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Anticancer, Anti-hepatitis A and Other Biological Activities of *Hyphaene Thebaica* and Its Application in Gluten-Free Biscuits

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ABSTRACT

Doum fruits (*Hyphaene thebaica*) are an important source of fiber and have demonstrated effectiveness against various diseases. This research aimed to investigate the properties of *H. thebaica* fruit powder as an antioxidant, antimicrobial, anticancer, antidiabetic, and anti-hepatitis A agent, as well as its application in the production of gluten-free biscuits. Fifteen phenolic acids and nine flavonoid compounds were identified and fractionated using HPLC. *H. thebaica* demonstrated inhibitory effects against both Gram-negative and Gram-positive bacteria. However, only slight inhibition was observed against *Salmonella typhimurium* (13mm). Additionally, antifungal activity against *Candida albicans* and *Aspergillus flavus* showed 15mm and 10mm inhibition zone, respectively. The results showed that the IC_{50} values for α-Amylase and α-Glucosidase inhibition were 18.73 and 2.92µg/ml, respectively. Moreover, *H. thebaica* significantly reduced HCT-116 cancer cell viability at a low concentration (31.25µg/mL). In Vero cells infected with HAV, the viability was reduced with an IC_{50} of 95.58µg/mL, suggesting that *H. thebaica* fruit powder may offer protection to virus-infected cells. Gluten-free biscuits were prepared using different levels of *H. thebaica* powder (25%, 50%, 75%, and 100%), and both sensory and chemical evaluations were performed. The results indicated that *H. thebaica* enhanced the nutritional value, particularly crude fiber, and prolonged shelf life. Furthermore, sensory evaluation revealed that biscuits with up to 50% and 75% H. thebaica were well accepted.

1. Introduction

While many plant-based products have been extensively researched, numerous rare and underutilized plants with unique nutritional and biochemical properties remain largely unexplored. One such plant is the wild doum palm (*Hyphaene thebaica*), which produces edible fruits rich in novel nutraceutical and pharmaceutical compounds. *H. thebaica* has demonstrated significant therapeutic potential, including antioxidant, antimicrobial, and cytotoxic/anticancer properties (Adenowo et al., 2024). Commonly known as the doum palm, *H. thebaica* is a highly nutritious plant indigenous to Egypt and northern Sudan. Its nutritional and medicinal value made it a sacred plant to ancient Egyptians and other African civilizations. Belonging to the *Arecaceae* family, doum is native to northern Africa and also grows in parts of the Arabian Peninsula. The fruit is rich in iron, proteins, carbohydrates, lipids, calcium, and phosphorus. It also contains a wide range of phytochemicals, including flavonoids, essential oils, hydroxycinnamates, amino acids, and niacin (El-Beltagi et al., 2018; Gibril et al., 2020). Despite its widespread traditional use in several African countries, limited scientific information is available on its bioactive components, biological activities, and potential applications (Saber et al., 2023). Studies have shown that *H. thebaica* fruit possesses antimicrobial and antihypertensive activities, largely attributed to its flavonoid content (El-Egami et al., 2001). The synergistic interaction of its phytochemicals and minerals may contribute to the development of innovative medicinal formulations (Amusan et al., 2024). *H. thebaica* fruits are a natural source of antioxidants, antimicrobials, and dietary fiber, offering low cost and nutritionally valuable compounds that are safe for human consumption. Low cost and nutritionally valuable compounds that are safe for human consumption. Their incorporation into food products can enhance

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both quality and functional value. Foods that provide additional physiological benefits beyond basic nutrition are known as functional foods. These foods deliver nutraceuticals with therapeutic effects that may protect against various diseases (Inuwa et al., 2023). According to Kumar et al. (2021a; 2021b), functional foods can offer medicinal properties that guard against both chronic and infectious diseases. The high fiber content of *H. thebaica* fruit powder makes it a promising ingredient for use in baked products such as biscuits and crackers, enabling the development of healthier functional foods (Aamer, 2015). Furthermore, it supports gastrointestinal health by preventing constipation and is regarded as a natural anti-colon cancer agent (Coimbra et al., 2011). Consumer demand for health-oriented bakery products with low sugar, low fat, and high fiber content is on the rise (Villemejane, 2013). Biscuits, in particular, are a widely consumed cereal-based products, valued for their low moisture content, high energy, long shelf life, and ease of handling characteristics that make them suitable for functional food development (Ajila et al., 2008). Integrating gluten free cereals, fruits, and vegetables into extruded gluten-free products that deliver physiologically active compounds presents a significant opportunity for food manufacturers to meet the needs of celiac patients. These consumers often lack access to fiber-rich gluten-free options (Stojceska et al., 2009). Moreover, many researchers believe that natural products, functional foods, and medicinal plants offer promising alternatives for developing new antibacterial agents to combat the rising threat of multidrug-resistant bacteria (Abdallah, 2011). The main objectives of this investigation are to evaluate *H. thebaica* fruit powder as a rich source of dietary fiber with potential antidiabetic, anticancer, and antiviral properties, and to assess its application as a functional ingredient in gluten-free biscuits. Also aims to examine the effect of replacing corn flour with *H. thebaica* powder on the sensory attributes of the resulting biscuits.

2. Materials and Methods

Materials

The powdered epicarp (outer layer) and edible mesocarp of *Hyphaene thebaica* fruits were obtained from the Medicinal and Aromatic Plants Department at the Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Corn flour was purchased from the South Cairo Mills Company, Giza, Egypt. Other ingredients used in biscuit preparation eggs, sugar, shortening, vanilla, and baking powder were sourced from the local market in Giza, Egypt. Solvents and the Folin–Ciocalteu reagent were obtained from El-Nassr Pharmaceutical Chemical Co., Cairo, Egypt. Quercetin, gallic acid, and 2,2-diphenyl-1-p icrylhydr azyl (DPPH) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All microbial strains used in this study, including Gram-negative *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (isolated strain), as well as Gram-positive *Staphylococcus aureus* (ATCC 6538) and *Listeria monocytogenes* (ATCC 19111), and the fungal strains *Candida albicans* (ATCC 14053) and *Aspergillus flavus* (isolated strain), were provided by the Animal Health Research Institute, Agricultural Research Center, Cairo, Egypt. Acarbose was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd., while α-glucosidase (from *Saccharomyces cerevisiae*) was purchased from Sigma-Aldrich. All other chemicals used were of analytical grade. Hepatitis A virus used in this study were provided by the Department of Microbiology, Faculty of Medicine for Girls, Ain Shams University, Cairo, Egypt.

Methods

Chemical composition

Proximate composition

The chemical composition of H. thebaica fruit powder and gluten-free biscuits prepared with 0% (control), 50%, and 75% H. thebaica fruit powder, were analyzed for moisture, protein, ash, crude fiber, and fat content according to the standard methods described by AOAC (2019). Available carbohydrates were calculated by difference.

Total phenolic and flavonoid contents and their fractionation

Total phenolic content (TP) in *H. thebaica* fruit powder was determined using the Folin–Ciocalteu reagent, following the method of Singleton et al. (1999). Results were expressed as milligrams of gallic acid equivalents per 100 grams of dry weight (mg GAE/100g DW). Total flavonoid content (TF) was quantified according to the procedure described by Eghdami and Sadeghi (2010) and expressed as milligrams of quercetin equivalents per 100 grams of dry weight (mg QE/100g DW). The phenolic compound profile of *H. thebaica* fruit powder was analyzed using high-performance liquid chromatography (HPLC) as described by Goupy et al. (1999).

Antioxidant activity

The radical scavenging activity of *H. thebaica* was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, following the method of Gyamfi et al. (1999). The antioxidant capacity was expressed as the percentage of inhibition, calculated using the following equation:

% DPPH (antioxidant activity)= $[(A_c-A_s)/A_c]\times100\%$

Whereas: Ac Control sample absorbance and As Test sample absorbance.

Fractionation of phenolic and flavonoid compounds in *H. thebaica* fruit powder were identified using High-Performance Liquid Chromatography (HPLC), following the method described by Goupy et al. (1999). Standard phenolic acids were prepared by dissolving them in the mobile phase and then injected into the HPLC system. The concentrations of phenolic compounds were determined based on retention time and peak area data.

