DTI
Drug Target Insights 2024; 18: 84-93
ISSN 1177-3928 | DOI: 10.33393/dti.2024.3169
ORIGINAL RESEARCH ARTICLE

Cytotoxic activity, selectivity, and clonogenicity of fruits and resins of Saudi medicinal plants against human liver adenocarcinoma

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ABSTRACT

Background: Edible fruits and resins provide various benefits to mankind including potential medicinal applications. This study aimed to determine the cytotoxicity, selectivity, and clonogenicity of fruits and exudates of certain Saudi medicinal plants (*Anethum graveolens* (BEP-09), *Opuntia ficus-indica* (L.) Miller (BEP-10), *Boswellia serrata* Roxb. ex Colebr. (BEP-11), and *Commiphora myrrha* (BEP-12)) against human liver adenocarcinoma (HepG2).
Methods: Initial cytotoxicity and cell line selectivity against different cell lines were screened using MTT assay. The most promising extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to determine the main phytoconstituents. Clonogenicity was checked for the most active extract.
Results: The selected plants' fruits and resins possess a significant cytotoxic activity estimated as IC_{50}. The fruit of BEP-10 was found to be the most active extract against liver cancer cells (IC_{50} = 2.82) comparable to both doxorubicin (IC_{50} = 1.40) and camptothecin (IC_{50} = 1.11). It showed a selectivity index of 4.47 compared to the normal human foetal lung fibroblast (MRC5) cells. BEP-10 showed a dose-dependent clonogenic effect against HepG2 cells comparable to the effect of doxorubicin. The GC-MS chromatogram of BEP-10 extract revealed the presence of eight small polar molecules, representing 73% of the total identified compounds and the rest three molecules (27%) were non-polar constituents. The furan derivatives represent the chief components in BEP-10 (16.3%), while the aldehyde 5-(hydroxymethyl)-2-furancarboxaldehyde was found to be the main molecule (13.2%).
Conclusion: The fruits of BEP-10 have a potential cytotoxic effect particularly against HepG2. The identified phytoconstituents in the tested plant extract might contribute to the investigated cytotoxic activity.

Keywords: Clonogenicity, Cytotoxicity, MTT assay, *Opuntia ficus-indica*, Saudi plants, Selectivity index

Introduction

Anethum graveolens L. is a member of the Apiaceae family locally known as Shabat-sanout. This plant has a long history of use as a spice in our food, where its seeds and leaves are used as flavouring agents. It is an erect, robust, and rather glabrous annual aromatic herb. The leaves are three to four pinnate, with the ultimate segments narrowly linear to filiform. The flowers are yellow, and appear in umbels, with an elliptic cremocarp. It has been recognized in different systems of traditional medicine for the treatment of different diseases and ailments of humans. The plant is used as an antispasmodic, carminative, and anti-inflammatory. It is also used as medicine for loss of appetite, cough and cold, menstrual cramps, liver problems, oral care, strengthening the immune system, protection against bone degradation, and urinary tract disorders (1). The antioxidant and anticancer activities of *A. graveolens* were investigated in human,

Received: June 16, 2024
Accepted: September 18, 2024
Published online: October 22, 2024

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lung, breast, and cervical carcinoma cell lines (2-4). Nam et al (5) studied the anti-inflammatory and protective properties of *A. graveolens* (dill seeds) on oesophageal mucosal damage in rats induced by reflux esophagitis and revealed good physiological activity and the possibility of being used as a medicinal, food, and functional resource for the prevention and therapy of gastro-oesophageal disorders. A systematic review and meta-analysis of randomized controlled trials investigated the effects of *A. graveolens* (dill) supplementation on lipid profile and glycaemic control, showing that *A. graveolens* could provide favourable effects on insulin resistance and serum low-density lipoprotein (6). The anthelmintic action of *A. graveolens* essential oil was found to be a promising alternative in the control of sheep gastrointestinal nematodes (7). Khare (8) reported that it was used for eye problems.

