

**Original Article**

**NOVEL FINDINGS OF *PHOENIX DACTYLIFERA*, DATES FRUIT EARLY-STAGE HABABUK FOR PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND CYTOTOXIC POTENTIALS**

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**ABSTRACT**

**Objective:** The present study aims to investigate the phytochemical constituents, antioxidant, and cytotoxic activity of the total ethanolic extract of the hababuk stage of date's fruits *Phoenix dactylifera* (PDHE).

**Methods:** Extracts PDHE obtained with shade dried and a powdered sample of hababuk stage of date fruits *Phoenix dactylifera* and subjected to phytochemical screening, *in vitro* antioxidant activity study by DPPH assay and ABTS assay, *in vitro* cytotoxic studies carried out for three different cell lines Lung Cancer (A549), Human cervical carcinoma (HeLa) and Breast Cancer (MCF)-7 cells by MTT method and brine shrimp lethality assay using standard procedure.

**Results:** The phytochemical study revealed the presence of important constituents like alkaloids, glycosides, phenolic, flavonoids, carbohydrate, proteins, amino acids, terpenoids, steroids, and saponins. The antioxidant studies of the PDHE compared with standard showed very excellent results IC<sub>50</sub> values for DPPH activity and nitric oxide. A good result was also shown by ABTS when compared with that of standard. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. The extract showed excellent LC<sub>50</sub> value, which correlates with cytotoxicity studies previously established. The MTT study results using the three different cell lines compared with the standard doxorubicin, promising results obtained for HeLa cell lines when compared with other cell lines.

**Conclusion:** Results obtained from the present work indicated that the hababuk stage of date fruit is a promising antioxidant and anticancer agent. Further study should be carried out to isolate the bioactive compound responsible for the activity.

**Keywords:** Date fruit, Ethanolic extract, Antioxidant, Hababuk, Brine shrimp, Phytochemical screening.

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**INTRODUCTION**

The potential medicinal properties of plant species have contributed significantly to the advance of abundant herbal therapies for a numeral of ailments across the world. The benefits of herbal medicine over allopathic medicine have aided medicinal plants to regain their reputation in the field of health and medicine [1]. The growth of awareness to cure diseases continued at an accelerating pace and some new plant-derived drugs increased likewise [2]. Medicinal plants are the richest bioresource of drugs in the traditional system of medicine also function as medicaments. These medicinal values of plants lie in some chemically active substance that produces a definite physiological action on the human body. Phytochemicals are biologically active and function to protect against disease and infection. Phytochemicals are broken into different classes, which include flavonoids, carotenoids, chlorophyll, and isothiocyanates [3]. Dates, *Phoenix dactylifera*, fruits historically important types of palm trees because they are a source of food that has supplied nutrition to millions of people over thousands of years. The plant and its various parts were evaluated for several pharmacological studies and found to have medicinal importance. Palm date fruits *Phoenix dactylifera* has been used for nutritional and medicinal purposes in Middle Eastern countries and is used in folk medicine for the treatment of liver diseases and highly recommended to be consumed by pregnant women before and after delivery [4].

The dates are believed to have many medicinal properties for example, to provide strength, fitness, and relief against many diseases and pains, including fever, stomach disorders, memory disturbances, nervous disorders, as well as an aphrodisiac and to boost immunity [5]. Nearly 400 different varieties of dates are available from Saudi Arabia, but only a limited number of date varieties have been utilized for scientific purposes [6]. Dates are of

religious importance for Muslims throughout the world and it is mentioned in many places in the Holy Quran. Dates are very rich in phenolics, in quality and quantity, which opens many fields of investigation in terms of new potential uses. The presence of several reports on the chemical composition and the nutritional value of dates, many other potentialities of the fruits remain to be explored. Many claims report on the antibacterial, antifungal, antitumor, antiulcer, and immunomodulatory properties of dates are reported [7]. Even more medicinal plants are available in the kingdom of Saudi Arabia, Date palm has its own powerful and unique importance. Knowing the useful nutrient and sources of food that should be beneficial for human mankind, we think of doing our research work in this plant. During our survey for the study, we came to know that work has been carried out in fruits, seed, and Dates pollen [8]. Hababuk stage, development of a small green colour fruit after a week of pollination was chosen for our research work and no study has been carried out in this stage before.

