Biocatalysis and Agricultural Biotechnology 20 (2019) 101199

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Ficus carica, Ficus sycomorus and Euphorbia tirucalli latex extracts: Phytochemical screening, antioxidant and cytotoxic properties

Azza M. Abdel-Aty[a,*], Mohamed Belal Hamed[a], Walaa H. Salama[a], Mamdouh M. Ali[b], Afaf S. Fahmy[a], Saleh A. Mohamed[a]

[a] Molecular Biology Department, National Research Centre, Dokki, Cairo, Egypt
[b] Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt

ARTICLE INFO

Keywords:
Latex
Ficus carica
Ficus sycomorus
Euphorbia tirucalli
Antioxidant
Cytotoxicity

ABSTRACT

Medicinal plants are important sources for discovering new drugs for many diseases. The aim of this study was to assess and compare the total phenolic and flavonoid contents, phytochemical compounds, antioxidant and cytotoxic activities of some medicinal plants: *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) latex extracts. A significant amount of phenolics and flavonoids was recorded in the FCE, FSE and ETE of 50.2, 88.0, 10.5 mg GAE/g latex and 12.5, 34.0, 4.3 mg CE/g latex, respectively. The phenolic content of FCE, FSE and ETE were effective in scavenging free radicals of DPPH and ABTS with IC_{50} values of 13.6, 7.0, 6.0 and 4.5, 6.4, 2.0 µg GAE/ml, respectively. The total antioxidant activity, using phosphomolybdenum assay, of FCE, FSE and ETE was also measured with EC_{50} values of 39, 25 and 6.5 µg GAE/ml, respectively. Various phytochemical compounds with potential therapeutic values were detected in each FCE, FSE and ETE latex extract using GC-MS analysis. Most of identified phytochemical compounds are found for first time in figs or euphorbia latex extracts. Various phenolic acids and flavonoids were identified and quantified in the FCE, FSE, and ETE latex extracts with diverse therapeutic properties using HPLC analysis. The phenolic content of ETE, FCE, and FSE latex extracts exerted a potent cytotoxic effect near to doxorubicin, a common anticancer drug, against acute myeloid leukemia HL-60, breast MCF-7 and liver HepG2 cancer cell lines, respectively. Additionally, all the tested extracts didn't exert any toxicity against human normal melanocyte HFB4 cell line with concentrations up to100 µg/ml. In conclusion, the phenolic content of FCE, FSE and ETE latex extracts could be used as therapeutic agents for fighting cancer.

1. Introduction

The use of plants to treat many diseases, extract therapeutic agents and improve the health backed hundreds of years ago and until now; plants are still an important source of novel active compounds and many blockbuster drugs. Malignant cancer is the second cause of death worldwide, which destroys the health and life of human beings. Epidemiological scientists reported that the consumption of some fruits and vegetables decreased the risk of many diseases like cancer (Abuajah et al., 2015). Over 50% of anticancer drugs were produced from natural sources, especially from plants (Mann, 2002; Kim and Park, 2002). The anticancer activity of some plants might be due to their antioxidant properties. Moreover, many plant antioxidants used as anticancer drugs and could induce cancer cells apoptosis (Michels et al., 2006).

Latex is a natural sticky polymer exuding from different plant parts and contains various phytochemicals and antioxidants. It shows diverse biological activities such as antifungal, antibacterial, antiviral, anti-inflammatory and anticancer (Santos and Van Ree, 2011; Upadhyay, 2011).

Ficus sycomorus and *Ficus carica* L., belong to *Moraceae* family, are short trees native to the continent of Africa. Their fruits are called figs which greatly used as a food and medicine throughout the world. Dry and fresh figs contain high levels of phenolic antioxidants, flavonoids and anthocyanins (Solomon et al., 2006). Traditionally, the fresh fig latex could treat warts, epilepsy, toothache, hemorrhoids, snake bites, and cough (Lansky et al., 2008). Also, it could inhibit the spontaneous and transplanted tumors growth in mice (Ullman, 1952; Ullman et al., 1952).

Euphorbia tirucalli L., belong to *Euphorbiaceae* family, is a small shrub native to Africa; however, it is excessively grown all over the world because of its uses in traditional medicines. Consequently, this has prompted scientific interest to study its pharmacological properties.

* Corresponding author. El Buhouth St, Dokki, Cairo, Egypt.
E-mail address: azzasdm@hotmail.com (A.M. Abdel-Aty).

https://doi.org/10.1016/j.bcab.2019.101199
Received 2 April 2019; Received in revised form 10 June 2019; Accepted 11 June 2019
Available online 13 June 2019
1878-8181/

E. tirucalli extracts are considered as a source of natural antioxidants. Traditionally, *E. tirucalli* fresh latex was used in treatment of some types of cancer (Munro et al., 2015; Waczuk et al., 2015).