Opuntia ficus-indica (L.) Miller is a member of the family of Cactaceae locally known as ElBarshoumy–El TeenElShawki. It is a shrub or arborescent. Leaves are subulate and deciduous. Fruits are ellipsoidal or obovoid, red, yellow to orange, fleshy, edible. The plant is widely distributed in the south and the southwest of Saudi Arabia. It is widely known for its beneficial properties (9). Historically it was used as food for humans and farm animals and in folk medicine due to its nutritional properties and beneficial activities (10). Traditional medicine has used many plant extracts for human and animal wellness, due to their beneficial properties in wound healing and skin. In this regard, the study of Trombetta et al (11) is most helpful. Traditionally it was used as a treatment for gastritis, hyperglycaemia, hypercholesterolaemia, arteriosclerosis, diabetes, and prostatic hypertrophy, and it also has hypolipidaemic action and immune regulation function in the gastrointestinal tract (12). The protective properties of various plant extracts on airway inflammation related to exposure to PM10 and diesel exhaust particles were evaluated in mice (13). The antioxidants of *O. ficus-indica* as important inhibitors of free radical formation were reported by Castañeda-Arriaga et al (14), as well as antioxidants and inhibition of the sugar digestive enzyme activities of polyphenols by in vitro experiments (15). The powder of peel and seed of the plant efficiently removes the aqueous manganese cations (16). The gums were used to improve the quality of breads and cakes (17). The phenolic phytoconstituents, antioxidant and antiacetylcholinesterase activities of *O. ficus-indica* peel and flower teas were evaluated after in vitro gastrointestinal digestion (18). It modulates the intestinal microbiome in obese women and improves host metabolism (19). Polysaccharides from *O. ficus-indica* showed a regulating effect on intestinal flora of cyclophosphamide-induced immunosuppressed mice by effectively increasing the white blood cell count index and improving their thymus and spleen, while effectively promoting the secretion of interleukin (IL)-4, IL-1beta, tumour necrosis factor (TNF)-alpha and interferon (IFN)-gamma (20). Indicaxanthin isolated from fruits enhances glucose dysmetabolism and reduces insulin resistance in mice fed the high-fat diet (21).

Boswellia serrata Roxb. ex Colebr. belongs to the Burseraceae family locally known as luban-Kundur. These are moderate to large deciduous trees. They have papery flakes of bark and "yellowish green resin" inside. Leaves are compound and alternate. Flowers are white and are distributed in southern Saudi Arabia.

The extract of *B. serrata* exhibited a potential effect in protecting the intestinal epithelium compared to lipopolysaccharide (LPS)-stimulated cells (22,23). The diuretic activity of gum extract in albino rats was investigated (24,25) and significant diuretic, kaliuretic, and natriuretic effects were observed. Synergistic antimicrobial activity of essential oil from *B. serrata* was studied with various azoles against azole-resistant strains of *Candida albicans* pathogens (26). The plant was used as a culture medium for micropropagation and as a natural source of nonsteroidal anti-inflammatory and antiarthritic agents (27). The antianaphylactic and mast cell stabilizing effects of boswellic acid have been assessed on passive paw anaphylaxis and revealed potential immunomodulatory activity (28). Recently, *Boswellia* spp. and its isolated bioactive phytoconstituents were traditionally used to treat chronic disease, inflammation, oral health, and microbial infection (29). Gum is traditionally used for the treatment of various inflammations that affect the skin, gums, eye, gastrointestinal tract (GIT) in addition to respiratory inflammation disorders such as bronchitis, asthma, laryngitis, etc. (30).

Commiphora myrrha (Nees) Engl. belongs to the Burseraceae family, locally known as El Murr Elihejazi. These are spiny, deciduous, almost shrub or small tree, with short thorns, producing a hard translucent yellowish gum resin. Leaves are green to greyish or glaucous, variable in shape, and minute in size. Native to Saudi Arabia, the plant is traditionally used as an anti-inflammatory and in the treatment of infectious diseases, making it a very popular and valuable alternative and traditional medicine (31,32). It was found to heal wounds, ulcers, and various diseases of the pulmonary, GIT, and urinary system (33).

Furanodienone and curzerene are bioactive components detected in the oil of the resinous exudate of *C. myrrha* that were tested and found to influence the spread of viruses by intervening at different stages of the virus life cycle (34). The antiosteoporotic effects of *C. myrrha* and its polysaccharide were inhibited through osteoclastogenesis (35). Sesquiterpenoids and its phytoconstituents isolated from the resinous exudate of *C. myrrha* were found to inhibit the migration of human hepatocellular liver carcinoma cells (HepG2) according to a dose-dependent pattern (36). A pilot study revealed that *C. myrrha* has significant analgesic properties (37). A combination of herbs (*Commiphora mukul*, *C. myrrha*, and *Terminalia chebula*) functions as an antioxidant, hypolipidemic, and antidiabetic substance; it could be recommended as a helpful herbal remedy for those with diabetes (38). The ethanolic extract of the resin of *C. myrrha* showed anti-obesity potential (39). It showed a hepatoprotective effect against D-GalN/LPS-induced liver injury in a rat model through multiple pathways (40). Murr (*C. myrrha*) is beneficial in treating eye diseases, as kahl forms in ulcers of the eye with other drugs. In Unani medicine, Murr is applied as a mixture with aabe mooli (radish juice) to eyes for cataracts, where the eyes are cleaned after dissolving murr in milk and in infraorbital haemorrhage (41-44).