**MATERIALS AND METHODS**

**Sampling**

The date's fruits, *Phoenix dactylifera* early-stage hababuk were collected during April 2019 from Unaizah near Buraidah in Al Qassim region, Saudi Arabia. The Sample drug has been identified and authenticated from the College of Food and Agricultural Sciences, King Saud University, Riyadh, Saudi Arabia. The voucher specimen number PD17569 was deposited in the department.

**Chemicals and reagents**

ABTS, 2,2-diphenyl-1-picrylhydrazyl, Sodium nitroprusside, Griess reagent from (Sigma chemicals), Dulbecco's modified Eagles medium, Dimethyl sulphoxide, DMEM (Sigma Aldrich).), the

antibiotic solution containing: Penicillin (100U/ml), Streptomycin, and Amphotericin B from NBS Eppendorf, India, MTT kit from Roche, India, Sodium bicarbonate, potassium persulfate and L-glutamine from Merck India Ltd and HiMedia.

### Extraction

Shade dried and powdered drug of *Phoenix dactylifera*, fruit early-stage hababuk was soaked in 95% ethanol in a round bottom flask and after soaking it for one day, it was refluxed for 3 h and the clear solution will be decanted off. The extraction was repeated thrice, the combined extracts were concentrated to a semisolid consistency. Thus, total ethanolic extract (TEE) was obtained and designated as PDHE (*Phoenix dactylifera* hababuk extract) [9].

### Qualitative chemical test

Various chemical tests were carried out for the PDHE to identify the presence of various phytochemical constituents. Chemical tests for phenolic (Ferric chloride test and Lead acetate test), chemical tests for flavonoids (Aqueous sodium hydroxide test and Shinoda test) and chemical test for carbohydrate (Fehling's Test, Molisch's Test, Benedict's Test, Tollen's test and Iodine test) [10].

### Antioxidant activity studies

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was carried as explained before [11]. Concisely, a 3 ml solution of DPPH (0.1 mmol) and 2 ml extract and standard ascorbic acid of each concentration were mixed and incubated in the dark for 30 min at room temperature. The absorbance of samples, standard, and blank (ethanol instead of DPPH) was measured at 517 nm. The equation given below was used to calculate DPPH radical scavenging activity and linear regression analysis of absorbance against concentration was done to compute EC<sub>50</sub> (representing 50% reduction) for DPPH value.

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of blank}} \times 100$$

The ABTS radical scavenging activity in PDHE was performed according to the method already reported [12]. In this assay, green ABTS<sup>•+</sup> is generated under appropriate oxidant and was blocked in the presence of antioxidants. 1.75 ml of potassium persulfate (2.45 mmol) with 100 ml of ABTS (7 mmol) in the ratio of 625:11 (V/V) were used to prepare ABTS<sup>•+</sup> stock solution. Working solutions were prepared by diluting the stock solution with 0.05M phosphate buffer (pH 7.4) till the absorbance was read at 734 nm. Different concentrations of extract and standard working solutions of 1:19 (V/V), were combined at room temperature and stored for 10 min in dark. Absorbance at taken 734 nm for 30 min against ethanol using Infinite M200 PRO microplate reader. The percentage ABTS radical scavenging activity (%) was calculated using the equation given. Linear regression analysis was done to compute EC<sub>50</sub> expressed as µg/ml. All the samples and standards were assayed in triplicates and averaged.

$$\text{ABTS radical scavenging activity} = \frac{(\text{Average OD of Control} - \text{Average OD of Test})}{\text{Average OD of Control}} \times 100$$

The concentration of the extract required to scavenge 50% of the radicals was calculated by plotting a graph of % inhibition vs concentration [13].

Nitric oxide scavenging assay carried out for PDHE using sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be measured at 546 nm spectrophotometrically in the presence of Griess reagent (1% sulphanilamide, 2% Phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) which convert nitrite into the deep purple azo compound. The scavengers of nitric oxide reduce the production of nitric oxide [14]. All the samples and standard were assayed in triplicates and averaged.

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

### Acute cytotoxic by brine shrimp lethality assay

Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conically shaped vessel (1L), filled with sterile artificial

seawater under constant aeration for 48 h. After hatching, active nauplii free from eggshells were collected from the brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml of the extract was added to 4.5 ml of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted with a hand lens. Experiments were conducted along with control (vehicle-treated), different concentrations (100-1000 µg/ml) of the substances in a set of three tubes per dose. Based on the percent mortality, the LD<sub>50</sub> of the test compound was determined using the probit scale [15].