Usually, the fresh latex of fig or *Euphorbia* was used externally or orally for cancer and other tumors treatment (Lansky et al., 2008; Caxito et al., 2017). Additionally, the screening of the bioactive compounds and the cytotoxicity of these latexes against human cancer cells was limited. Therefore, this study aimed to assess and compare the total phenolic, flavonoid contents, antioxidant capacity and phytochemical compounds of the latex extracts from locally grown *F. carica*, *F.* sycomorus and *E. tirucalli* plants. The evaluation of the cytotoxicity of phenolic content of the latex extracts against different human cancer cell lines was also studied.

2. Materials and methods

2.1. Collection of plant latex

The latex of the tested plants were collected into sterile containers by cutting the ends of *Ficus carica* and *Ficus* sycomorus fruits, while it was collected by cutting the ends of *Euphorbia tirucalli* branches. All these plants were collected from Ministry of Agriculture, Sharkia government, Egypt. All the tested plants were authenticated by Dr. M. Salah (Professor, Botany department, Agriculture Research Center (ARC), Cairo, Egypt). Voucher specimens were deposited in the herbarium of the Botany department under the numbers of 255996#, 255997#, and 255998#.

2.2. Extraction of bioactive compounds from latex

The latex of each tested plant was collected on equal volume of petroleum ether according to Ashok et al. (2011). Then the petroleum ether layer was separated from aqueous layer by separating funnel. The aqueous samples were dried in the oven at 50 °C overnight. The dried samples (5 g) were extracted in 50 ml of 80% methanol with shaking at 140 rpm at 30 °C overnight and then filtered with filter paper Whatman No.1. Each filtrate is designated as a methanol latex extract. For cytotoxic activity measurement, each methanol latex extract was dried in the oven at 50 °C overnight and dissolved in a least volume of 0.1% dimethyl sulfoxide (DMSO).

2.3. Total phenols measurement

The total phenolic content was measured by the method of Velioglu et al. (1998). The reaction mixture includes: 0.1 ml methanol extract, 0.1 ml Folin-Ciocalteu reagent and 0.8 ml distilled water were incubated for 5 min at room temperature. Then 0.5 ml sodium carbonate (20%) was added and incubated at room temperature for 30 min. The blue colour was measured at 750 nm. Gallic acid (GA) was used as a standard.

2.4. Total flavonoids measurement

The total flavonoid content was measured by the method of Zhishen et al. (1999). Incubation of 0.25 ml methanol extract, 1.25 ml distilled water and 0.075 ml of 5% $NaNO_2$ for 6 min, then add 0.15 ml of 10% $AlCl_3$. After 5 min, 0.5 ml of 1.0 M NaOH and 0.275 ml distilled water were added. The change of colour was measured at 510 nm. Catechin was used as a standard.

2.5. Antioxidant assays

2.5.1. DPPH assay

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) method was used for determination of the antioxidant activity of the latex extracts (Ao et al., 2008). The reaction mixture includes: 0.1 ml methanol extract and 0.9 ml of 0.1 mM DPPH dissolved in methanol were incubated for 30 min at room temperature and dark. The absorbance of the colour was measured at 517 nm. DPPH scavenging percent = [(O.D. control – O.D. sample)/O.D. control] x 100.

Table 1
Total phenolic and flavonoid contents of *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) methanol latex extracts.

Extract	Total phenolic (mg GAE/g latex)	Total flavonoid (mg CE/g latex)
FCE	50.20 ± 2.50[a]	12.50 ± 1.10[a]
FSE	88.00 ± 5.20[b]	34.00 ± 2.20[b]
ETE	10.50 ± 1.20[c]	4.30 ± 0.50[c]

GAE, gallic acid equivalent, CE, catechin equivalent. Values are presented as means ± S.E. (n = 4). Values with different superscript letters within the same column indicate significant differences at level $p < 0.01$.

2.5.2. ABTS assay

ABTS (2, 2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) reagent was prepared and used for determination of the antioxidant activity of the latex extracts (Re et al., 1999). The reaction mixture includes: 1.0 ml of ABTS reagent and 0.1 ml of the extract were incubated for 1 min at room temperature and the reduction of absorbance was measured at 734 nm. ABTS scavenging percent = [(O.D. control – O.D. sample)/O.D. control] x 100.

2.5.3. Phosphomolybdenum complex assay

The total antioxidant activity of the latex extracts was also evaluated by formation of a phosphomolybdenum complex by the method of Prieto et al. (1999). The reaction mixture includes: 4 mM ammonium molybdate, 28 mM sodium phosphate, 600 mM sulfuric acid, and 50 µl of the extract were incubated for 90 min at 95 °C. After cooling, the absorbance of colour was read at 695 nm. EC_{50} is defined as a concentration of the phenolic compounds gives absorbance of 0.5.