Antimicrobial activity evaluation

The aqueous extract of *H. thebaica* fruit powder was prepared by soaking the powder in hot water at a ratio of 1:5 (w/v) for 12 hours at a controlled temperature of 22 ± 2 °C, as described by Aamer (2016). The antimicrobial activity of the resulting extract was evaluated against Gram-negative bacteria [*Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (isolated strain)], Gram-positive bacteria [*Staphylococcus aureus* (ATCC 6538) and *Listeria monocytogenes* (ATCC 19111)], and fungi [*Candida albicans* (ATCC 14053) and *Aspergillus flavus* (isolated strain)]. The disk diffusion agar method was employed to assess the antagonistic effects of the aqueous extract, as described by Nurmahani et al. (2012) and Abd-ELmageed et al. (2019). Briefly, 100 µL of the microbial suspension was evenly spread over the surface of an agar plate using a sterile glass rod. After allowing the surface to dry for 5 minutes, sterile filter paper disks (6mm diameter, Whatman No. 1) saturated with 20µL of the *H. thebaica* extract were placed on the inoculated agar surface. Plates were then incubated at 37°C for 24 hours. After incubation, antimicrobial activity was measured as the diameter of the inhibition zone (mm).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the aqueous extract of *H. thebaica* fruit powder was determined by preparing serial dilutions of the extract at concentrations of 200, 100, 50, 25, and 12.5mg/mL. The MIC was defined as the lowest concentration that completely inhibited the visible growth of the tested microorganisms, following the methods described by Greenwood (2007) and Eloff (1998).

Antidiabetic assay

α-Amylase Inhibitory Activity

The α-amylase inhibition assay was carried out using the 3,5-dinitrosalicylic acid (DNSA) method, as described by Wickramaratne et al. (2016). The inhibitory activity was expressed as a percentage of inhibition, calculated using the following formula:

% α-Amylase inhibition=100(Abs 100% Control−Abs Sample)/Abs100% control.

α-Glucosidase inhibitory assay

The α-glucosidase inhibitory activity of the aqueous extract of *H. thebaica* fruit powder was evaluated according to the method described by Pistia-Brueggeman and Hollingsworth (2001), with slight modifications. The percentage of α-glucosidase inhibition was calculated using the following equation:

Glucosidase inhibition % =100-(OD Blank-OD Sample)/OD Blank x 100)

Where: OD Blank is absorbance of Enzyme without samples, but OD Sample is absorbance of enzyme with sample.

One unit of α-glucosidase activity was defined as the amount of enzyme required to catalyze the formation of 1 µmol of *p*-nitrophenol from the substrate *p*-nitrophenyl-α-D-glucopyranoside per minute under the assay conditions.

The IC_{50} value (the concentration of extract required to inhibit 50% of the enzyme activity) was determined by plotting the percentage of inhibition against various concentrations of the extract (ranging from 1.95 to 1000 µg/mL). A regression equation was generated from the dose–response curve and used to calculate the IC_{50} for the different extracts and fractions.

Determination of anticancer impact

The anticancer activity of *H. thebaica* fruit powder extract was evaluated against human colorectal cancer cells (HCT-116) using the MTT assay [3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazoliumbromIde], as described by Ugwu and Conradie (2023). Cancer cells were treated with various concentrations of the extract (ranging from 32.25 to 1000 µg/mL) and incubated for 24 hours. After treatment, MTT reagent was added, and cell viability was measured by recording absorbance at 680 nm. The percentage of cell inhibition was calculated, and the IC_{50} value was derived from the dose–response curve.

Detection of antiviral role

Determination of cytotoxic impact on Vero cells

The maximum non-toxic concentration (MNTC) of *H. thebaica* fruit powder extract on Vero cells was determined to evaluate its potential antiviral properties. MNTC was assessed by plotting the extract concentration on the X-axis and the percentage of cell damage on the Y-axis. This enabled identification of the highest concentration at which the extract maintained host cell viability without any observable cytotoxic effects compared to the untreated control. Additionally, a regression analysis was performed to determine the cytotoxic concentration that reduced cell viability by 50% (CC_{50}), providing a quantitative measure of cytotoxicity (Fouda et al., 2022).

Antiviral activity

Evaluation of anti-HAV activity

The antiviral activity of *H. thebaica* fruit powder against hepatitis A virus (HAV) was evaluated based on its ability to suppress viral infectivity. The difference in optical density between HAV-infected and uninfected cells was used to assess the degree of cellular damage and calculate the anti-HAV effect, following the method described by Fouda et al. (2022).

Preparation of gluten-free biscuits

Gluten-free biscuits were prepared by substituting *H. thebaica* fruit powder for corn flour at levels of 25%, 50%, 75%, and 100%. The base recipe included: 1000 g corn flour, 300g sugar, 300g shortening, 4 eggs, 5g vanilla, and 10g baking powder, following the method of Man et al. (2014) with slight modifications. The dough was divided into two groups:

- Control biscuits prepared with sugar
- Experimental biscuits (containing *H. thebaica* powder) prepared without sugar, based on preliminary sensory evaluations which indicated acceptable taste even without added sugar.

Sensory evaluation of gluten-free biscuits

Sensory evaluation was performed using the 9-point hedonic scale method described by Rathod and Annapure (2017). Ten trained panelists from the Food Technology Research Institute, Agricultural Research Center (Giza), participated in the assessment. Biscuit samples were evaluated for color, appearance, flavor, texture, and overall acceptability, where 1 represented "extremely poor" and 9 indicated "excellent." All samples were coded and presented in a randomized order to minimize bias.

Color measurement of gluten-free biscuits

Color attributes of the gluten-free biscuits were measured using a Hunter Lab color analyzer. Calibration was performed using a standard white tile as the illuminant reference. Measurements were recorded for L^* (lightness), a^* (red-green), and b^* (yellow-blue) values to assess the impact of *H. thebaica* substitution on biscuit color setting, the values L^* (0=black, 100=white), a^* (+value=red, -value=green), and b^* (+value=yellow, -value=blue) (Saricoban and Yilmaz, 2010).

Texture profile analysis (TPA) of gluten free biscuits

Texture analysis of gluten-free biscuits

The texture of gluten-free biscuits was evaluated using a universal testing machine (Conetech, B type, Taiwan), equipped with dedicated software, following the method of Gomez et al. (2007). A 25mm diameter aluminum cylindrical probe was used in a Texture

Profile Analysis (TPA) double compression test. The test was performed at a speed of 1 mm/s, penetrating to 50% of the biscuit's height. Texture parameters such as firmness (N), gumminess (N), chewiness (N), cohesiveness, and springiness were calculated from the TPA curve.

Total Microbial Count

Microbial analysis was conducted on control biscuits and those containing 50% and 75% *H. thebaica* fruit powder. Samples were tested for total bacterial count (TBC) and total yeast and mold count using conventional laboratory methods based on ISO (2013) guidelines. Microbial assessments were performed at storage intervals of 2, 4, and 6 months.

Statistical Analysis

All analytical data were processed using SPSS software version 16.0. Descriptive statistics, including means and standard deviations, were calculated. One-way analysis of variance (ANOVA) followed by a multiple range test was used to determine significant differences among samples. A significance level of $P \leq 0.05$ was considered statistically significant.

3. Results and Discussions

Chemical Composition of *H. thebaica* Fruit Powder

The proximate composition of *H. thebaica* fruit powder is presented in Table 1. The fat content was 0.91%, which aligns with previous findings reported by Nwosu et al. (2008) and Siddeeg et al. (2019), who recorded fat contents of 0.8% and 0.95%, respectively, in *H. thebaica* pulp and palm fruit mesocarp. The moisture content was found to be 11.23%. Protein content was 5.99%, slightly lower than the 6.39% reported by Nwosu et al. (2008) for *H. thebaica* pulp. These differences may be attributed to variations in postharvest handling, cultivar, and regional climatic conditions. Ash content was the highest among the proximate components at 6.65%, although lower than the 8.1% reported by Nwosu et al. (2008). Crude fiber content was 13.13%, suggesting significant potential for application in bakery products to enhance texture, flavor, and nutritional value. The high fiber content also contributes to digestive health and may aid in preventing gastrointestinal disorders, particularly constipation, making it a potential natural anti-colon cancer agent (Siddeeg et al., 2019). The available carbohydrate content was 62.09%, indicating that *H. thebaica* fruit powder could serve as a low-cost alternative carbohydrate source in food formulations. These results support the observations by Aboshora et al. (2014), who described *H. thebaica* as a nutrient-rich fruit with notable functional properties.

