *n*-hexane extract	Agar well diffusion method	The highest activity towards *K. pneumoniae* with DZI ranged from 15.10 ± 0.51 mm to 2.0 ± 0.55 mm MIC and MBC = of 100 mg/mL & 200 mg/mL, respectively	(Ewansiha et al., 2021)
	Fruit aqueous extract	Agar-well diffusion technique	DZI = 20.33 mm towards *S. aureus*	(Abd-ELmageed et al., 2019)
	Endocarp 80% methanol extract	Bioassay technique	Most potent activity against *S. pneumonia* and *B. subtilis*	(Atito, et al. 2019)
	Fruits with 80% methanol extract	The disc diffusion assay	At a concentration of 500 µg fruit extract showed antifungal activity towards *Candida albicans* with IZ = 9.0 ± 0.0 mm	(Taha et al. 2020)
	Fruits with 80% methanol extract	Agar-well diffusion technique	Inhibition zones of 16.0 ± 1.0 mm and 18.5 ± 0.5 mm against *S. aureus* and *P. aeruginosa*, respectively MIC = 62.5 mg/mL and 125 mg/mL for *S. aureus* and *P. aeruginosa,* respectively	(Abdallah, 2021)
	Fruits with 80% methanol extract	MIC assay by the dilution method	MIC = 31.25 and 250.00 mg/mL, and MBC = 31.25 and 125.00 mg/mL, *S. aureus* & *Proteus mirabilis,* respectively	(Abdallah et al. 2021)
Cytotoxic activity	Fruit extract	Modified cytotoxic trypan blue-exclusion technique	IC_{50} = 3 µg/mL towards acute myeloid leukemia (AML) cell line	(Faten 2009)
	Bark methanol extract	MTT assay	IC_{50} values of 32 ± 0.9 and 38 ± 1.2 µg/mL against A549 (lung carcinoma cell line) and MCF-7 cell line, respectively	(Fayad et al., 2015)
	80% methanol extract from leaves	Sulforhodamine B (SRB) colorimetric assay	Leaves, male parts, and fruits showed IC_{50} = 3.08, 1.14 and 3.07 µg/mL towards HepG-2 cell line and IC_{50} = 2.07, 1.15 and 2.76 µg/mL, respectively against A549 cell line	(Taha et al. 2020)
Antidiabetic activity	Fruit aqueous extracts	In vitro insulin secretion assay	Myricetin, luteolin, and apigenin promote insulin release by the human pancreatic cells by 20.9 ± 1.3, 13.74 ± 1.8, and 11.33 ± 1.1 ng/mL, respectively	(Shady et al. 2021)
	80% methanol extract of the endocarp, mesocarp, and coat samples	α-Amylase inhibitory activity	IC_{50} = 87.06, 81.20, 81.83 µg/mL, respectively	(Atito et al. 2019)
	Flower methanol extract	α-Glucosidase inhibitory activity	It exhibited inhibitory activity of 2% at a concentration of 25 ppm	(El-Manawaty and Gohar. 2018)

creatinine were significantly decreased, while insulin and C-peptide levels significantly increased. Cystatin C and neutrophil gelatinase-associated lipocalin decreased. Regarding histopathological observation, collagen fiber deposition was elevated, associated with apparent thickening of the parietal layer of Bowman's capsules and the basal lamina of convoluted tubules.

Different extracts of doum palm fruits were investigated for their antidiabetic activity in the STZ-induced diabetic model at a dose of 60 mg/kg. The aqueous extracts considerably lowered high blood glucose and enhanced the relative expression of insulin. On the other hand, a significant reduction of the inflammatory mediators (TNFα and TGFβ) was observed. Regarding the histopathological examination, the aqueous extract significantly reverted the β-cell necrosis generated by STZ. The noticed potential antidiabetic activity of the aqueous extracts would be attributed to the presence of flavonoids; apigenin (**5**), luteolin (**8**), chrysoeriol (**18**), and myricetin (**19**) as major bioactive compounds. The molecular docking results revealed that they targeted the SUR1 binding site attaining binding energy ratings ranging from − 9.9 to − 10.3 kcal/mol (Shady et al. 2021).

Anti-inflammatory Activity

An in vivo anti-inflammatory study on different fractions from different parts of *H. thebaica* at a dose of 200 mg/kg b.wt. revealed that the chloroform and ethanol extracts of the seeds showed the most potent anti-inflammatory activity of 27% and 22%, respectively, as compared with the standard drug aspirin at a dose of 100 mg/kg b.wt. (Eltayeb et al. 2009).