Tumour-related destructive autoimmune responses can affect the eye, where autoantibody-mediated destruction

of retinal cells is induced by ectopic expression of peripheral tumour-related ocular antigens (45). Neuroendocrine tumours can metastasize to the orbits of the eyes of the midgut carcinoid (46). In the Philippines, the majority of conjunctival, eyelid, and orbit tumours were benign, and retinoblastoma was the most prevalent type of intraocular tumour, while the majority of them were malignant (47). An update is needed to reorient the way to predict the prognosis of paediatric cancers, such as rhabdomyosarcoma and retinoblastoma, and also adult cancers, such as uveal melanoma and lymphomas, and the benefit of targeted therapies, immunotherapy, or even chemotherapy (48).

Fruits and resins are usually used as nutritional supplements and are rarely used for medical purposes. Attempts are being made to look for the constituents of the plant that can prevent and reverse cancer. In this study, in vitro anticancer activity and cell line selectivity of two fruits and two resins were studied in three different cell lines, while clonogenicity was investigated against HepG2. Furthermore, the most promising extract was subjected to gas chromatography-mass spectrometry (GC-MS) analysis to determine the main active phytoconstituent(s).

Materials and methods

Phytochemical studies

Identification of plant materials

Four plants – *A. graveolens* (fruit, coded as BEP-09), *O. ficus-indica* (fruit, BEP-10), *B. serrata* (resin, BEP-11), and *C. myrrha* (resin, BEP-12) (Fig. 1) – were identified and taxonomically classified by an expert taxonomist (Dr. Mohamed, HAA, Department of Biology, Faculty of Sciences, Al-Baha University) and were compared to herbarium materials and different volumes of the flora of Saudi Arabia (49-51). Voucher herbarium specimen numbers (BUH-76,77,78, and 79) were deposited at the Department of Biology of the Faculty of Science of Al-Baha University.

FIGURE 1 - *Opuntia ficus-indica* (L.) Miller (BEP-10) grows in the Al-Baha area, KSA.

Collection and extraction of plant materials

Plant specimens were collected from different sites in Baljurashi province (Wadi El khaitan), Al-Baha area, in April 2021. Fruits (1 kg) and resins (1 kg) were shade-dried and then powdered using a mechanical grinder. The dried materials were macerated in 80% ethanol v/v) for 1 week at room temperature. The resulting residues were filtered, pooled, and evaporated to dryness to provide viscous green to brownish syrups. The crude extracts, so obtained, were transferred to a Petri plate, allowed to dry, and finally weighed.

The percentage of yield was calculated using the formula: yield% = (Afforded extract weight)/(Air-dried weight) × 100.

The plants yielded extracts weighing 1.53, 1.73, 1.62, and 2.17 g, respectively.

GC-MS analysis

The dried fruits of *O. ficus-indica* (L.) Miller (BEP-10) were dissolved in methanol to reach a concentration of 1 mg/mL and diluted 1:10 v/v in methanol (100 µg/mL). The diluted sample was analysed using a GC-MS instrument (Thermo Scientific, USA) attached to a trace ultra-GC and ISQ detector and an AS 3000 autosampler. The separation of components was carried out using a TR-5MS column (Thermo Scientific, USA) with a length of 30 cm, a diameter of 0.25 mm, and a film thickness of 0.25 mm. Helium was used as a carrier gas at 1.2 mL/min with constant flow. The injection port was set at 32°C for 5 minutes, followed by a ramp to 205°C at a rate of 5°C/min and a hold time of 5 minutes. This was followed by a ramp to 280°C at a rate of 5°C/min and hold time of 5 minutes and at the end to 300°C at a rate of 5°C/min and a hold time of 5 minutes. The maximum oven temperature was set at 320°C. A volume of 2 µL diluted extract was injected into the system in split mode with the mass spectrometer run in electron ionization mode with 0.6 scan periods throughout the mass range of 60-900 amu (minutes). Both the temperature of the MS ion source and the transfer line were adjusted to 320°C and 350°C, respectively, using a 1 kV electron multiplier voltage.