2.6. GC-MS analysis of the latex extracts

Each latex extract was analyzed by GC–MS Spectrometer (PerkinElmer, USA) equipped with 30 m × 0.25 mm Elite-1MS column. The carrier gas is helium. The temperatures were adjusted as follows: 50 °C hold for 5 min, raised to 250 °C for 10 min. The injector and detector temperatures were adjusted at 280 °C. The ion source and interface temperatures were adjusted at 200 and 250 °C, respectively. The mass range was scanned from 50 to 300 amu. The control of the GC–MS system and the data peak processing were controlled by means of Turbo Mass, version 5.4.2.1617 software. Compound identification was verified based on the relative retention time and mass fragmentation pattern spectra with those of standards and the NIST 2008. LIB.

2.7. HPLC analysis of the latex extracts

The high performance liquid chromatography (HPLC) analysis was performed for the extracts according to Kim et al. (2006) using an Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The determination and separation were performed on XDB-C18 column (150 × 4.6 µm). The samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. The column was eluted by acetonitrile (solvent A) and 2% acetic acid (v/v) (solvent B) at a flow rate of 1 ml/min. The peaks were monitored simultaneously at 280, 320 and 360 nm. The obtained peaks were identified by congruent retention times and UV spectra and compared to commercial phenolic compounds as standards.

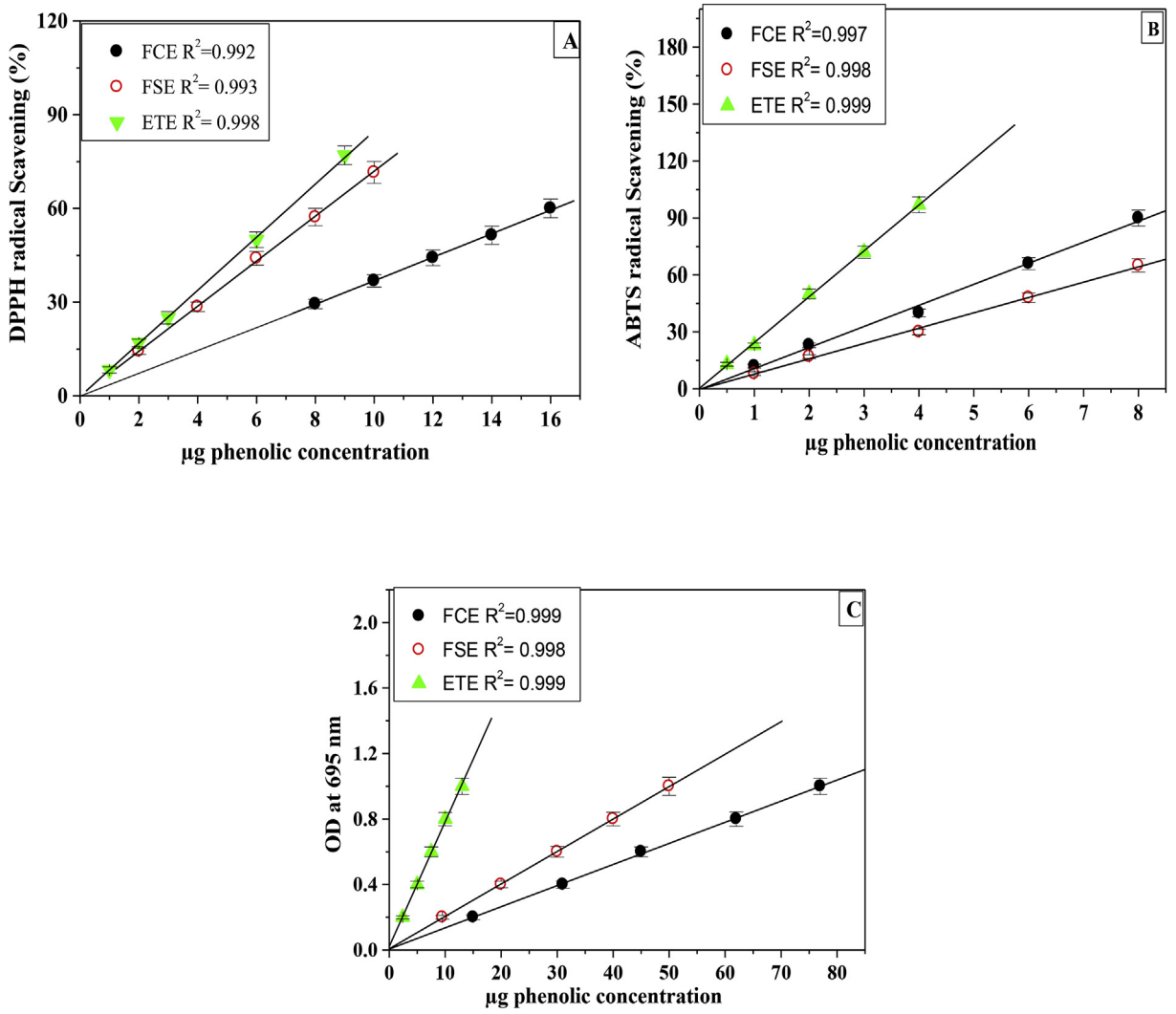


Fig. 1. Correlation between different concentrations of the phenolic contents of *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) methanol latex extracts and their antioxidant capacity as determined by DPPH (A), ABTS (B) and phosphomolybdenum (C) assays. Values are presented as means ± S.E. (n = 4).