Miscellaneous Activities

The protective effect of *H. thebaica* fruits extract (500 and 1000 mg/kg b.wt.) on liver/kidney functions was evaluated in mercuric chloride-induced hepatotoxicity in rats. The pre-treatment with *H. thebaica* extract elevated the hepatic antioxidant system (GSH-Px, GST, and CAT). A reduction in ALT and AST levels and decrease in proinflammatory cytokines (TNF-α and IL-1β) and hepatic MDA levels were detected as compared with the healthy control group (Shehata and Abd El-Ghffar 2017).

The water extract of *H. thebaica* fruits at doses of 20 and 40 mg/kg b.wt. exhibited a significant reduction in the total cholesterol, triglycerides, and LDL concentrations as compared with atorvastatin as the positive control group (40 mg/kg b.wt.). Also, the level of HDL was significantly increased. The histopathological examination of the liver revealed an improvement in the tissue alterations caused by a high level of cholesterol when compared with the control positive group. A significant reduction in liver weight at two doses (20 and 40 mg/kg) by 14.79% and 7.30%, respectively (Alharbi and Sindi 2020). Another report revealed that different fractions of *H. thebaica* fruits (aqueous, 80% methanol, ethyl acetate, chloroform) exhibited hypocholesterolemic effects in vivo at different doses (1.8, 3.5, 4, 2.5, 0.5, 7 and 3 g/kg b.wt.). All fractions significantly reduced total cholesterol and non-HDL cholesterol levels. The fractions improved the lipid profile after 2 weeks of treatment (Hetta and Yassin 2006).

Salihu et al. (2019) reported the in vivo effect of the alkaloids-rich fraction of *H. thebaica* fruits at doses of 100 and 250 mg/kg b.wt. in high fat-fed obese Wistar rats as compared with atorvastatin. The alkaloid fraction significantly increased catalase activity, glutathione peroxidase, and superoxide dismutase levels while MDA (malondialdehyde) and AChE (acetylcholinesterase) levels were significantly decreased. Also, the levels of total cholesterol, triglyceride, and LDL cholesterol were decreased and the level of HDL was increased.

The 70% ethanol extract of the fruits (1.0 g/kg b.wt.) was evaluated in vivo for its neuroprotective effect against Alzheimer's disease (AD) induction. The results of biochemical analyses revealed that the level of reduced glutathione GSH was increased by 39.49%. The lipid peroxidation MDA and AChE levels were significantly decreased as compared with the group with AD-induced group and showed improvement by 66.98% and 58%, respectively. Moreover, the improvement in the total cholesterol level reached 96.59%. On the genetic level, the increase in the 8-hydroxy-2-deoxyguanosine 2-deoxyguanosine (8-OHdG/2-dG) ratio in the AD-induced group was partially improved due to *H. thebaica* extract. AD induction in rats significantly elevated the expression of the amyloid precursor protein (APP) gene in brain tissue which was improved by using *H. thebaica* extract (Farrag et al. 2020).

The aqueous and ethanolic extracts of *H. thebaica* (200 mg/kg) were investigated for hypoglycemic, hypolipidemic, and antioxidant activities in albino rats. The aqueous and ethanolic extracts of *H. thebaica* decreased blood glucose levels (from 382.4 to 145.2–157.4 mg/dL) and HbA1c (from 14.08 to 6.5–6.98 g/100 g). The liver (AST, ALT, and ALP) and kidney function markers were also improved, which was attributed to the antioxidant activity. The lipid profile was enhanced, which was represented by a decrease in LDL and TGs and an increase in HDL (El-Hadary 2022).

Hussein et al. (2022) studied the effect of feeding diabetic rats on *H. thebaica* fruit powder on their lipid profile and vital organ functions. Feeding rats on doum powder for 8 weeks resulted in a significant reduction in blood glucose level (from 649 to 118 mg/dl) and a notable enhancement in lipid profile represented by a decrease in LDL, VLDL, and TG and an increase in HDL compared with control groups. A significant improvement in liver and kidney function markers was observed including ALT, AST, urea, creatinine, and uric acid (Table 4).