Identification of phytoconstituents

Xcalibur software was used for mass spectral data analysis and the fragmentation patterns of each constituent were matched with MS data in the instrument database and built-in libraries including MAINLIB, NIST, and REPLIB. The phytocompounds present in the extract were identified by comparing them with the structures available in the computer library, and the percent abundance of each component was determined using the peak area as reference. The reported biological properties of the detected compounds are based on data from Duke's Phytochemical and Ethnobotanical Database (52).

Cancer cell studies

Cancer cell culture

In this study, three cancer cell lines, MCF7 (human breast adenocarcinoma), HT29 (human colorectal adenocarcinoma),

and HepG2 (human liver adenocarcinoma), were used, in addition to MRC5 (normal human foetal lung fibroblast), all were from American Type Culture Collection (ATCC), USA. Three cancer cells were subcultured in RPMI-1640 medium (10% foetal bovine serum (FBS)), while MRC5 was preserved in Eagle's Minimum Essential Medium (EMEM, 10% FBS) – all at 37°C, 5% CO_2, and 100% relative humidity, for a maximum of 5-10 passages.

Cytotoxicity and selectivity studies

The cytotoxic effect of four extracts, in addition to doxorubicin and camptothecin, was evaluated by the MTT assay, as reported by Alsanosy et al (53) and Abdalla et al (54). Each cell line was cultured separately in 96 wells (3×10^3/well) and incubated with each of the extracts or doxorubicin at a final concentration of 0-100 µg/mL, for 3 days at 37°C overnight (dimethyl sulfoxide (DMSO) 0.1%; n = 3 of three independent experiments). After 3 days of incubation, the cytotoxicity of each extract was evaluated using an MTT assay. MTT was added to each well in culture medium at a concentration of 0.5 mg/mL and incubated for 3 hours at 37°C. The MTT solution was removed and the formazan granules were dissolved by DMSO. The absorbance was read on a multiplate reader (BIORAD, PR 4100, Hercules, CA, USA). The optical density of the purple formazan A_{550} is proportional to the number of viable cells. The extract concentration causing 50% inhibition (IC_{50}), compared to the control group, 100% cell growth, was estimated using GraphPad Prism. The selectivity index (SI) for the five extracts was calculated by dividing its IC_{50} for MRC5 cells by the IC_{50} for MCF7, HT29, or HepG2 cells.

Clonogenic assay

The clonogenic assay measures tumour cell survival and subsequent proliferative ability after drug exposure (55). The extract (BEP-10) was selected for a further clonogenic test, as it showed the highest selectivity to the normal cell line MRC5. Exponentially growing HepG2 cells in DMEM (supplemented with 10% FBS and 1% penicillin/streptomycin) were seeded in duplicates at a density of 200 cells/well in a 6-well plate and allowed to attach overnight and then exposed to an increasing concentration of BEP-10 (0, 0.75, 1.5, 2.25 µg/mL) for 72 hours. The wells containing the extract were then replaced with fresh media without the extract. The cells were left to grow at 37°C, 5% CO_2, and 100% humidity. Daily wells were checked and the cells that form colonies were roughly counted. After 14 days, plates were rinsed in phosphate-buffered saline and fixed with pre-chilled methanol at room temperature for 20 minutes, then stained with 0.5 methylene blue in 1:1 methanol/H_2O (v/v) for 10 minutes, washed thoroughly in dH_2O, and air dried. Cell colonies were counted and recorded macroscopically.

Ethics approval of the study

According to the standards of Al-Baha University, all funded project proposals have undergone a critical review followed by approval by relevant scientific research committees before acceptance.

Results

Phytochemical studies

The four plant extracts produced the following yields: BEP-09 (15.3%), BEP-10 (17.3%), BEP-11 (16.2%), and BEP-13 (21.7) from fruits and resins (Fig. 2).

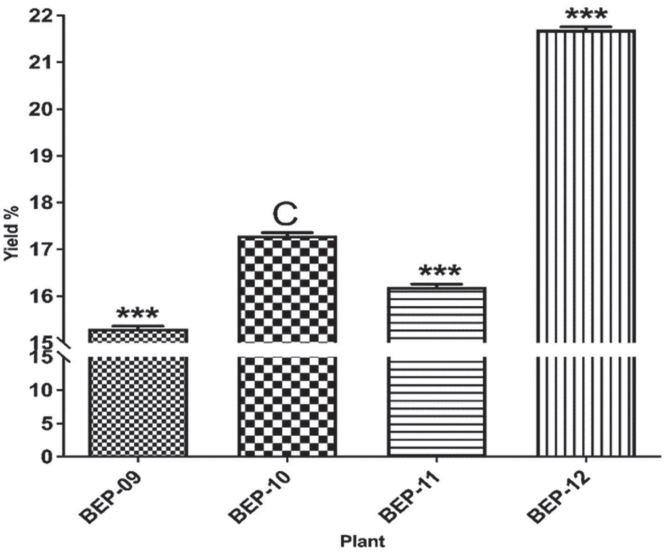

FIGURE 2 - Yield % of dry extracts obtained after ethanolic extraction and evaporation of four different selected plants. C; control, ***; $p \leq 0.001$.