Table 2
The antioxidant activity of phenolic content of *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) methanol latex extracts.

Antioxidant assay	FCE µg GAE/ml	FSE µg GAE/ml	ETE µg GAE/ml
DPPH (IC_{50})	13.60 ± 1.20^a	7.00 ± 0.30^b	6.00 ± 0.25^c
ABTS (IC_{50})	4.50 ± 0.72^a	6.40 ± 0.32^b	2.00 ± 0.13^c
Total antioxidant capacity (EC_{50})	39.00 ± 2.20^a	25.00 ± 1.20^b	6.50 ± 0.30^c

IC_{50} value is the concentration of the phenolic content required to scavenge 50% of either DPPH or ABTS free radical. EC_{50} is the effective concentration of extract at which the absorbance was 0.5. Values are presented as means ± S.E. (n = 4). Values with different superscript letters within the same row indicate significant differences at level $P > 0.01$.

2.8. Cell lines and culturing

Acute myeloid leukemia (HL-60), breast MCF-7, liver HepG2, colon HCT116 and lung A549 cancer cell lines and human normal melanocyte (HFB4) cell line were obtained from (Rockville, MD, USA). The cells were preserved in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated fetal calf serum (GIBCO), streptomycin (100 µg/ml) and penicillin (100 U/ml) in humidify atmosphere with 5% CO_2 at 37 °C. Cells at a concentration of 0.50 × 106 were grown in a flask (25 cm^2) in 5 ml of culture medium.

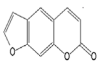
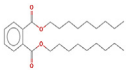
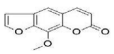
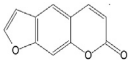
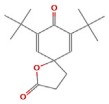
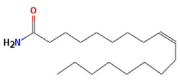
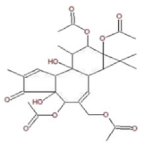
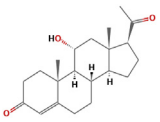
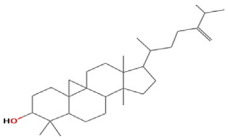
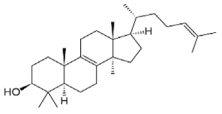
2.9. In vitro cytotoxic activity

The cytotoxic activity was determined *in vitro* using Sulfo-Rhodamine-B stain (SRB) assay according to Skehan et al. (1990). Cells were inoculated in 96-well microtiter plate (10^4 cells/well) for 24 h before adding the phenolic extracts to allow attachment of cells to the wall of the plate. The tested extracts were dissolved in 0.1% DMSO immediately before adding to the cell culture. Different concentrations of the tested extracts (12.5–100 µg GAE/ml) and doxorubicin, a reference anticancer drug, were used. Triplicate wells were prepared for each concentration. The tested extracts and the cells were incubated in atmosphere of 5% CO_2 at 37 °C for 48 h. The cells were washed and stained with 0.4% SRB (w/v) dissolved in 1% acetic acid for 30 min. The excess of dye was eliminated by 4 washes with 1% acetic acid and the stained cells were recovered by Tris-EDTA buffer. The colour intensity was recorded by an ELISA reader. The concentration of the phenolic content of latex extract required for 50% inhibition of cell viability (IC_{50}) was recorded.

2.10. Statistical analysis

The results were statistically analyzed by a one-way analysis of

Table 3
Major phytochemical compounds identified from *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) methanol latex extracts using GC-MS analysis.

Extract	Compound name	Compound structure	Retention time (min)	Molecular weight	Pharmacological action	References
FCE	4-hydroxy-3-methoxbenzoic acid, methyl ester		27.2	182	Antioxidant, anti-cancer and anti-apoptotic	Kumar et al. (2003)
	1(2H)-Naphthalenone, 3,4-dihydro-2,5,8-trimethyl		33.3	188	Anti-fungal	Sandeep and Prakash (2014)
	Psoralen		35.18	186	Antioxidant, anticancer, antimicrobial, Bone-modifying therapeutic agent for bone metastases treatment	Wu et al. (2013)
	Phthalic acid		36.6	166	Antioxidant, cytotoxic on human cancer cell lines and anti-apoptotic, anti-inflammatory	Kok et al. (2008)
	Dibutyl phthalate		38.8	278	Antioxidant, potent antimicrobial agent	Roya et al. (2006)
	Xanthotoxin		40.7	216	*Anti*-leukodermal and antitumor properties	(Abdel Hafez et al., 2009)
	Benzoic acid, 2-hydroxy-, methyl ester		16.7	152	Antioxidant, antimicrobial and anti-proliferative agent	Jaganathan and Mandal (2009)
	Psoralen		35.18	186	Antioxidant, anticancer, antimicrobial, Bone-modifying therapeutic agent for bone metastases treatment.	Wu et al. (2013)
FSE	7,9-Di-*tert*-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione		37.8	276	Antioxidant, anti-mineralocorticoid, anti-androgen agent	Rukhsana et al. (2015)
	9-Octadecenamide		55.2	281	Antioxidant, antimicrobial agent.	Rukhsana et al. (2015)
	4H- Cyclopropa [5′,6']benz [1′,2':7,8]azuleno[5,6-*b*] oxiren-4-one		54.13	520	New compound	
ETE	Pregn-4-ene-3,20-dione, 11-hydroxy		57.4	330	Steroid compound Therapeutic drug	Meher et al. (2013)
	9,19-Cyclo-9.beta.-lanostane-3.beta.,25-diol		60.7	444	Cytotoxicity against prostate cancer cell line	Shamsabadipour et al. (2018)
	Lanosterol		62.7	426	Chemo-preventive activity against colon carcinogenesis, cytotoxicity against some human cancer cell lines.	Ma et al. (2013)