### Antiproliferative by MTT assay

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent (isopropanol) and the released; solubilized formazan reagent is measured spectrophotometrically. Optical density will be read at 540 nm using DMSO as blank.

All the cell lines A549 (lung cancer), HeLa (cervical cancer), and MCF-7 (Breast cancer) cell lines were initially procured from National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's modified Eagles medium, DMEM. The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate, and the antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37 °C in a humidified 5% CO<sub>2</sub> incubator. Two days old confluent monolayer of cells were trypsinized and the cells were suspended in a 10% growth medium, 100 µl cell suspension (5x10<sup>3</sup> cells/well) was seeded in 96 well tissue culture plate. 1 mg of sample was weighed and dissolved in 1 ml DMEM using a cyclomixer. The sample PDHE solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. After 24 h the growth medium was removed, freshly prepared each compound in DMEM were five times serially diluted by two-fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500 µl of DMEM) and each concentration of 100 µl was added in triplicates to the respective wells and incubated at 37 °C in a humidified 5% CO<sub>2</sub> incubator. Non treated control cells were also maintained.

The entire plate was observed after 24 h of treatment in an inverted phase-contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation was recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation, and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

After the incubation period, the supernatant was removed and 100 µl of MTT solubilization Solution (Dimethyl sulphoxide, DMSO) was added and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm [16]. The percentage of growth inhibition was calculated using the formula:

$$\text{Percentage of viability} = \frac{\text{Mean OD samples}}{\text{Mean OD of the control}} \times 100$$

### Statistical analysis

Data were presented as mean±Standard Deviation for triplicates. For detailed information on data set and to gain insight into the distribution of phytochemicals and their bioactivities. The IC<sub>50</sub> values for all the extracts were determined by ED<sub>50</sub> PLUS V1.0 Software [17].

## RESULTS AND DISCUSSION

### Preliminary phytochemical evaluation

The Preliminary phytochemical evaluation of the PDHE showed the presence of alkaloids, glycosides, phenolics, flavonoids,

carbohydrates, proteins, amino acids, terpenoids, steroids, and saponins. The results of each chemical tests are recorded and tabulated in the following table 1. The phytochemicals present in the PDHE justified the results obtained for antioxidant and cytotoxicity evaluation. The antioxidant activity studies like DPPH, ABTS, and Nitric oxide were carried for the ethanolic extract of *Phoenix dactylifera* hababuk extract, PDHE. Acute cytotoxic study of the extract was done by Brine shrimp lethality assay and MTT assay by using A549, HeLa, and MCF-7 cancer cell lines were examined. The qualitative analysis of the PDHE extracts evidenced that the immature fruits contain most of the important bioactive principles, which readily accounts for its folklore medicinal claims [18].

**Table 1: Phytochemical evaluation of PDHE**

Phytochemicals	Inference
Alkaloids	+
Glycosides	+
Phenolic	+
Flavonoids	+
Carbohydrates	+
Proteins and amino acids	+
Terpenoids	+
Steroids	+
Saponins	+

+indicates the phytochemicals present.

#### Antioxidant activity

The antioxidant activity of PDHE extracts was evaluated for its various antioxidant studies like DPPH assay, ABTS assay, and Nitric

oxide scavenging assay was carried out. Because a single assay is not satisfactory to evaluate the free radicals scavenging capability of all antioxidants. Since oxidative stress has been implicated in the pathology of many diseases like cancer, the results propose the potential of these plants in the treatment of cancer. Medicinal plants, being the potential source of natural antioxidants, have been widely studied because some synthetic antioxidants are harmful to human health [13]. The scavenging activities have expressed the concentration of an inhibitor where the response is reduced by half ( $IC_{50}$ ). A sample extract with the lowest  $IC_{50}$  value had strong antioxidant activity.

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 516 nm induced by antioxidants. DPPH assay is one of the most simple and commonly used antioxidant techniques [19] that estimates the capacity of antioxidant compounds to react and scavenge the DPPH free radical [20]. In the studied sample, the  $IC_{50}$  value for DPPH activity was 21.403 and for standard ascorbic acid 16.7741  $\mu\text{g/ml}$  respectively. The results obtained for the extract are excellent when compared with the standard ascorbic acid. The PDHE was the total ethanol extract and not the fractionated one or isolated compound, even showing almost equal activity that of the standard was depicted in table 2. This indicates that the hababuk stage has significant potential to scavenge free radicals [21].