Phenolic and Flavonoid Contents of *H. thebaica* Fruit Powder

Table 1 also presents the total phenolic and flavonoid contents and antioxidant activity of *H. thebaica* fruit powder. The total phenolic content was 36.00 mg GAE/100g dry weight, while total flavonoids were 15.37mg QE/100g. The DPPH radical scavenging activity was 79.2%. These findings are comparable to those reported by Aamer (2015), who recorded phenolic and flavonoid contents of 49.82mg/100g and 6.98mg/100g, respectively. Additionally, Seleem (2015) reported a higher DPPH antioxidant activity of 86.00%. HPLC analysis revealed the presence of 15 phenolic acids and 9 flavonoid compounds. Among the phenolic acids, *e*-vanillic (65.02mg/100g), vanillic (61.46mg/100g), caffein (49.81mg/100g), and protocatechuic acid (45.94mg/100g) were the most abundant. The lowest concentrations were recorded for *p*-coumaric (14.40mg/100g), caffeic (7.15mg/100g), isoferulic (6.70mg/100g), α-coumaric (5.47mg/100g), and cinnamic acid (2.12mg/100g). Among the flavonoids, hesperidin (29.70mg/100g) and hesperetin (13.52mg/100g) were the most prominent, followed by rutin (9.29mg/100g) and naringin (7.02mg/100g). Quercetin, rosmarinic acid, apigenin, and kaempferol were present in lower concentrations. HPLC also confirmed the presence of 23 polyphenolic compounds, including caffeic acid, protocatechuic acid, rhamnetin, catechin, quercitrin, vanillic acid, kaempferol-3-O-acetyl glucoside, cinnamic acid, apigenin-7-glucoside, intricatin, hydroxytyrosol, luteolin, quercetin, naringenin, kaempferol, vanillic acid 4-β-D-glycoside, coumaric acid, ferulic acid, luteolin-6-arabinose-8-glucose *p*-coumaroyl malic acid, eriocitrin, apigenin, and hesperetin (Hussein et al., 2021). Additionally, chlorogenic acid was reported by Taha et al. (2020). These findings suggest that *H. thebaica* fruit powder is a valuable natural source of antioxidants with potential applications in nutraceuticals, dietary suplements, and functional food products.

Table 1. Proximate composition and Phytochemical of *H. thebaica* fruit powder

Chemical Composition for *H. thebaica* fruit powder	Moisture	Protein	Ash	Crude fiber	Fat	Available carbohydrate
	11.23±0.4	5.99±0.60	6.65±0.35	13.13±0.8	0.91±0.05	62.09±0.5

Total Phenols and Flavonoids and Antioxidant activity			
Phytochemical *H. thebaica* fruit powder	Total phenolic content Equivalent Gallic acid (mg/100g)	Total flavonoids content Quercetin Equivalent (mg/100g)	Antioxidant activity DPPH %
	36.00±0.41	15.37±0.28	79.2±2.35 %

Phytochemical fractionation by HPLC			
Phenols	mg/100g	Flavonoids	mg/100g
Caffein	49.81	Kaempferol	0.44
Isoferuilc	6.70	Hesperetin	13.52
Caffeic	7.15	Naringenin	0.65
p- coumaric	14.40	Quercetin	1.49
Ferulic	30.13	Rosmarinc	1.03
Vanillic	61.46	Rutin	9.29
Catechein	30.44	Hesperidin	29.70
Protocatechuic	45.94	Naringin	7.02
Ellagic	32.61	Apigenin	0.49
α- coumaric	5.47		
e-vanillic	65.02		
p-oh benzoic	33.84		
Salysilic	26.68		
Cinnamic	2.12		
Coumarin	21.06		

Antimicrobial of *H. thebaica* fruit powder Extract

The antimicrobial potential of *H. thebaica* fruit powder aqueous extract was tested against two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19111), two Gram -negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhimurium*), and two fungal strains (*Candida albicans* ATCC 14053 and *Aspergillus flavus*). The results, presented in Table 2, indicated that the aqueous extract exhibited varying degrees of inhibition. The growth inhibition zone diameter for the Gram-positive bacteria were 16.6% for *S. aureus* and 12.2% for *L. monocytogenes*. For Gram-negative bacteria, *E. coli* and *S. typhimurium* showed inhibition zone of 18mm and 13mm, respectively. Fungal strains demonstrated moderate inhibition, with C. albicans and A. flavus exhibiting inhibition 15 and 10mm, respectively. These antimicrobial effects are likely attributed to the water soluble phenolic compounds present in the *H. thebaica* fruit powder aqueous extract, as previously suggested by Hsu et al. (2006). Similar results were reported by Mohamed et al. (2010), who found that *H. thebaica* aqueous extracts inhibited both Gram-positive (*S. aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Salmonella typhi*), although the extract showed only slight inhibition against *L. monocytogenes* and no inhibition against *E. coli.* Additionally, Irobi and Adedayo (1999) demonstrated antifungal activity of *H. thebaica* water extract against several fungal strains, including Aspergillus niger and Candida albicans. Overall, these findings support the hypothesis that *H. thebaica* fruit pulp possesses antimicrobial properties effective against both Gram-positive and Gram-negative bacteria, as well as common pathogenic fungi. Therefore, regular consumption of *H. thebaica* may provide antimicrobial benefits and support general health and well-being (Abdallah, 2021).

Table 2. Antimicrobial of *H. thebaica* fruit powder extract

Strain	Inhibition zone diameter (mm)
Gram-Positive Bacteria	
Staphylococcus aureus (ATCC 6538)	21±0.6
Listeria monocytogenes(ATCC19111)	16±0.4
Gram-negative Bacteria	
Escherichia coli(ATCC25922)	18±0.5
Salmonella typhimurium (isolated strain)	13±0.4
Yeast and Mold	
Candida albicans (ATCC14053)	15±0.5
Aspergillus flavus (isolated strain)	10±0.3

Data are mean ± SD, N = 3

Minimum inhibitory concentration (MIC) of *H. thebaica* fruit powder extract

Table 3 presents the minimum inhibitory concentration (MIC) values of *H. thebaica* aqueous extract against selected microbial strains. The results revealed that *Staphylococcus aureus* and *Salmonella typhimurium* were susceptible to the extract at a concentration of 25mg/mL. In contrast, *Listeria monocytogenes* and *Escherichia coli* required a higher MIC of 50 mg/mL to achieve complete growth inhibition. These differences may be attributed to the structural composition of bacterial cell walls and the nature of the active antimicrobial constituents in *H. thebaica* extract. Despite their relatively thin peptidoglycan layers, Gram-negative bacteria possess an additional outer membrane rich in lipopolysaccharides, which acts as a formidable barrier to many antimicrobial agents. In comparison, Gram-positive bacteria lack this outer membrane but have thicker peptidoglycan layers, which may respond more readily to certain phenolic compounds (Silhavy et al., 2010). Previous studies support these findings. Abdallah (2021) reported that an 80% methanolic extract of *H. thebaica* exhibited strong antibacterial activity against resistant strains, including *Staphylococcus aureus* (MIC = 31.25mg/mL) and the multidrug-resistant Gram-negative bacterium *Proteus mirabilis* (MIC = 250.00 mg/mL). Similarly, Auwal et al. (2013) found that the aqueous extract from *H. thebaica* pericarp had MIC values of 25mg/mL against *S. aureus*, *Streptococcus pyogenes*, and *Salmonella typhi*, and 50mg/mL against *E. coli* and *Shigella dysenteriae*.