variance (ANOVA). The data were considered as means ± S.E. (n = 4). Differences were significant at $P < 0.01$.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the *Ficus carica* (FCE), *Ficus sycomorus* (FSE) and *Euphorbia tirucalli* (ETE) latex extracts were 50.2, 88, 10.5 mg GAE/g latex and 12.5, 34, 4.3 mg CE/g latex, respectively (Table 1). Among analyzed methanol extracts, the FSE showed significantly highest total phenolic and flavonoid contents ($P < 0.01$), which it contained about two and three times more phenolic and flavonoid contents than FCE, respectively. The lowest total phenolic and flavonoid levels were detected in ETE. Variations in total phenolic and flavonoid contents (21.5–77 mg GAE/g and 0.012–28 mg CE/g, respectively) were previously reported for different Euphorbia parts extracts (Jahan et al., 2011; Ben Mohamed Maoulainine et al., 2012; Munro et al., 2015). Furthermore, low contents in total phenolics and flavonoids (21.9 mg GAE/g and 8.58 mg CE/g, respectively) were recorded in *F. carica* fruit extract (Amessis-Ouchemoukh et al., 2017). Low phenolic contents were also reported for seventy six fig accessions from the Eastern Mediterranean region of Turkey (0.69–2.2 mg GAE/g) (Caliskan and AytekinPolat, 2011). Moreover, in *F. sycomorus* extracts, the previously recorded phenolic and flavonoid contents (3.43–81.56 mg GAE/100 g and 0.1–0.527 mg CE/100 g, respectively) (Veberic et al., 2008; Al-matani et al., 2015) were much lower than our obtained results. Therefore, the data of the present study indicated that the latex of FCE and FSE contained higher phenolic and flavonoid contents than other plant parts of figs that reported above.

Table 4
Phenolic compounds composition of the *Ficus carica* (FCE), *Ficus sycomorus* (FSE), and *Euphorbia tirucalli* (ETE) methanol latex extracts using HPLC technique.

Compounds	RT	Phenolic compound %		
		FCE	FSE	ETE
Gallic	5.7	ND	ND	20
Protochatechuic	9.9	8	40	ND
p-hydroxybenzoic	15.1	ND	3	ND
Catechin	18.6	8	ND	ND
Chlorogenic	20.6	59	5	ND
Vanillic	24.8	1	ND	ND
Caffeic	31.6	3	1	ND
Ferulic	32.4	ND	ND	21
Sinapic	33.8	1	4	1
Rutin	36.2	20	ND	3
p-coumaric	37.2	ND	35	ND
Cinnamic	42.8	ND	5	ND
Quercetin	43.6	ND	ND	55
Apigenin	46.0	ND	7	ND

ND: No Detection; RT: Retention Time.

3.2. Antioxidant activity

Natural antioxidants could reduce the rate of mortality associated with many danger diseases such as cancer by their scavenger effect on free radical species (Marrelli et al., 2012; Amessis-Ouchemoukh et al., 2017). In addition, natural antioxidants are different in their types, structures, mode of action and reactivity, therefore, three methods DPPH, ABTS and phosphomolybdenum were used to screen and compare the antioxidant activity of the three methanol latex extracts. In all tested antioxidant assays, Fig. 1 shows strong correlation coefficients (R^2) (0.992–0.999) between the concentration of phenolic content of latex extracts and antioxidant activity. The IC_{50} and EC_{50} values were also determined from Fig. 1, where a low value indicates the high antioxidant activity. The antioxidant activity of the phenolic content of FCE, FSE and ETE latex extracts was evaluated on the basis of their ability to scavenge free DPPH and ABTS radicals, which exhibited IC_{50} values of 13.6, 7.0, 6.0 µg/ml and 4.5, 6.4, 2.0 µg/ml, respectively (Table 2). For DPPH or ATBS assays a significant difference ($P < 0.01$) between the radical scavenging potential of FCE and FSE within the same family, as well as ETE of different family was found as presented in Table 2.