Identification of phytoconstituents using GC-MS

Investigation of the GC-MS chromatogram (see the supplementary file) of the fruits of the Miller plant *O. ficus-indica* (L.) (BEP-10 extract) indicated the presence, mainly, of eight small polar molecules (18) (Tab. 1 and Fig. 3).

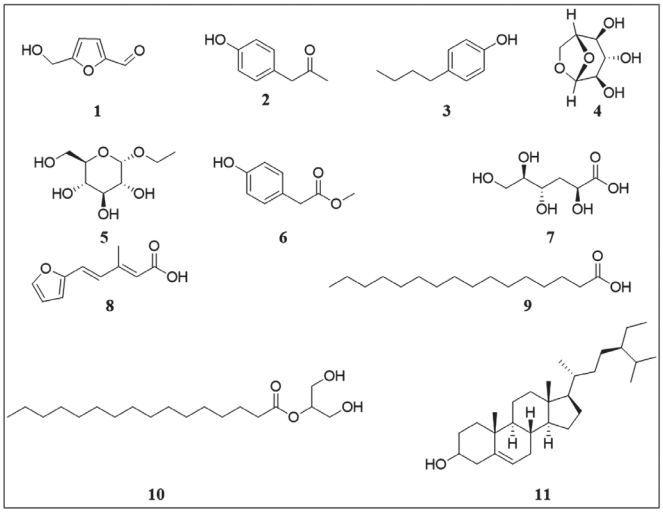

FIGURE 3 - Structures of chemical constituents identified by gas chromatography-mass spectrometry for the fruits of *Opuntia ficus-indica* (L.) Miller (BEP-10 extract).

TABLE 1 - Phytoconstituents identified by GC-MS analysis of the extract of *Opuntia ficus-indica* (L.) Miller (BEP-10)

Compound	Formula	Molecular weight	Peak area (%)	Retention time (minutes)	Biological activity
(1) 5-(hydroxymethyl)-2-furancarboxaldehyde	$C_6H_6O_3$	126.11	13.2	5.875	Known to be associated with antimicrobial properties (56) used as an antifungal (57)
(2) 1-(4′-Hydroxyphenyl)-2-propanone	$C_9H_{10}O_2$	150.17	2.52	7.752	Exhibits a myriad of pharmacological actions, such as antimicrobial, antitussive, antispasmodic, and anticancer properties (58)
(3) 4-Butyl-phenol	$C_{10}H_{14}O$	150.22	2.93	8.011	No significant report
(4) 1,6-Anhydro-beta-D-glucopyranose	$C_6H_{10}O_5$	162.14	5.31	8.328	No significant report
(5) Ethylalpha-d-glucopyranoside	$C_8H_{16}O_6$	208.09	4.60	9.245	Maintenance and improvement of skin homeostasis and moisturizing functions (59)
(6) Benzeneacetic acid, 4-hydroxy-, methyl ester	$C_9H_{10}O_3$	166.06	4.66	9.438	-
(7) 3-Deoxy-d-mannonic acid	$C_6H_{12}O_6$	180.16	6.43	9.651	-
(8) 5-(2-Furyl)-3-methyl-penta-2,4-dienoic acid	$C_{10}H_{10}O_3$	178.18	3.1	9.843	-
(9) n-Hydroxydecanoic acid	$C_{16}H_{32}O_2$	256.42	1.89	11.347	As anti-inflammatory (60), cytotoxic activity (61)
(10) Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50	3.01	14.656	-
(11) Stigmast-5-en-3-ol	$C_{29}H_{50}O$	414.71	1.54	23.313	Apoptotic and antiproliferative effects (62)

GC-MS = gas chromatography-mass spectrometry.

These molecules represent 73% of the total identified compounds. The rest (27%) were the non-polar constituents represented by compounds **9–11**. Therefore, polar molecules constitute >40% of the peak area % relative to the total peak area % of the components that existed in BEP-10 extract. In contrast, non-polar residues represented only 8% of the total peak area % of the components that existed in BEP-10 extract. 5-(Hydroxymethyl)-2-furancarboxaldehyde (**1**) was found to be the main molecule in BEP-10 extract (13.2%). The other furan derivative (**8**) was found to have a peak area % of 3.1. Thus, furan derivatives represent the main component of BEP-10 extract (Fig. 4) while phenolic derivatives represented by compounds **2, 3,** and **6** came at the second level (peak area % = 10.05) with carbohydrates **4** and **5** (peak area % = 9.91).