Table 3. Minimum inhibitory concentration (MIC) of *H. thebaica* fruit powder extratct

Microorganism	MIC (mg/ml)
Staphylococcus aureus (ATCC6538)	25±2.7
Listeria monocytogenes (ATCC19111)	50±3.1
Escherichia coli (ATCC25922)	50±3.0
Salmonella typhimurium (isolated strain)	25±2.1
Candida albicans (ATCC14053)	25±2.4
Aspergilus flavus (isolated strain)	25±2.7

Antidiabetic assay of *H. thebaica* fruit powder

Diabetes mellitus is a metabolic disorder characterized by impaired glucose and insulin homeostasis, resulting from defects in insulin secretion, action, or both. This leads to chronic hyperglycemia and associated complications (Nyenwe et al., 2011).

α-Amylase Inhibition

α-Amylase is a key digestive enzyme that catalyzes the hydrolysis of starch into simpler sugars. Inhibiting this enzyme slows down carbohydrate digestion and glucose absorption, thereby aiding in blood sugar control especially for individuals with type 2 diabetes (Kumar et al., 2021b; Wang et al., 2021).

The *in vitro* antidiabetic activity of *H. thebaica* fruit powder was evaluated using the α-amylase inhibition assay. The inhibitory potential was compared to that of acarbose, a commercial antidiabetic drug, used as a standard. The assay was conducted using extract concentrations ranging from 1.97 to 1000µg/mL. At the highest tested concentration (1000 µg/mL), *H. thebaica* extract exhibited 85.5% inhibition of α-amylase activity, while acarbose demonstrated 95.0% inhibition. The calculated IC_{50} values (the concentration required to inhibit 50% of enzyme activity) were 18.73µg/mL for *H. thebaica* and 4.32µg/mL for acarbose. These findings indicate that *H. thebaica* possesses significant α-amylase inhibitory activity. As α-amylase plays a major role in the digestive conversion of starch into glucose, its inhibition can delay postprandial blood glucose spikes (Zhu et al., 2020). The results align with those of Albishi and Alsabi (2024), who also reported strong α-amylase inhibition by *H. thebaica* extracts. In conclusion, *H. thebaica* fruit powder demonstrates promising antidiabetic potential, supporting its application as a natural agent in the management of hyperglycemia. Its ability to reduce α-amylase activity may contribute to early stage diabetes mellitus intervention and help minimize associated vascular complications (Shady et al., 2021).

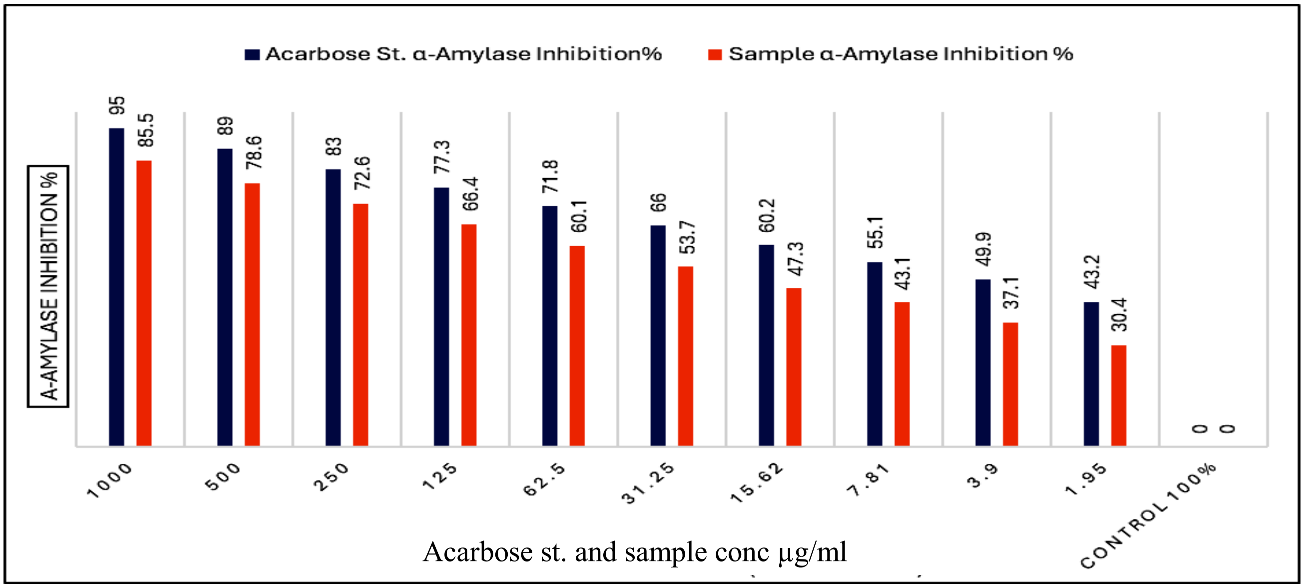

Figure 1. Effect of *H. thebaica* fruit powder and acarbose (control) on α-amylase inhibition

α-amylase calculated IC_{50}% inhibition		
Acarbose	IC_{50}	4.32
H. thebaica	IC_{50}	18.73

IC_{50} (half maximal inhibitory concentration)

α-glucosidase inhibitory

α-Glucosidase is a carbohydrate-hydrolyzing enzyme located on the brush border of the small intestinal mucosa. It plays a key role in the final steps of carbohydrate digestion by hydrolyzing glycosidic bonds to produce absorbable monosaccharides. This enzymatic activity contributes significantly to postprandial blood glucose elevation (Daub et al., 2020; Attjioui et al., 2020). To evaluate the α-glucosidase inhibitory potential of *H. thebaica* fruit powder, an *in vitro* assay was conducted using acarbose as a standard reference inhibitor. A range of concentrations (1.97–1000µg/mL) of the extract was tested. As shown in Figure 2, *H. thebaica* exhibited substantial inhibitory activity, with an IC_{50} value of 2.92 µg/mL, compared to acarbose, which showed an IC_{50} of 1.99µg/mL. These findings suggest that *H. thebaica* fruit powder possesses strong α-glucosidase inhibition comparable to that of the pharmaceutical standard. The observed inhibitory effect may be attributed to the high flavonoid content in the fruit. Oduje et al. (2016) previously reported that aqueous extracts of *H. thebaica* demonstrated significant α-glucosidase inhibition, largely due to their rich flavonoid and phytochemical profiles.

However, contrasting findings have been reported. El-Manawaty and Gohar (2018) found that methanolic extracts of *H. thebaica* flowers exhibited only weak α-glucosidase inhibition, with just 2% inhibition at 25 ppm. On the other hand, Khallaf et al. (2022) demonstrated that ethanolic leaf extracts of *H. thebaica* were highly effective, reporting an IC_{50} value of 52.40µg/mL. These differences may be due to the specific plant parts used (fruit, flower, leaf), extraction solvents, or variations in phytochemical composition. Nevertheless, the present findings support the potential application of *H. thebaica* fruit powder as a natural α-glucosidase inhibitor for managing postprandial hyperglycemia and as a functional food ingredient in diabetic diets.