According to previous studies [22], ABTS assay was useful in both organic and inorganic solvent systems to evaluate the antioxidant capacity and was considered a better method compared to DPPH assay. The  $IC_{50}$  value 877.358  $\mu\text{g/ml}$  obtained for the extract and the standard ascorbic acid 189.798  $\mu\text{g/ml}$  respectively, were depicted in table 3. Further antioxidant capacity was carried out by nitric acid scavenging assay.

**Table 2: Antioxidant activity by DPPH assay**

Sample	Sample concentration ( $\mu\text{g/ml}$ )	Optical density*	% Inhibition	$IC_{50}$ ( $\mu\text{g/ml}$ )
Control	-	0.9527	100	16.7741
Ascorbic acid (standard)	12.5	0.6040 $\pm$ 0.0105	36.60 $\pm$ 0.0061	
	25	0.2670 $\pm$ 0.0223	71.23 $\pm$ 0.0129	
	50	0.1930 $\pm$ 0.011	78.58 $\pm$ 0.0063	
	100	0.1070 $\pm$ 0.0113	88.24 $\pm$ 0.0066	
	200	0.0470 $\pm$ 0.0091	94.85 $\pm$ 0.0053	
Control	-	0.9579	100	21.4053
PDHE	12.5	0.7067 $\pm$ 0.0116	25.44 $\pm$ 0.0067	
	25	0.38 $\pm$ 0.0036	59.91 $\pm$ 0.0021	
	50	0.2197 $\pm$ 0.0057	76.82 $\pm$ 0.0033	
	100	0.1794 $\pm$ 0.0011	81.07 $\pm$ 0.0006	
	200	0.1307 $\pm$ 0.0011	86.21 $\pm$ 0.0006	

\*expressed as mean $\pm$ SD, n=3

**Table 3: Antioxidant activity by ABTS assay**

Sample	Sample concentration ( $\mu\text{g/ml}$ )	Optical density*	% Inhibition	$IC_{50}$ ( $\mu\text{g/ml}$ )
Control	-	0.9527	0.00	189.798
Ascorbic acid (standard)	125	0.624 $\pm$ 0.0272	35.13 $\pm$ 0.0158	
	250	0.305 $\pm$ 0.0079	68.29 $\pm$ 0.0046	
	500	0.197 $\pm$ 0.0281	79.52 $\pm$ 0.0163	
	1000	0.097 $\pm$ 0.0121	89.91 $\pm$ 0.0070	
	2000	0.023 $\pm$ 0.0043	97.60 $\pm$ 0.0025	
Control	-	0.962	100	877.358
PDHE	125	0.724 $\pm$ 0.0261	24.26 $\pm$ 0.0152	
	250	0.6 $\pm$ 0.0125	37.23 $\pm$ 0.0072	
	500	0.521 $\pm$ 0.02	45.50 $\pm$ 0.0116	
	1000	0.46 $\pm$ 0.0115	51.88 $\pm$ 0.0067	
	2000	0.21 $\pm$ 0.0130	78.03 $\pm$ 0.0076	

\*expressed as mean $\pm$ SD, n=3

The Nitric oxide assay is one of the most extensively utilized methods to determine the antioxidant potential. A potential determination of oxidative damage is the oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments. Nitric oxide or reactive nitrogen species formed during its reaction with oxygen or with superoxides such as NO<sub>2</sub>, N<sub>2</sub>O<sub>4</sub>, N<sub>3</sub>O<sub>4</sub>,

nitrate, and nitrite are very reactive. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. This assay offers the ability of sample extract to scavenge 452.017 and for the standard gallic acid 140.713 µg/ml [23].

**Table 4: Antioxidant activity by Nitric oxide scavenging assay**

Sample	Sample concentration (µg/ml)	Optical density*	% Inhibition	IC <sub>50</sub> (µg/ml)
Control	-	0.9527	0.00	140.713
Gallic Acid (standard)	125	0.05685±0.0009	33.19±0.0005	452.017
	250	0.0416±0.0041	51.11±0.0024	
	500	0.0203±0.0017	76.14±0.0010	
	1000	0.013±0.0013	84.72±0.0007	
	2000	0.0049±0.0007	94.24±0.0004	
Control	-		100	
PDHE	125	0.0684±0.0040	22.53±0.0023	
	250	0.0578±0.0043	34.54±0.0025	
	500	0.041±0.0072	53.56±0.0041	
	1000	0.03±0.0006	66.02±0.0003	
	2000	0.0218±0.0050	75.31±0.0029	