Cytotoxicity and cell line selectivity studies

The four extracts showed a variable IC_{50} ranging from 0.75 to 19.32 µg/mL. The most active extract was BEP-10 against HepG2 cells, and showed ~4.5-fold selectivity compared to normal MRC5 cells. The selectivity of the extract BEP-10 was greater than that of doxorubicin and camptothecin (Tabs. 2 and 3).

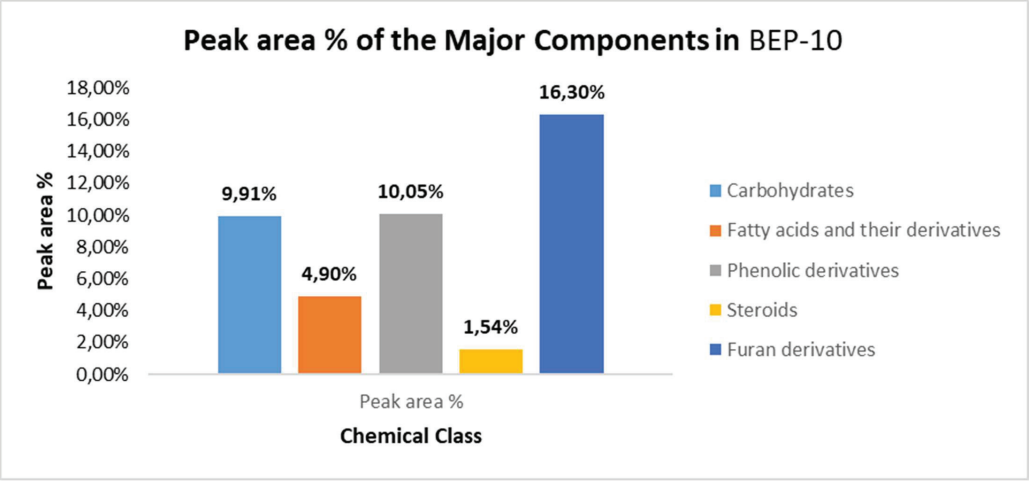

FIGURE 4 - Peak areas (%) for the major components of *Opuntia ficus-indica* (L.) Miller (BEP-10 extract).

TABLE 2 - Cytotoxic activity of the four extracts, doxorubicin and camptothecin, against three cell lines, and normal fibroblast (MTT 72 hours, IC_{50}, µg/mL ±SD, n = 3)

Extract	MCF7	HT29	HepG2	Average* IC_{50}	MRC5
BEP-09	6.00±1.61	8.20±0.57	5.20±0.58	6.47	2.94±0.71
BEP-10	1.85±0.73	5.85±0.23	**0.75±0.11****	**2.82****	3.34±0.41
BEP-11	16.93±0.66	16.07±0.11	14.07±1.04	15.69	10.77±0.54
BEP-12	16.53±0.43	19.32±0.64	9.60±0.82	15.15	8.72±1.56
Doxorubicin	0.07±0.01	1.98±0.10	2.15±0.15	1.40	5.86±0.35
Camptothecin	0.08±0.01	2.50±0.26	0.76±0.07	1.11	1.18±0.10

*Average cytotoxicity (IC_{50}) of each extract against the three cancer cells. **$p \leq 0.01$.

TABLE 3 - Selectivity index of the five extracts, doxorubicin, and camptothecin, against normal MRC5 cells

Extract	MRC5	HT29	HepG2
BEP-09	0.49	0.36	0.57
BEP-10	1.80	0.57	4.47
BEP-11	0.64	0.67	0.77
BEP-12	0.53	0.45	0.91
Doxorubicin	78.57	2.96	2.73
Camptothecin	13.80	0.47	1.55

Clonogenic effect of the extract BEP-10 against HepG2 cells

The extract BEP-10 was tested for its possible clonogenic effect against HepG2 liver cancer cells. The extract revealed a dose-dependent clonogenic activity against a dose-dependent effect against HepG2 cells that was comparable to the effect of doxorubicin on the same cancer cells (Fig. 5).

Discussion

Fruits and resins have interesting medicinal uses. In vitro anticancer activity, cell line selectivity, and clonogenicity were considered as a useful trend to scavenge for a useful natural therapeutic agent(s) with putative anticancer property.