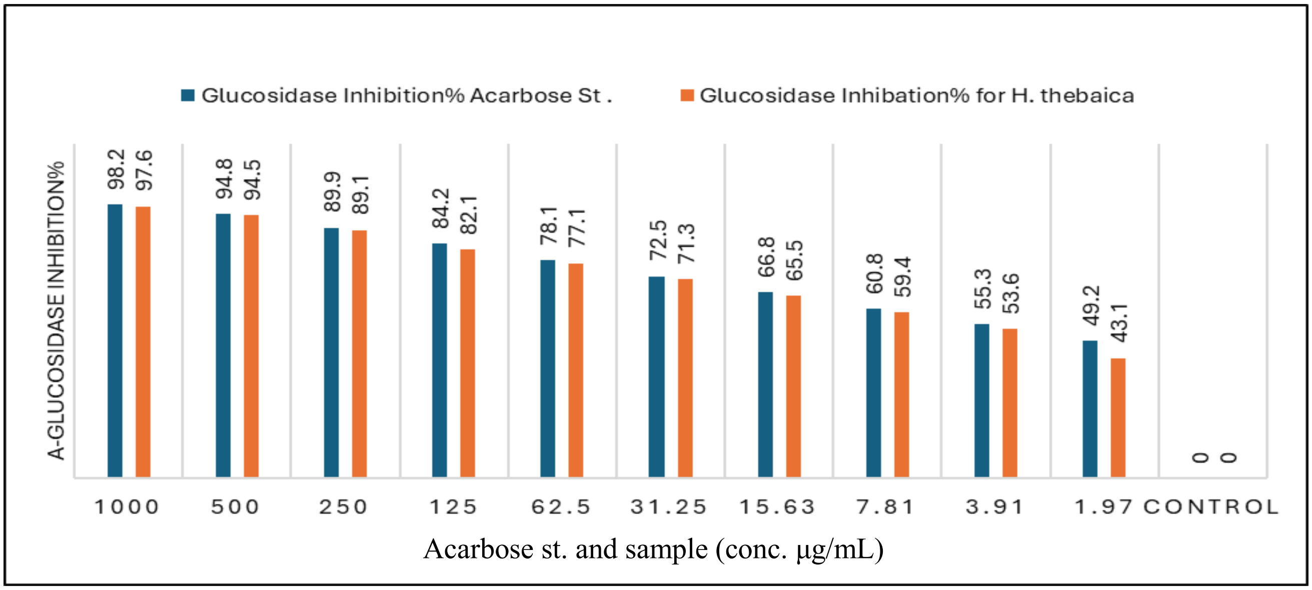

Figure 2. Inhibitory effect of *H. thebaica* fruit powder and acarbose (control) on α-glucosidase

α-glucosidase calculated IC_{50} inhibition		
Acarbose	IC_{50}	1.99
H. thebaica	IC_{50}	2.92

IC_{50} (half maximal inhibitory concentration)

Anticancer Impact

The anticancer potential of *H. thebaica* fruit powder was evaluated using human colorectal carcinoma (HCT-116) cells. The extract exhibited dose-dependent cytotoxicity, as illustrated in Figures 3 and 3a. Notably, significant inhibition of cancer cell viability was observed at lower concentrations (31.25–62.5µg/mL), with an IC_{50} value of 86.26±0.51µg/mL, indicating moderate antiproliferative activity. These findings are consistent with previous reports on *H. thebaica*. Abou-Elalla (2009) reported that *H. thebaica* fruit extract exerted strong cytotoxic effects against acute myeloid leukemia (AML) cells, with an IC_{50} of 3 µg/mL. Similarly, Fayad et al. (2015) found that methanolic extracts of *H. thebaica* bark inhibited the proliferation of several cancer cell lines, including MCF-7 (breast cancer) and A549 (lung carcinoma), with inhibition rates of 87% and 89%, respectively, at a concentration of 100 µg/mL. The same study showed a 28% inhibition of HCT-116 colon cancer cells.

Taha et al. (2020) also demonstrated the cytotoxic potential of *H. thebaica* fruits, where 80% methanolic extracts significantly inhibited HepG-2 (human hepatocellular carcinoma) and A549 (lung carcinoma) cells, with IC_{50} values of 3.07 µg/mL and 2.76 µg/mL, respectively at a concentration of 100µg/mL. The same study showed a 28% inhibition of HCT-116 colon cancer cells. Taha et al. (2020) also demonstrated the cytotoxic potential of *H. thebaica* fruits, where 80% methanolic extracts significantly inhibited HepG-2 (human hepatocellular carcinoma) and A549 (lung carcinoma) cells, with IC_{50} values of 3.07µg/mL and 2.76µg/mL, respectively. The anticancer activity observed in this study may be attributed to the high content of polyphenolic compounds and flavonoids in

H. thebaica fruit powder. As reported earlier, major flavonoids identified include hesperidin, hesperetin, rutin, and naringin. These compounds have been extensively studied for their biological roles, including antioxidant, anti-inflammatory, and anticancer effects (Albuquerque de Oliveira Mendes et al., 2020; Madureira et al., 2023). In particular, hesperidin and naringenin have been shown to modulate apoptotic pathways and suppress tumor proliferation. In conclusion, *H. thebaica* fruit powder demonstrates promising antiproliferative properties against colorectal cancer cells, likely due to its rich phytochemical profile. These findings support its potential as a functional food ingredient with chemopreventive benefits.

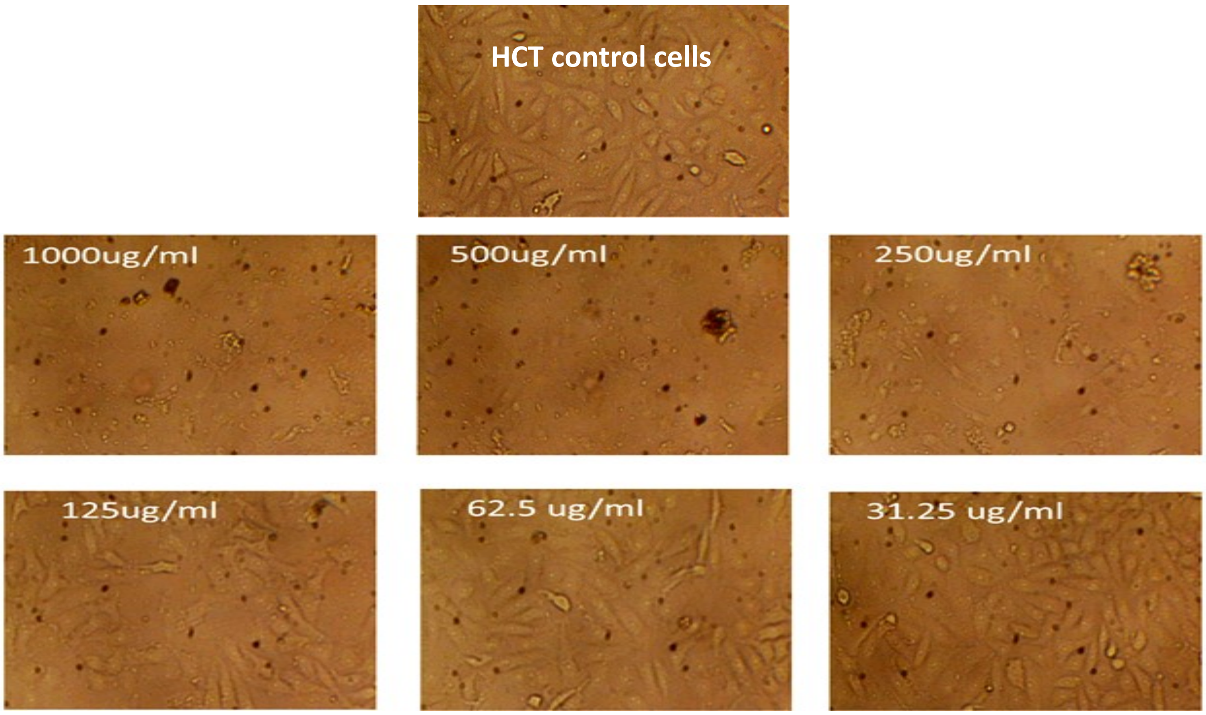

Figure 3. The anticancer impact of *H. thebaica* fruit powder on HCT cells was determined at various levels using an inverted microscope. (Magnification=40X)