Table 4 In vivo studies of *Hyphaene thebaica*

Type of activity	Used part/extract	Used technique	Dose	Potency or efficiency	Ref
Antidiabetic activity	Fruit water–soluble fraction	Alloxan-induced diabetic rats	20 mg/kg b.wt	Improvement in glucose, insulin tolerance, and kidney function as well as, ↓ Glycosylated Hb	(Salib et al. 2013)
	Fruit aqueous extract	STZ-induced diabetic rats	1 g/kg b.wt	Improved hyperglycemia ↓ Serum total lipids, cholesterol, triglycerides, LDL, and HDL	(Tohamy et al. 2013)
	Fruit extract	STZ-induced diabetic nephropathy	150 mg/kg b.wt	↓ Blood glucose, urea, creatinine, cystatin C, and neutrophil gelatinase-associated lipocalin ↑ Insulin and C-peptide levels	(AbdEl-Moniem et al. 2015)
	Fruit aqueous extract	STZ-induced diabetic rats	60 mg/kg b.wt	↓ High blood glucose Enhanced relative expression of insulin	(Shady et al. 2021)
Anti-inflammatory activity	The chloroform and ethanol seed extracts	Anti-inflammatory study in comparison with Aspirin	200 mg/kg b.wt	↓ Inflammation by 27% and 22%, respectively	(Eltayeb et al. 2009)
Protective effects on liver/kidney functions	Fruit extract	Mercuric chloride-induced hepatotoxicity in rats	500, 1000 mg/kg b.wt	↑ Hepatic antioxidant system ↓ Proinflammatory cytokines	(Shehata and Abd El-Ghffar 2017)
Hypocholesterolemic effects	Fruit aqueous extract	High-fat diet model	20 and 40 mg/kg b.wt	↓ Total cholesterol, triglycerides, and LDL concentrations	(Alharbi and Sindi 2020)
	Fruits (aqueous, 80% methanol, ethyl acetate, chloroform)	-		↓ Total cholesterol and non-HDL cholesterol levels Improved the lipid profile after 2 weeks of treatment	(Hetta and Yassin 2006)
Antioxidant and hypolipidemic activities	Fruit alkaloid fraction	High fat–fed obese Wistar rats	100 and 250 mg/kg b.wt	↑ HDL level, CAT, GPx, and SOD ↓ MDA, total cholesterol, triglyceride, and LDL cholesterol	Salihu et al. (2019)
Neuroprotective effect	70% ethanol extract from the fruits	Aluminum chloride–induced Alzheimer's disease	1.0 g/kg b.wt	↑ Glutathione by 39.49% ↓ Lipid peroxidation MDA and AChE levels	(Farrag et al., 2020)

Doum Pharmaceutical Formulations

Recent studies focused on the implication of doum extracts in different formulations. El-Said et al. (2018) prepared encapsulated doum liposomes intending to fortify yogurt products. The high antioxidant activity and high bioavailability were observed in these products. Interestingly, these biological properties were not affected by milk proteins.

Mohamed et al. (2019a) utilized green synthesis to prepare silver nanoparticles of aqueous extract of the doum fruits. Promising biological potentials were revealed including antioxidant, antibacterial, antifungal, cytotoxic, and inhibition of protein kinases.

Other nano-formulae were prepared from doum fruit extracts to include copper oxide, chromium oxide, cerium oxide nanoparticles, and bismuth vanadate nanorods (Mohamed et al. 2021, 2019b, 2020; Ahmed Mohamed et al. 2020). To physically characterize these formulae, different techniques were performed such as X-ray diffraction, Fourier-transformer infrared, and energy dispersive spectroscopy. The prepared doum fruit's formulae demonstrated significant biological properties as antioxidant, antimicrobial, and cytotoxic agents.

Applications in Chemical, Veterinary, and Food Industries

Chemical/Industrial Applications

Mannan polysaccharides are regarded as safe constituents with myriad applications in pharmaceuticals, food industries, cosmetics, and textiles (Singh et al. 2018). The powdered doum seeds were found to be an excellent source of mannan production as compared to commercial mannan. Mannan was extracted using an alkaline solution of NaOH (Gibril et al. 2020). Also, the physical characteristics of doum seeds powder were determined using gravimetric, elemental, microscopical, and X-Ray diffraction analyses.

Bsheer (2020) was able to utilize doum leaves to extract cellulose, which was then chemically converted to carboxymethyl cellulose. This application offers good stability and appropriate production yield when compared to other conventional methods. Additionally, Elnasri et al. (2013) reported the potential of doum fruit to be actively carbonized showing high adsorption properties which could be used as a purification bed of ferrous ions in water treatment systems.