Also, the results appeared no much differences between DPPH and ABTS IC_{50} values for FSE, while the DPPH IC_{50} values were three times more than ABTS IC_{50} values in case of FCE and ETE. These results suggests that the phenolic content of FSE have the same susceptibility toward DPPH and ABTS free radicals, while the phenolic content of other latex extracts have different abilities toward these radicals. This is due to that each phenolic compound has different contribution to the antioxidant activity (Mohamed et al., 2016). In phosphomolybdenum assay, the ETE also possessed a significant high reducing ability ($P < 0.01$) with EC_{50} value of 6.5 µg/ml, while FCE and FSE possessed lower reducing ability with EC_{50} values of 39 and 25 µg/ml, respectively (Table 2). Therefore, the phenolic content of ETE latex extract possessed a potent antioxidant activity; this is proved by the three antioxidant assays. However, *E. tirucalli* branches aqueous extract concentrations (1–150 µg/mL) exhibited weak DPPH radical scavenging activity (Waczuk et al., 2015). Further, the DPPH radical scavenging activity of *E. royleana* methanol and water extracts was 580 and 1130 µg/ml, respectively (Ashraf et al., 2015). Several studies reported that figs extracts could be a rich source of natural antioxidants with variations in their amounts. These variations might be attributed to the type of solvent, phenolic contents, and interaction between extract components (Feng et al., 2015; Amessis-Ouchemoukh et al., 2017).

3.3. GC-MS analysis of the latex extracts

To obtain more information about the potential healthy phytochemicals of the FCE, FSE and ETE latex extracts, the GC-MS analysis was performed. Table 3 screens the major phytochemical compounds of the three latex extracts and their pharmacological actions using GC-MS technique. The results showed that the FCE contains 4-hydroxy-3-methoxbenzoic acid, methyl ester, 1(2H)-Naphthalenone, 3, 4-dihydro-2, 5, 8-trimethyl, Psoralen, Phthalic acid, Dibutyl phthalate and Xanthotoxin. The FSE contains Benzoic acid 2-hydroxy- methyl ester, Psoralen, 7,9-Di-*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione and 9-Octadecenamide. Moreover, the ETE contains 4H- Cyclopropa [5′,6']benz[1′,2':7,8]azuleno[5,6-*b*]oxiren-4-one, Pregn-4-ene-3,20-dione-11-hydroxy, 9,19-Cyclo-9.beta.-lanostane-3.beta-25-diol and Lanosterol. Most of identified compounds are oxygenated hetero-cycles. Many naturally occurring oxygenated hetero-cycles are biologically active compounds and have therapeutic actions such as anticancer activity (Kaur et al., 2013). Psoralen is a furocoumarin compound with a potent pharmacological action. Figs are one top sources of psoralen (Wu et al., 2013) and our results indicated that both figs latex (FCE and FSE) are natural sources of psoralen. However, the rest of identified bioactive compounds are found for first time in figs and euphorbia latex extracts. Besides, most of these bioactive compounds have potential therapeutic actions in different clinical settings such as antioxidant, anticancer, antitumor, anti-apoptotic, anti-inflammatory and antimicrobial as mentioned in Table 3.

3.4. HPLC analysis of the latex extracts

To identify and quantify the phenolic compounds of the tested latex extracts, the HPLC technique was carried out. In Table 4, the HPLC analysis of the FCE, FSE, and ETE latex extracts showed a considerable variation in phenolic acids as well as some flavonoids. The chlorogenic acid was the major phenolic compound of the FCE latex extract followed by rutin and represented 59 and 20% of total phenolic content, respectively. In the FSE latex extract, the protochatechuic acid and *p*-coumaric acid were the dominating phenolic compounds and represented 40 and 35% of total phenolic content, respectively. A high concentration of quercetin was detected in the ETE latex extract followed by ferulic acid and gallic acid and represented 55, 21, and 20% of total phenolic content, respectively. Most of the identified phenolic compounds of the three latex extracts have diverse therapeutic properties besides their antioxidant, antitumor, anticancer anti-proliferative and cytotoxic effects against different cancerous tissues and human cancer cell lines (Jiang et al., 2000; Garrait et al., 2006; Lakhanpal and Rai, 2007; Semaming et al., 2015; Ganeshpurkar and Saluja, 2017).

3.5. In vitro cytotoxic activity

Fig. 2 shows the cytotoxicity of different concentrations ranged 12.5–100 µg/ml of phenolic content of FCE, ETE and FSE latex extracts against HepG2, MCF7, A549, HL-60 and HCT116 cancer cell lines as well as human normal melanocyte, HFB4. Further, the doxorubicin, a reference anticancer drug, and DMSO were used as controls for cancer cells. The cancer cells appeared normal growth in the culture medium and DMSO didn't have any effect on the cell growth (data not shown). In addition, the phenolic content of the FCE, ETE and FSE didn't exhibit any effect against the growth of the normal HFB4 cell line.