The extract of *O. ficus-indica* (fruits; BEP-10) yielded 17.3%. This indicates the high amounts of constituents that are expected to be available in these fruits.

The MTT assay of the four extracts showed a variable IC_{50} ranging from 0.75 to 19.32 µg/mL comparable to both standards: doxorubicin (IC_{50} = 1.40) and camptothecin (IC_{50} = 1.11), respectively. Fruits appear to be more effective than resins, because they showed lower IC_{50} values than those produced by standard drugs. These results were consistent with those of Castañeda-Arriaga et al (14) who studied the antioxidant effect of this plant and found that its chelating compounds can reduce the harmful effects caused by the most reactive free radical existing immediately.

The resulting selectivity (~4.5 fold) of the most active extract BEP-10 against HepG2 cells compared to normal MRC5 cells was found to be higher than that of doxorubicin and camptothecin. The extract BEP-10 was considered for more cytotoxic and mechanistic studies. Selectivity indicates the ability of the extract to have a maximum effect on cancerous cells and a lesser effect on normal cells. This indicates both its safety and efficacy, and thus it can serve as a promising and useful drug candidate (63).

Due to its high selectivity for HepG2 liver cancer cells, the extract BEP-10 was chosen to test its possible clonogenic effect and showed a dose-dependent clonogenic effect comparable to the effect of doxorubicin in the same cells. The macroscopically counted cell colonies indicate the suppression ability of the active extract, which can be taken as evidence to support the preliminary cytotoxicity and selectivity effects. A study by Terzo et al (21) revealed that the *O. ficus-indica* fruit extract exerted significant antioxidant and anti-inflammatory effects.

Correlating the cytotoxic activity of the most promising BEP-10 extract with its phytochemical constituents, GC-MS was performed and different classes of phytoconstituents were detected, including polar molecules (73%) and lipophilic constituents (27%). These have been reviewed as anti-inflammatory (64), antioxidant (65), and anticancer agents (66).

Our results showed that in compound **1**, aldehyde 5-(hydroxymethyl)-2-furancarboxaldehyde, the furan derivative was the main compound of the BEP-10 extract. The literature revealed that the medicinal properties of furan include anticancer, antidepressant, antianxiolytic, analgesic, anti-inflammatory, muscle relaxant, antihypertensive, anti-arrhythmic, antimicrobial like antibacterial, antifungal, or antiviral (67), anti-ageing agents, anti-ulcer, antihistaminic, anticholinergic, antiparkinsonian, antidiuretic, and inhibition of sickle cell formation (68).

However, the GC-MS chromatogram showed three phenolic derivatives (compounds **2, 3**, and **6**) that were classified as second contents in the BEP-10 extract. These findings were consistent with various studies such as the anticancer (69), anti-trypanosomal activity (70), antileishmanial, anti-inflammatory and antimicrobial activities (71), and anti-neuroinflammatory and neuroprotective activities (72).

Conclusion

The study concludes that the *O. ficus-indica* fruit (BEP-10) is widely distributed in the Al-Baha area and is locally considered a popular fruit. Its extract showed a significant cytotoxic