Food Industry Applications

Fortifying foods have gained more attention with the purpose to enhance the health-benefiting effects, especially for children, and women to protect them against malnutrition as well as vitamin and mineral deficiencies (Olson et al. 2021). Doum fruits' powder was involved in bread baking such as toast bread and gluten-free pan. These fortified products were superior to plain white flour bread in terms of nutritive values (proteins, carbohydrates, minerals, and vitamin contents) in addition to the healthy content of antioxidant and antimicrobial compounds (Aboshora et al. 2016; Shahin and Helal 2021; El-Hadidy and El-Dreny 2020). In another study, the doum fruit powder was incorporated to fortify cake and tahina, thus enhancing their sensory, nutritive, and healthy properties (Siddeeg et al. 2019). Other doum food products also include biscuits, crackers, syrups, jelly, and ice creams. Noteworthy, Ismail et al. (2020) reported lower acceptability (65.00%) in the sensory evaluation of ice creams with increasing the percentage of doum fruit syrups and pomegranate peels (5% and 0.5% respectively). A detailed list of the doum-based functional foods is shown in Table 5.

Animal Nutrition

Doum fruits were implicated in some animal/poultry feeding aspects. The dried mesocarp of doum fruits was utilized as a constituent of livestock feed formulations, where it exhibited good nutritive food alternatives in terms of caloric content but was better to be fortified with a protein source (Nwosu et al. 2008). In the same context, Makinde et al. (2018) reported the nutritive use of the dried doum mesocarp as a good substitute for a broiler chicken diet. Nevertheless, the authors recommended first minimizing the anti-nutritional factors mostly present in doum before using it in bird feed resulting in increased WBCs count. The supplementation of the highly nutritive and antioxidant-rich doum powder to the rabbits' diet resulted in a significant increase in their body weight, with improved semen characteristics, sperm concentration and motility, and accordingly higher fertility in addition to decreased abortion and mortality compared to the control groups (Hassanien et al. 2020).

Conclusions, Challenges, and Future Perspectives

The current review represents the first comprehensive review on *H. thebaica* integrating its chemical and biological data in addition to its food and pharmaceutical applications for further exploitation of this interesting plant. Here, we report on the technology of extraction, and analytical methods, in addition to food and industrial applications of *H. thebaica*. It was reported that several technological methods of extraction were adopted

Table 5 Incorporation of Doum (*H. thebaaica*) into food applications

Product	Preparation	The scientific base for doum's incorporation	Product characterization/ investigation	Significance	Reference
Bread	Doum fruit flour partially substituted wheat flour (5–20%) in bread baking	Good nutritive profile (carbohydrates, fibers, vitamins, and minerals) High phenolic and flavonoid content imparting high antioxidant and antimicrobial activity	Sensory and texture profile analysis (TPA) Moisture content, ash content, total protein, crude fiber, and fat content Carbohydrate, mineral content Total phenolic (TPC) up to 33.4 mg GAE/g DW Flavonoid content (TFC) up to 9.48 mg RE/g DW Antioxidant (DPPH)	Nutritive alternative for bread with higher fibers, vitamins, and antioxidant compounds	(Aboshora et al., 2016)
Gluten-free pan bread	Doum fruit flour was added as a supplement to different gluten-free bread formulas	High nutritional value health promotive effect of the rich antioxidant content of phenolics and flavonoids	Physical properties and texture profile analysis (TPA) Color and sensory analysis Chemical composition; moisture content, ash content, total protein, fat content, crude fiber, and carbohydrate content Mineral content	The good component of the gluten-free pan for better nutrition of coeliac disease patients	(Shahin and Helal, 2021)
Toast bread	Doum fruit powder (0–30%) substituted wheat flour	Good nutritive characteristics, to improve physical characteristics	Sensory analysis Proximate analysis; moisture content, ash content, total protein, fat content, crude fiber, and carbohydrate content Mineral analysis (increase in Ca, K, Mg, and Fe)	Enhanced dough properties (development time, dough softening, and water absorption) Nutritive, healthy, and palatable toast	(El-Hadidy and El-Dreny, 2020)
Biscuits	Doum fruit powder (0–10%) was mixed with wheat flour	Good nutritive profile High phenolic and flavonoid content Antioxidant activity	Physical characteristics: diameter, thickness, and spread ratio color and sensory evaluation *Antioxidant assays*: ABTS (up to 18.34 mM Trolox equivalent/100 g) DPPH (EC_{50} up to 5.36 mg/ml) Total polyphenol up to 2.9 mg GAE/g	Improved dough properties (development time, mixing tolerance index, and water absorption) Nutritive and antioxidants properties	(Aboshora et al. 2019)