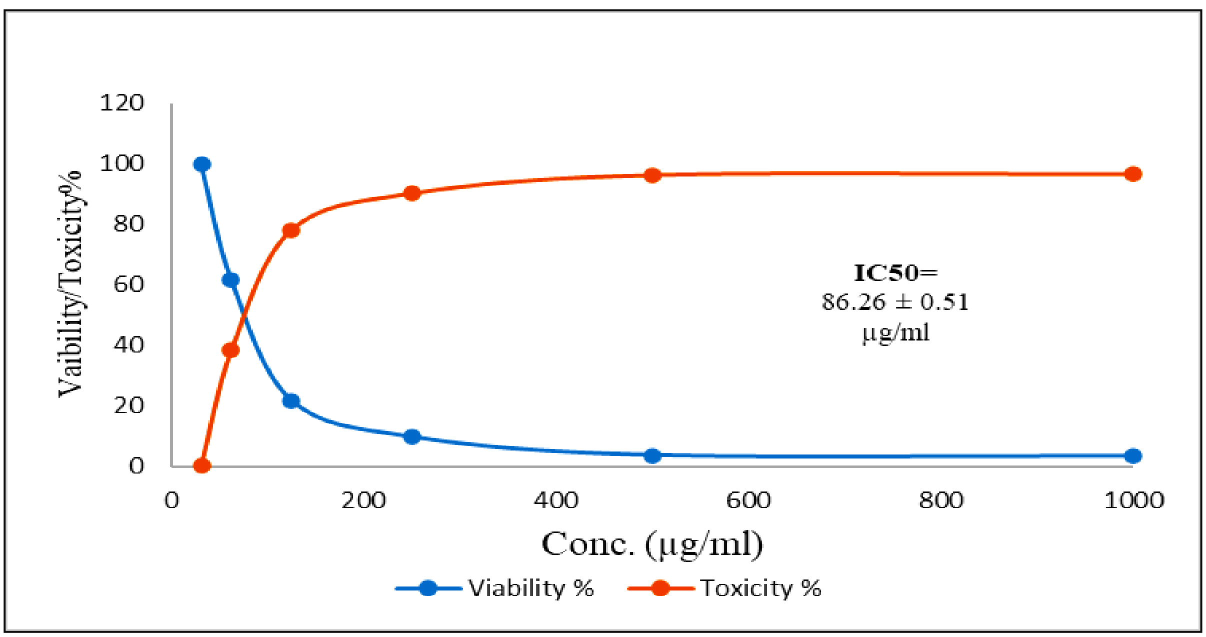

Figure 3a. Statistical analysis for the anticancer impact of *H. thebaica* fruit powder towards HCT cells (data are represented as means ± SD)

Antiviral impact

To investigate the antiviral potential of *H. thebaica* fruit powder, its cytotoxicity toward Vero cells (normal host cells) was initially assessed, and the results are presented in Figure 4. An ideal antiviral agent should demonstrate efficacy against viral infections while exhibiting minimal cytotoxicity to host cells. The cytotoxicity assay revealed that *H. thebaica* fruit powder exhibited dose-dependent toxicity in Vero cells at concentrations ranging from 1000 to 125 µg/mL. The calculated half-cytotoxic concentration (CC_{50}) was 360.41±2.16 µg/mL (Table 4, Figures 4 and 4a). The maximum non-toxic concentration (MNTC) was determined to be 125µg/mL, as no significant cytotoxic effects were observed at this level. Therefore, this concentration was selected to evaluate the antiviral efficacy of *H. thebaica* against the hepatitis A virus (HAV). As shown in Figure 4b, Vero cells infected with HAV and subsequently treated with the MNTC of *H. thebaica* exhibited reduced viral viability compared to untreated infected controls. The extract demonstrated notable antiviral activity with an IC_{50} value of 95.58 ± 1.86 µg/mL, indicating a protective effect on virus-infected cells. This antiviral activity may be largely attributed to the phenolic and flavonoid content of *H. thebaica* fruit powder. Kumar and Goel (2019) stated that phenolic acids have antioxidant, antiviral, and anticancer properties, which may help minimize the risk of illness. The phytochemical analysis revealed the presence of nine flavonoid compounds, with hesperidin, hesperetin, rutin, and naringin being the most prominent. These flavonoids have been previously reported to exert antiviral properties against various viral pathogens, including HSV and SARS-CoV-2 (Choi et al., 2022; Alhemaly et al., 2024).

Table 4. The maximum non-toxic concentration [MNTC] of the sample = 125µg/ml (data are represented as means ± SD)

ID	µg/ml	Viability %	Toxicity %	CC_{50} ± SD
Vero	--------	100.0	0	µg/ml
H. thebaica fruit powder	1000	5.50	94.50	360.41 ± 2.16
	500	20.36	79.64	
	250	73.50	26.50	
	125	99.91	0.09	
	62.5	100.0	0	
	31.25	100.0	0	

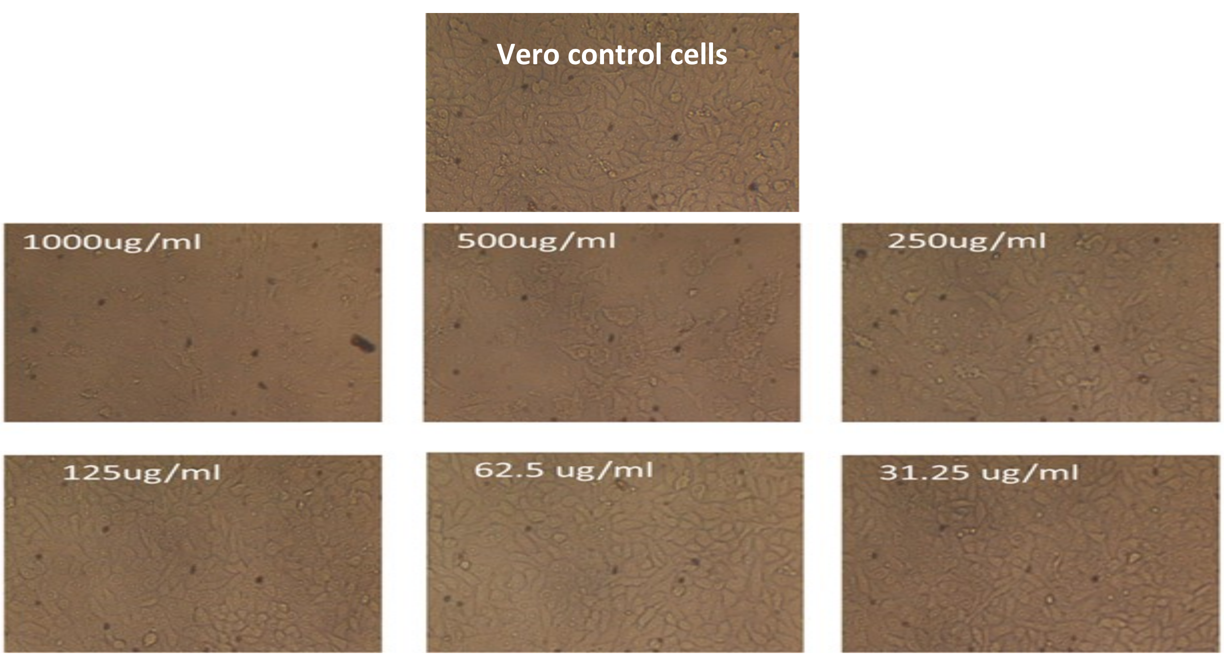

Figure 4. The cytotoxic impact of *H. thebaica* fruit powder towards Vero cells at various levels using an inverted microscope. (Magnification=40X)

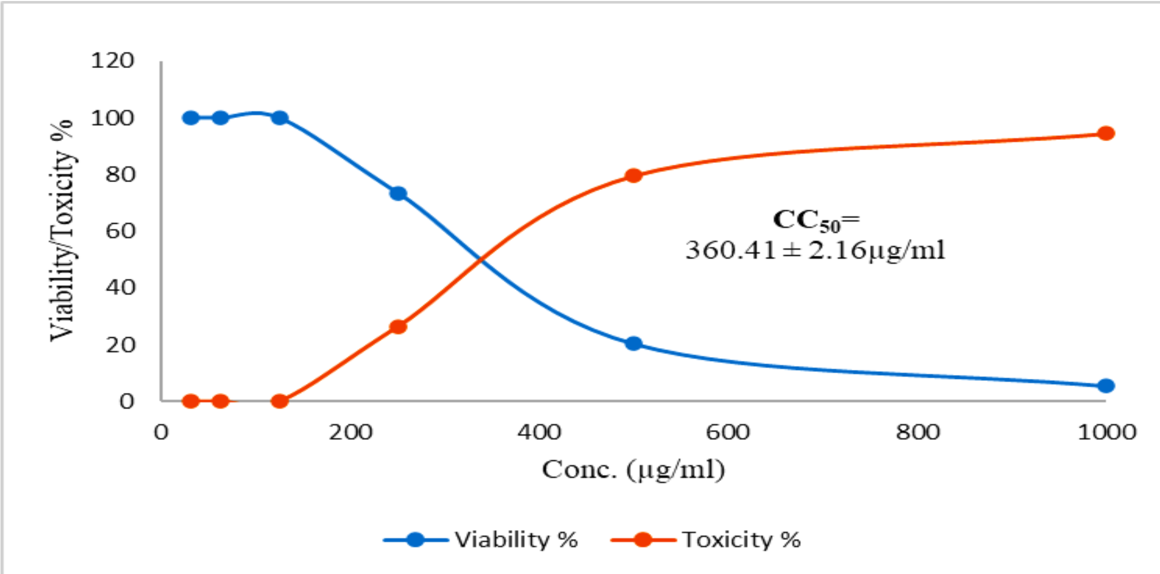

Figure 4a. The cytotoxic impact of *H. thebaica* fruit powder towards Vero cells at various levels using an inverted microscope (data are represented as means ± SD; CC_{50} = 360.41 ± 2.16 µg/ml)