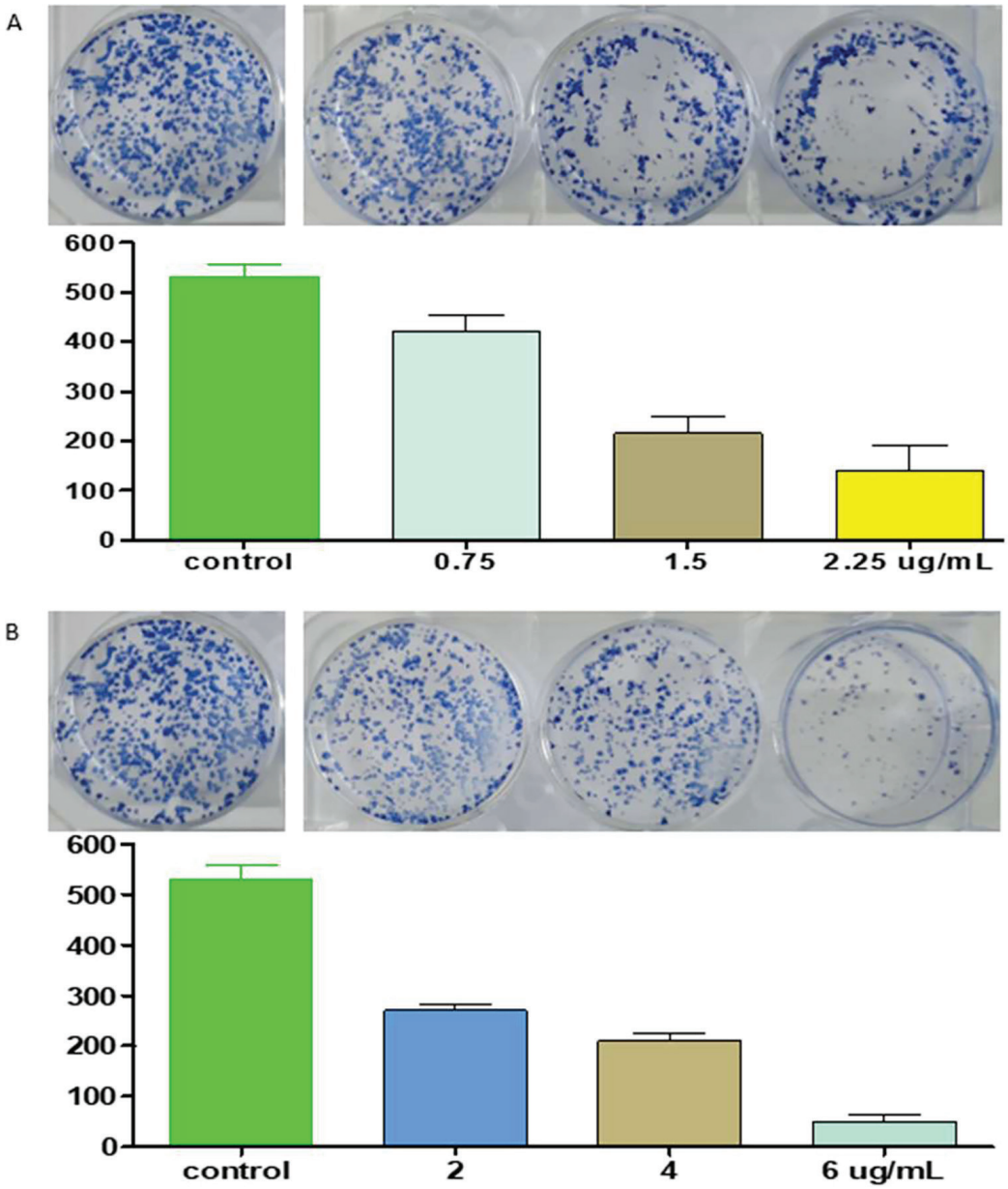

FIGURE 5 - Colonies of HepG2 cells treated with A) extract BEP-10 (0, 0.75, 1.5, 2.25 µg/mL; n = 2), and B) doxorubicin (0, 2, 4, and 6 µg/mL; n = 2) for 72 hours in 6-well plates followed by a 14-day period of incubation without extract. Bar graph showing x-axis: extract concentrations (BEP-10) or doxorubicin concentrations; and y-axis: colony number. Results are expressed as cell number ± standard deviation of two independent experiments.

effect, particularly against HepG2 liver cancer cells with high cell selectivity.

Polar and lipophilic phytoconstituents were identified in the plant extract and could contribute to the investigated cytotoxic activity. The furan derivatives that are present as the main compound may play a vital role in the activity studied. Further research is required to obtain the profile of the drug candidate.

Acknowledgements

This article is a part of the funded project: Investigation of Medicinal Plants with Putative Ocular Effects from Al-Baha Area, Southwestern, Saudi Arabia, Grant number (MOE-BU-4-2020); thus, the funder body is highly acknowledged for their financial support.

Disclosures

Data Availability Statement: All data generated or analysed during this study are available with Dr. Ali as the correspondence author and can be provided upon request.

Conflict of interest: The authors declare that they have no competing interests.

Funding: The author(s) disclosed receiving the following financial support for the research, authorship, and/or publication of this article. This work was supported by the Department of Research and Innovation of the Ministry of Education of Saudi Arabia (grant number: MOE-BU-4-2020). Sponsors did not play a role in the design of the study, the collection and analysis of data, or the preparation of the manuscript.

Authors' contribution: Ali conceived the original idea. Mahadi, Hiadar, and Aimun designed the study. Ashraf, Mohamed EA, Riyad A, and Bassem R conducted the experimental work and collected

the data. Aimun analysed the data and drafted the manuscript. Ali and Asaad, revised it. All approved the final version that was submitted. All authors equally contributed to the whole work preparation. All authors approved the version to be published and agreed to be accountable for all aspects of the work.

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