The results revealed that the FCE displayed a significant potent cytotoxic activity ($P < 0.01$) against MCF-7 with IC_{50} concentration of 25.30 ± 2.11 µg/ml near to the doxorubicin (IC_{50}: 24.50 ± 1.72 µg/ml) after 48 h of incubation, Table 5. The ethanol extract of *F. carica* fruits exhibited cytotoxic activity against MCF-7 breast cancer cell line (IC_{50}: 31.2 µg/ml) after 72 h of incubation (Jasmine et al., 2015). In addition, the FCE showed a moderate activity against HepG2 and HCT116 cancer cell lines with IC_{50} values 32.25 ± 3.66 and

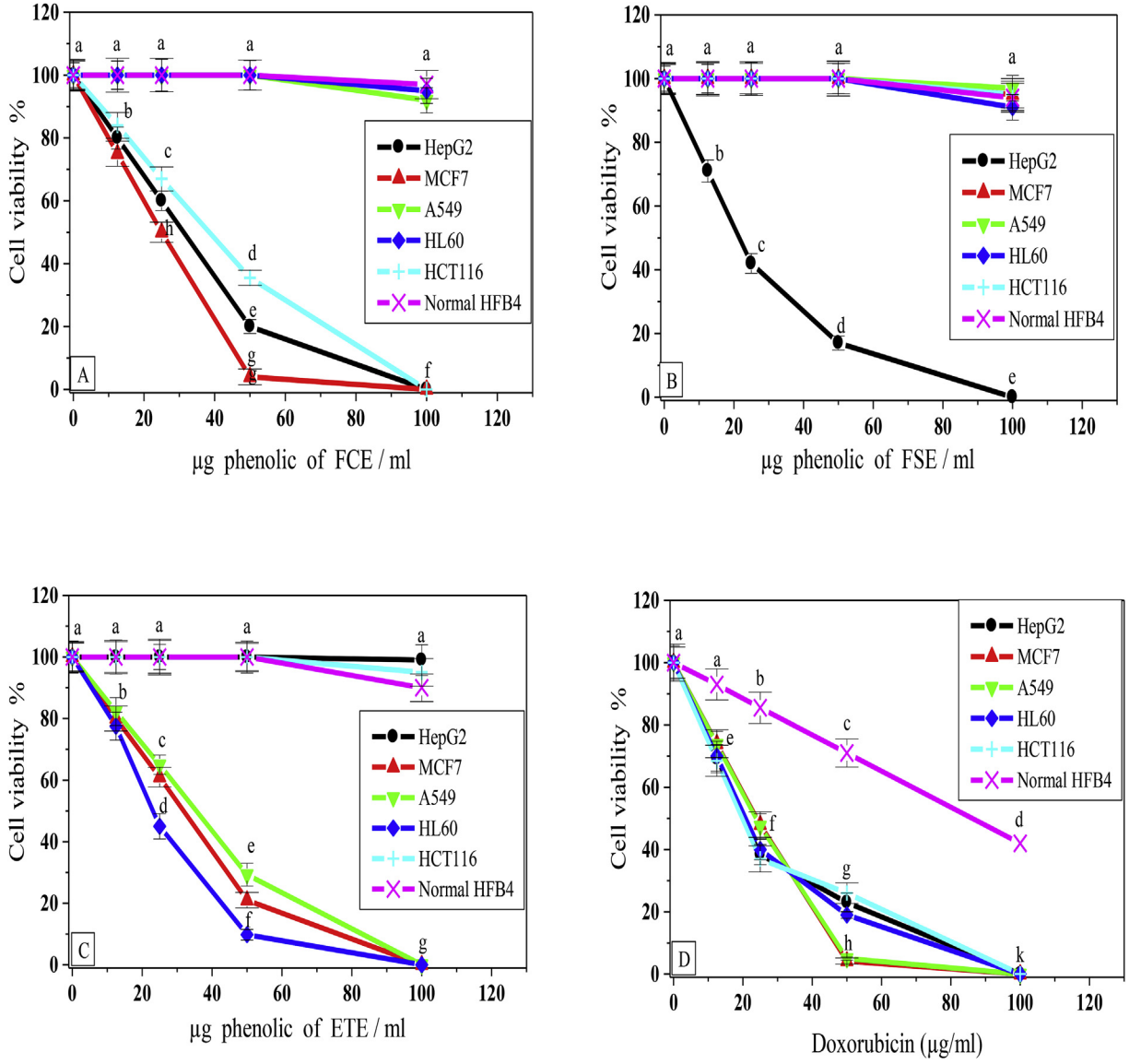


Fig. 2. Effect of different concentrations of (A) *Ficus carica* (FCE), (B) *Ficus sycomorus* (FSE), (C) *Euphorbia tirucalli* (ETE) latex extracts and (D) doxorubicin, a reference anticancer drug, on liver HepG2, breast MCF-7, lung A549, acute myeloid leukemia HL-60 and colon HCT116 cancer cell lines, as well as human normal melanocyte HFB4. Values are presented as means ± S.E. (n = 4). Values with different superscript letters within the same curve indicate significant differences at level $P < 0.01$.