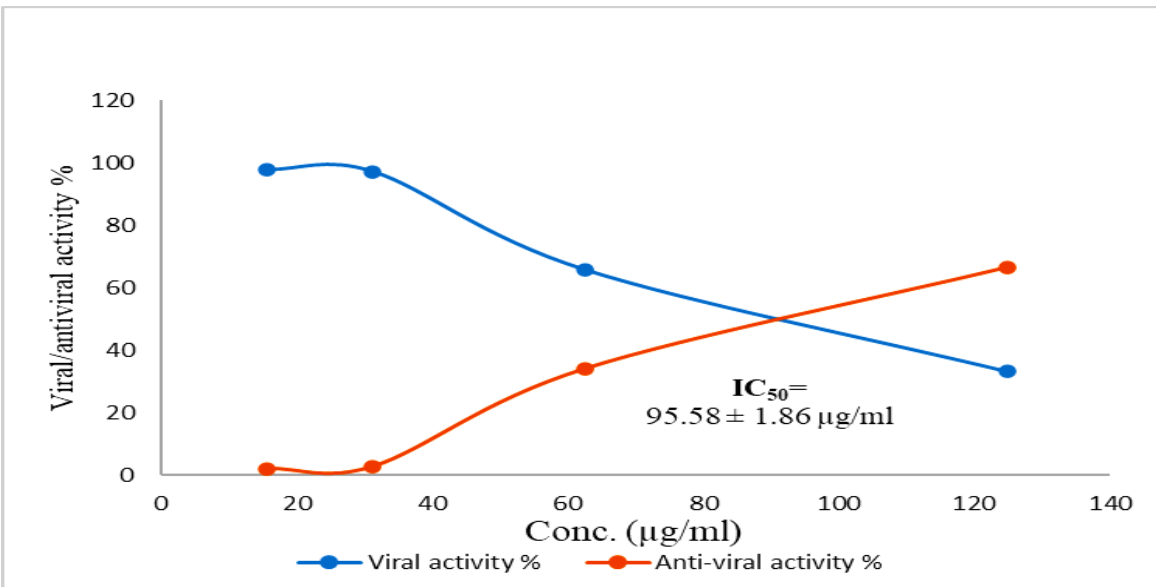

Figure 4b. The antiviral action of *H. thebaica* fruit powder towards HAV (data are represented as means ± SD; IC_{50} = 95.58 ± 1.86µg/ml)

Sensory evaluation of *H. thebaica* fruit powder gluten-free substituted biscuits

The sensory evaluation results for gluten-free biscuits enriched with *H. thebaica* fruit powder are presented in Table 5. The data revealed that biscuit samples incorporating *H. thebaica* powder were highly accepted by panelists up to a 75% substitution level. Compared to the control, biscuits containing 50% and 75% *H. thebaica* showed a favorable increase in taste, flavor, and overall acceptability, which can be attributed to the fruit powder's naturally pleasant sweet flavor profile. However, at 100% substitution, the biscuits exhibited unfavorable sensory characteristics, including a noticeably dry texture and dark color, which negatively affected mouthfeel (taste) and color at this concentration. The brownish-orange hue of the natural pigments in the fruit rind (mesocarp) may contribute to these effects. Additionally, the high polyphenol content (particularly tannins and flavonoids) may undergo Maillard-like reactions during baking, further intensifying the color Ramadan et al. (2019).

Additionally, texture analysis showed that 75% *H. thebaica* inclusion significantly increased biscuit hardness relative to the control. This increase in firmness could be explained by the higher water absorption capacity of the fiber-rich fruit powder, leading to reduced moisture retention and a denser structure (Ajila et al., 2008). These findings are in line with previous studies that employed *H. thebaica* fruit powder in bakery applications. El-Hadidy and El-Dreny (2020) reported improved nutritional value and enhanced antioxidant and antimicrobial properties in toast and gluten-free pan bread supplemented with *H. thebaica*. Similarly, Siddeeg et al. (2019) demonstrated that fortifying cake and tahini with *H. thebaica* improved both their sensory quality and health-promoting properties. Based on the overall sensory performance, biscuit formulations with 50% and 75% *H. thebaica* substitution were selected for further evaluation of chemical composition and microbial stability in comparison with control samples.

Table 5. Sensory evaluation of *H. thebaica* fruit powder gluten-free substituted biscuits

Blends	Color	Texture	Taste	Flavor	Overall acceptability
Control	$9.0^{a}\pm0.71$	$9.0^{a}\pm0.64$	$9.0^{a}\pm0.81$	$9.0^{a}\pm0.85$	$9.0^{a}\pm0.71$
25%	$7.0^{c}\pm0.35$	$7.0^{c}\pm0.32$	$7.0^{c}\pm0.28$	$7.0^{c}\pm0.46$	$7.0^{c}\pm0.34$
50%	$8.0^{b}\pm0.51$	$8.0^{b}\pm0.41$	$8.0^{b}\pm0.46$	$8.0^{b}\pm0.68$	$8.0^{b}\pm0.36$
75%	$8.5^{ab}\pm0.42$	$8.5^{ab}\pm0.39$	$8.5^{ab}\pm0.76$	$8.5^{ab}\pm0.74$	$8.5^{ab}\pm0.52$
100%	$4.0^{d}\pm0.12$	$6.0^{d}\pm0.13$	$5.0^{d}\pm0.14$	$4.0^{d}\pm0.14$	$4.0^{d}\pm0.14$

Values in the same column with different letters are significantly different ($P \leq 0.05$)

Chemical composition of *H. thebaica* fruit powder gluten-free substituted biscuits

Table 6 presents the proximate composition of gluten-free biscuits formulated with 50% and 75% *H. thebaica* fruit powder substitution, compared to control biscuits. The results indicated a gradual increase in moisture content, from 5.29% in control biscuits to 6.06% in biscuits with 75% *H. thebaica*. This increase is likely attributed to the higher water absorption capacity of the fiber-rich *H. thebaica* powder, which requires more water during dough formation (Choudhury et al., 2015). A slight reduction in protein content was observed, decreasing from 5.64% in the control to 5.27% in the 75% substitution level. This may be attributed to the relatively low protein content of *H. thebaica* fruit powder, as previously reported. Ramadan et al. (2019) observed that incorporating 20% *H. thebaica* into biscuits led to a reduction in protein content compared to the control (wheat flour biscuits with 72% extraction). In contrast, the fat content showed a slight increase, rising from 20.56% in the control to 21.45% at the highest substitution level. This variation may be due to a shift in the compositional balance caused by the addition of *H. thebaica*, which, although low in fat, may affect fat retention during baking. The ash content, which serves as an indicator of mineral content, increased significantly with the inclusion of *H. thebaica*, from 0.85% in control biscuits to 5.36% at the 75% substitution level. This is consistent with the findings of Jan et al. (2015), who reported that higher ash content correlates with increased mineral concentrations in functional food products.