\*expressed as mean±SD, n=3

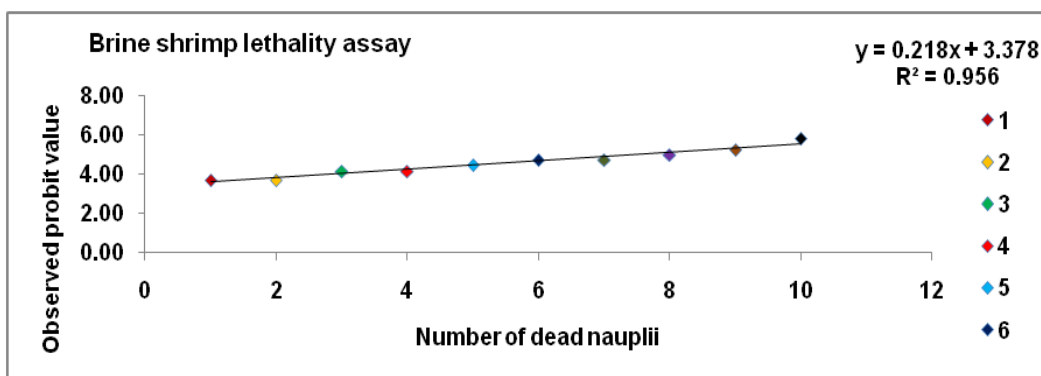
**Table 5: Brine shrimp lethality assay concentration, number of live nauplii, number of dead nauplii, probit value, and LC<sub>50</sub>**

Concentration of PDHE (µg/ml)	Number of nauplii live	Number of nauplii dead	Observed probit values	LC <sub>50</sub> (µg/ml)
Control	9	1	-	34.50 µg/ml
100	8	2	3.72	
200	8	2	3.72	
300	7	3	4.16	
400	6	4	4.16	
500	5	5	4.48	
600	5	5	4.75	
700	4	6	4.75	
800	3	7	5	
900	2	8	5.25	
1000	1	9	5.84	

**Acute cytotoxicity by Brine shrimp lethality assay**

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases compares practically well with cytotoxic and antitumor properties. In the present study, the brine shrimp lethality of extract PDHE was determined using the procedure of the previous study [24]. The LC<sub>50</sub> values of the brine shrimp obtained for extracts and that of the control are given in table-5. Ethanolic extract of PDHE showed the most prominent LC<sub>50</sub> values 34.50 µg/ml. The degree of lethality was found to

be directly proportional to the concentration of the extract. Maximum mortalities took place at a concentration of 1000 µg/ml, whereas the least mortalities were at 100 µg/ml concentration compared to that of control. The LC<sub>50</sub> values of the plant extracts were obtained by a plot of the percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line was obtained from the data using regression analysis. This significant lethality PDHE extracts to brine shrimp are indicative of the presence of potent cytotoxic components, which warrants further investigation. Fig. 1 illustrates the calculation of dead nauplii against observed probit value.



**Fig. 1: The LC<sub>50</sub> calculation graph number of dead nauplii against observed probit value**

**In vitro cytotoxic activity study by MTT assay**

The antiproliferative study of the PDHE of the Plant *Phoenix dactylifera* using different cancer cell lines particularly to discover the novel anticancer drugs. MTT assay is an established method of determining the antiproliferative and cytotoxic activity [25, 16]. The current study investigated the antiproliferative activity of date fruit early-stage hababuk, *Phoenix dactylifera*, PDHE in A549 (Lung Cancer), HeLa (Cervical cancer) and MCF (Breast Cancer). The methodical evaluation of detailed cytotoxicity cell proliferation

effects in human cancer cells revealed that all compounds have dose-dependent cytotoxicity cell growth inhibitory activity. The cytotoxicity test performed using MTT assay methods shows a significant reduction of yellow tetrazolium dye to a purple formazan product, as shown in table 6, 7, and 8. This indicates the ability of the PDHE extract to induce cytotoxicity and decrease cell lethality. Considering the results obtained, it can confirm that the plant contains essential phytochemical constituents and possess active antioxidant and cytotoxic property. However, a majority of the extracts confirmed to be toxic and thus possess anticancer activity.