Table 5 (continued)

Product	Preparation	The scientific base for doum's incorporation	Product characterization/ investigation	Significance	Reference
Biscuits, crackers, and budding	Doum fruit flour	Good nutritive profile High phenolic and flavonoid content Antioxidant activity	Organoleptic and sensory analysis Proximate analysis: moisture content, ash content, total protein, and fat content, crude fiber, and mineral content Carbohydrate content β-carotene (0.502 mg/100 g) Vitamin B complex content (HPLC) (B3, B6, and B1) TPC-TFC (49.82 mg tannic acid equivalent/100 g and 6.98 mg/100 g, respectively) HPLC (19 phenolics and 11 flavonoids) Antioxidant activities (DPPH)	Palatable and nutritive functional food with antioxidant activity	(Aamer 2015)
Tahina and cake	Doum powder replaced wheat flour in cake (5–15%) Doum powder (15–35%) replaced sesame in tahina making	The rich content of nutrients, minerals, and antioxidants	Proximate analysis; moisture content, protein content, fiber content, and ash content minerals, fat content, and total carbohydrate content Sensory evaluation The water absorption capacity (WAC) and fat absorption capacity (FAC)	Excellent supplementation for healthy functional food with high nutritional value	(Siddeeg et al., 2019)
Cake	Doum fruit powder was added (0–20%) to wheat flour	The rich content of nutrients and antioxidants made doum an excellent supplement for cake fortification with vitamins, minerals, and antioxidants	Sensory and texture profile analysis (TPA) Physical measurements: weight, volume, specific volume, and density Rheological properties color evaluation proximate analysis; moisture, protein, total fat, ash, and crude fibers Mineral content Total phenolic content: 34.86–66.92 mg Gallic acid/100 g DPPH antioxidant activity: up to 56.71% inhibition	Highly nutritive cake and health-promoting, with good taste, texture, and color	(Seleem 2015)

Table 5 (continued)

Product	Preparation	The scientific base for doum's incorporation	Product characterization/ investigation	Significance	Reference
Drink, syrup, concentrate, and jelly	Doum fruit aqueous extract	The high level of phenols and flavonoids with high antioxidant and anti-cancer activities, in addition to being a good source for vitamin B complex (B1, B2, B3, B6, and B9)	Sensory evaluation (color, odor, taste, appearance, overall acceptability)	Palatable, nutritive, and protective drinks and sweets Adequate intake of vitamin B complex and antioxidant compounds	(Aamer, 2016)
Ice cream	Doum fruit syrup (fruit bulb soaked in warm water (70 °C) 1 for 1 h, filtered) and concentrated) was added to ice cream	High antioxidant and antimicrobial properties High nutritive properties Good taste and high palatability	Sensory evaluation Chemical analysis: 70% TSS, 53.27% carbohydrates, 12.42% crude fat, 3.86% protein, 1.54% ash content, and 0.27% crude fiber pH and acidity 45.69% DPPH inhibition Mineral content Microbial count	Nutrient-enriched and more palatable ice cream with high antioxidant activity and increased microbial safety Good source of minerals (K, Ca, Na, P, Mg, Fe, and Mn)	(Ismail et al., 2020)

to prepare different extracts from this species. The phytochemical composition of *H. thebaica* was investigated by several analytical methods such as GC–MS, HPLC, UPLC, and NMR analyses. *H. thebaica* extracts showed important in vitro and in vivo biological properties ranging from antidiabetic, anti-inflammatory, antimicrobial, and antioxidant effects. Including the doum in various food products was mainly correlated with improved rheological and physical properties in addition to being fortified with antioxidants. These studies should be up-scaled to find out the possibility for further industrial applications.

The reported biological effects should be further investigated in future studies to highlight the pharmacodynamic and pharmacokinetic parameters involved. Also, molecular docking of *H. thebaica* bioactive compounds should be performed to identify the target proteins related to their biological potential. Moreover, to validate the use of *H. thebaica* formulations, more in-depth biological studies with mechanistic approaches and pre-clinical and toxicological studies must be evaluated. The proteomics profile of doum should be also investigated so the doum fruits can be incorporated into more products based on solid scientific data. Another proposed challenge is to apply response surface methodology (RSM) to improve the phenolic yield of the plant.

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