Table 6. Chemical composition of selected *H. thebaica* fruit powder gluten-free substituted biscuits

Components	Control	50%	75%
Moisture	$5.29^{b}\pm0.31$	$5.88^{ab}\pm0.23$	$6.06^{a}\pm0.43$
Protein	$5.64^{a}\pm0.26$	$5.44^{b}\pm0.53$	$5.27^{c}\pm0.15$
Fat	$20.56^{b}\pm0.45$	$21.13^{ab}\pm0.14$	$21.45^{a}\pm0.19$
Fiber	$3.22^{c}\pm0.37$	$4.67^{b}\pm0.22$	$5.84^{a}\pm0.29$
Ash	$0.85^{c}\pm0.12$	$3.60^{b}\pm0.67$	$5.36^{a}\pm0.30$
Available Carbohydrate	$64.44^{a}\pm0.23$	$59.28^{b}\pm0.34$	$56.02^{c}\pm0.20$

Values in the same column with different letters are significantly different ($P \leq 0.05$)

Color measurements of selected *H. thebaica* fruit gluten-free substituted biscuits

The color of a food product significantly influences consumer perception and acceptability; therefore, it is essential to assess how formulation modifications affect color parameters. The color characteristics of gluten-free biscuits were evaluated using the Hunter Lab color scale, where L* indicates lightness, a* indicates redness, and b* indicates yellowness. As shown in Table 7, the control biscuits exhibited the highest L* values (lightness) compared to biscuits formulated with *H. thebaica* fruit powder. A consistent decrease in L* values was observed with increasing levels of *H. thebaica* powder, likely due to the natural browning effect of the fruit powder. The a* values (redness) showed a slight increase with higher concentrations of *H. thebaica*, indicating enhanced reddish tones in the final product. Similarly, b* values (yellowness) gradually increased with the addition of *H. thebaica* fruit powder, reflecting a shift toward a more yellow hue. These color changes are attributed to the natural pigments present in *H. thebaica* as well as the Maillard reaction that occurs during baking. These findings are consistent with previous research by Seleem (2015), who reported comparable L*, a*, and b* values in cake samples enriched with *H. thebaica*. Furthermore, it has been well-documented that increased polyphenol content can contribute to darker coloration, aside from Maillard browning reactions during thermal processing (Takeungwongtrakul and Benjakul, 2017).

Table 7. Color parameters of *H. thebaica* fruit powder gluten-free substitute biscuits

Blends	*L**	*a**	*b**
Control	70.23 [a] ± 2.51	12.21 [a] ± 0.07	18.23 [c] ± 0.03
50%	48.00 [b] ± 1.53	13.17 [a] ± 0.09	30.84 [b] ± 0.06
75%	40.21[c] ± 0.04	14.56 [b] ± 0.07	35.39 [a] ± 1.39

Values in the same column with different letters are significantly different ($P \leq 0.05$)

Texture profile analysis of the selected *H. thebaica* fruit powder gluten-free substitute biscuits

Texture is a critical factor influencing the overall eating experience. It refers to the physical characteristics of food that are perceived by the senses of touch, including the fingers, tongue, palate, or teeth (Vaclavik and Christian, 2003). Texture profile analysis was conducted on selected gluten-free biscuit samples substituted with *H. thebaica* fruit powder, and the results are presented in Table 8. The control biscuits exhibited the lowest values for hardness, gumminess, and cohesiveness, recorded at 4.52N, 0.64g, and 0.08, respectively. As the proportion of *H. thebaica* powder increased, these texture parameters progressively rose. Hardness ranged from 10.53 to 12.12 N, gumminess from 1.02 to 1.11g, and cohesiveness from 0.53 to 0.72. Hardness, also referred to as firmness, is a primary sensory characteristic in texture profile analysis and is defined as the force required to achieve a specific deformation (Garrido et al., 2014). The observed increase in hardness with higher *H. thebaica* content may be attributed to the fruit powder's high fiber content, which contributes to a denser and more compact biscuit structure. Additionally, the reduction in free water availability in fiber-enriched formulations further strengthens the texture (Raymundo et al., 2014). Cohesiveness, which reflects the internal bonding strength of the biscuit matrix, also increased with higher *H. thebaica* concentrations. This increase is likely due to the structural integrity imparted by the fruit powder's fibrous components. Higher hardness and cohesiveness levels often correspond to increased gumminess and chewiness, as more energy is required to compress and break the biscuit structure (Wu et al., 2015). Accordingly, both gumminess and chewiness followed the same increasing trend with higher levels of *H. thebaica* substitution.

Table 8. Texture profile of *H. thebaica* fruit powder gluten-free substituted biscuits

Blends	Hardness (N)	Gumminess (g)	Cohesiveness	Chewiness (mJ)
Control	4.52 ^{c} ± 0.15	0.64 ^{c} ± 0.01	0.08 ^{c} ± 0.01	8.52 ^{c} ± 0.19
Blend 50%	10.53 ^{b} ± 0.21	1.02 ^{b} ± 0.03	0.53 ^{b} ± 0.02	12.46 ^{b} ± 0.25
Blend 75%	12.12 ^{a} ± 0.24	1.11 ^{a} ± 0.05	0.72 ^{a} ± 0.02	14.56 ^{a} ± 0.29

Values in the same column with different letters are significantly different (P ≤0.05)

Total microbiological count of selected *H. thebaica* fruit powder gluten-free substituted biscuits during six- month of storage

Table 9 presents the total bacterial, yeast, and mold counts in the control biscuits and those substituted with 50% and 75% *H. thebaica* fruit powder at zero, two, four, and six months of storage. The results indicate that no detectable microbial growth neither bacterial nor fungal was observed during the first two months across all samples. However, by the fourth month, bacterial counts had emerged in the control and 50% *H. thebaica* samples, ranging from 4.50 to 1.20 $\log_{10}$ CFU/g, respectively. Notably, the 75% *H. thebaica* sample exhibited no detectable bacterial growth at this stage. After six months of storage, the bacterial counts slightly increased, reaching 1.78 and 1.65 $\log_{10}$ CFU/g for the 50% and 75% *H. thebaica* biscuits, respectively still significantly lower than the control, which reached 3.80 $\log_{10}$ CFU/g. This marked reduction in microbial load in the *H. thebaica*-enriched biscuits, particularly at the 75% substitution level, can be attributed to the high concentration of bioactive compounds such as phenolics and flavonoids, known for their antimicrobial properties. Similar trends were observed for yeast and mold counts, which followed the same pattern as bacterial growth. These findings suggest that the incorporation of *H. thebaica* not only enhances the antimicrobial potential of the product but also contributes to prolonged shelf life. These results are consistent with those reported by Saber et al. (2023), who highlighted the antibacterial and antioxidant activities of *H. thebaica* flour due to its rich content of flavonoids and other phenolic compounds. Likewise, Abd Rabou and AlSadek (2018) observed that incorporating *H. thebaica* fruit flour into gluten-free cakes improved both nutritional quality and shelf stability, noting that formulations with 30% *H. thebaica* maintained extended microbial safety during storage.

Table 9. Total microbial count of *H. thebaica* fruit powder gluten free substituted biscuits during storage

H. thebaica concentration / Storage period	Total bacterial count (Log_{10} cfu/gm)				Total mold/yeast count (Log_{10} cfu/gm)			
	Zero time	Two Months	Four months	Six months	Zero time	Two Months	Four months	Six months
Control	ND	ND	4.50	6.45	ND	ND	1.27	3.87
50%	ND	ND	1.20	3.80	ND	ND	1.00	1.78
75%	ND	ND	ND	2.85	ND	ND	ND	1.65

4. Conclusion

Hyphaene thebaica (doum palm) fruit powder is a rich source of phenolic and flavonoid compounds that exhibit significant biological activities. The present study demonstrated its potent antimicrobial effects against various bacterial and fungal strains, as well as its notable inhibition of α-glucosidase and α-amylase enzymes, indicating promising antidiabetic potential. Furthermore, *H. thebaica* fruit powder exhibited cytotoxic effects against colon cancer (HCT-116) cells and provided protective effects on virus-infected cells, supporting its potential antiviral activity. Incorporating *H. thebaica* fruit powder into gluten -free biscuit formulations enhanced their antioxidant and antimicrobial properties, improved sensory attributes, and extended product shelf life. Although doum palm may be considered economically costly, it is locally available in Egypt and possesses valuable health-promoting constituents, making it a promising candidate for functional food development.

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