**Table 6: MTT assay of A549 cell lines**

Sample	Sample concentration (µg/ml)	Optical density*	% viability*	LC <sub>50</sub> (µg/ml)
Doxorubicin	6.25	0.3764±0.0008	59.18±0.0004	5.27961
	12.5	0.2978±0.0004	67.70±0.0002	
	25	0.1911±0.0005	79.25±0.0003	
	50	0.1445±0.0019	84.33±0.0011	
	100	0.1028±0.0007	88.88±0.0045	
PDHE	6.25	0.7972±0.007	6.897±0.0043	139.577
	12.5	0.7135±0.006	16.67±0.0038	
	25	0.6804±0.008	20.53±0.0048	
	50	0.6111±0.007	28.63±0.0045	
	100	0.5426±0.002	36.62±0.0012	

\*expressed as mean±SD, n=3

**Table 7: MTT assay of HeLa cell lines**

Sample	Sample concentration (µg/ml)	Optical density*	% viability*	LC <sub>50</sub> (µg/ml)
Doxorubicin	6.25	0.477±0.0065	59.08±0.0037	5.28854
	12.5	0.476±0.0282	65.54±0.0164	
	25	0.283±0.0011	74.07±0.0006	
	50	0.193±0.0054	82.32±0.0031	
	100	0.163±0.0008	85.05±0.0005	
PDHE	6.25	1.013±0.0137	7.34±0.0079	47.1542
	12.5	0.986±0.001	9.78±0.0005	
	25	0.704±0.0029	35.53±0.0017	
	50	0.546±0.0023	50.05±0.0013	
	100	0.505±0.0033	53.72±0.0019	

\*expressed as mean±SD, n=3

The selection of the various cell lines like A549 (lung cancer), HeLa (human cervical cancer), and MCF-7 (breast cancer) for our study to identify which cell lines help us to continue our work for the further. The results obtained were 5.27961, 5.28854, and 6.26755 µg/ml for the standard doxorubicin and 139.577, 47.1542, and 68.1826 µg/ml for A549, HeLa, and MCF-7 respectively. Promising results obtained for HeLa cell lines when compared with that of the standard. Our results seem consistent

with the correlation previously established between cytotoxicity and brine shrimp lethality in plant extracts. All the activity study results showed the importance of the extract for further development for the anticancer research. This preliminary study given by the cell lines is excellent even though our extract is the total extract and not a fraction or isolated compound. The activity study showed good results confirming the presence of rich phytoconstituents in the hababuk stage of the fruit.

**Table 8: MTT Assay of MCF-7 cell lines**

Sample	Sample concentration (µg/ml)	Optical density*	% viability*	LC <sub>50</sub> (µg/ml)
Doxorubicin	6.25	0.797±0.0074	6.89±0.0004	6.26755
	12.5	0.713±0.0065	16.67±0.0038	
	25	0.680±0.0083	20.53±0.0048	
	50	0.611±0.0078	28.63±0.0045	
	100	0.54266±0.0020	36.62±0.0012	
PDHE	6.25	0.376±0.0008	59.18±0.0004	68.1826
	12.5	0.297±0.0004	67.70±0.0002	
	25	0.1911±0.0005	79.27±0.0003	
	50	0.144±0.0019	84.33±0.0011	
	100	0.102±0.0007	88.85±0.0004	

\*expressed as mean±SD, n=3

**CONCLUSION**

The phytochemical evaluation confirmed the presence of alkaloids, glycosides, phenolic, flavonoids, carbohydrates, proteins, amino acid,

terpenoids, steroids, saponins. The antioxidant activity studies DPPH assay, ABTS assay, and nitric oxide also showed excellent results due to the high number of potential constituents. It may be prudent to further explore the hababuk extract for its usefulness in the

oxidative stress-induced disorders and cancer. Based on the results, it can be concluded that the extract PDHE exhibits varying degrees of cytotoxicity activities against brine shrimp larvae. Finally, the cytotoxicity test performed using MTT assay methods using different cancer cell lines A549, HeLa, and MCF-7, HeLa cell lines showed a significant reduction of yellow tetrazolium dye to a purple formazan product. This indicates the ability of the PDHE extract to induce cytotoxicity and decrease cell viability in cervical cancer.

Considering the results obtained, it can be concluded that the plant contains essential phytochemical constituents and possess active antioxidant and cytotoxic property. Thus, it can be attributed that the presence of these active components can be the basis of the aforesaid properties. Further studies would be required to isolate the bioactive compounds which are responsible for the antioxidant and anticancer activity and determine their underlying molecular mechanism of action to find out novel lead candidates. All the studies were very interesting and recommending in such a manner to continue for our further research work.

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Nil

#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICT OF INTERESTS

Declared none

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