Table 5
The IC_{50} values of phenolic content of *Ficus carica* (FCE), *Ficus sycomorus* (FSE), *Euphorbia tirucalli* (ETE) latex extracts and doxorubicin, a reference anticancer drug, against different human cell lines measured by SRB assay.

Sample	IC_{50} (µg GAE/ml)					
	HepG2	MCF-7	A549	HL-60	HCT116	HFB4
DMSO	NA	NA	NA	NA	NA	NA
Doxorubicin	20.30 ± 2.34[a]	24.50 ± 1.72[a]	23.84 ± 2.43[a]	21.87 ± 2.31[a]	19.83 ± 2.11[a]	86.20 ± 8.88
FCE	32.25 ± 3.66[b]	25.30 ± 2.11[a]	NA	NA	38.75 ± 4.74[b]	NA
FSE	21.35 ± 2.11[a]	NA	NA	NA	NA	NA
ETE	NA	31.65 ± 3.67[b]	35.36 ± 3.82[b]	22.76 ± 2.85[a]	NA	NA

IC_{50} value is the concentration of the phenolic content required for 50% inhibition of cell viability. Values are presented as means ± S.E. (n = 4).
NA is no activity. Values with different superscript letters within the same column indicate significant differences at level $P < 0.01$.

38.75 ± 4.74 µg/ml compared to the doxorubicin with IC_{50} values 20.30 ± 2.34 and 19.83 ± 2.11 µg/ml, respectively. Additionally, it did not exert any activity against HL-60 and A549 cancer cells Table 5.

The FSE displayed a significant potent cytotoxic activity ($P < 0.01$) against HepG2 with IC_{50} value 21.35 ± 2.11 µg/ml near to the doxorubicin IC_{50} value 20.30 ± 2.34 µg/ml. It did not exert any cytotoxicity against the rest of the cancer cell lines, Table 5. The results appeared that the FCE and FSE IC_{50} concentrations were much lower than that reported previously. The cytotoxic activity of *F. sycomorus* fruit ethyl acetate extract showed a high IC_{50} value (26.82 mg/ml) (Al-

matani et al., 2015). Furthermore, the *F. carica* fruit extract didn't exert any cytotoxic effect on U87 glioblastoma cells at the concentration ranged from 250 to 1000 μg/ml (Amessis-Ouchemoukh et al., 2017). We can say this is the first report shows the cytotoxic activity of the FCE and FSE latex extracts against MCF-7, HepG2 and HCT116 cancer cell lines.

The results evidenced that a relative low concentration of the phenolic content of ETE displayed a significant potent cytotoxic activity ($P < 0.01$) against HL-60 with IC_{50} value of 22.76 ± 2.85 μg/ml near to the reference drug, doxorubicin with IC_{50} value 21.87 ± 2.31 μg/ml. Furthermore, the ETE appeared a moderate cytotoxic activity against MCF-7 and A549 cancer cell lines with the IC_{50} values of 31.65 ± 3.67 and 35.36 ± 3.82 μg/ml compared to the doxorubicin with IC_{50} values of 24.50 ± 1.72 and 23.84 ± 2.43 μg/ml, respectively. Additionally, ETE did not exert any activity against HepG2 and HCT116 cancer cells, Table 5. The leaves and stems methanol extracts of the *E. tirucalli* exerted a high growth inhibition capacity against the MiaPaCa-2 pancreatic cancer cell line (Munro et al., 2015).The stem ethanol extract of the *E. tirucalli* also inhibited the growth of HL-60 cancer cell line with relatively high IC_{50} values (113–300 μg/ml) (Caxito et al., 2017). Further, tannins were isolated from some Euphorbia species (dried whole plant) showed antitumor activity (Khanbabaee and Ree, 2001). However, relative high concentrations of *E. tirucalli* branches aqueous extract (100–150 μg/ml) causing genotoxicity and cytotoxicity in normal human cells. It was associated with changes in gene expression of some antioxidant resulting in overproduction of reactive oxygen species (ROS) and decreasing the level of cell tolerance to chemical components of this plant (Waczuk et al., 2015).

Indeed, the cancer cells have ability to absorb any component more than the healthy cells. So it can be concluded that the cancer cells were highly oxidized or affected by the identified oxygenated phytochemical and phenolic compounds of each FCE, FSE and ETE latex extract compared to the healthy cells. Besides, most of these compounds have antioxidant, anticancer, antitumor and anti-apoptotic activities, which supports their strong influence on some tested cancer cell lines.

4. Conclusion

From above results, it can be concluded that although the lower of phenolic and flavonoid contents were detected in ETE latex extract, it possessed higher antioxidant activity compared with FCE and FSE. The GC-MS and HPLC analysis of the FCE, FSE, and ETE latex extracts revealed that they are promising sources for several bioactive compounds. These identified compounds have potent therapeutic properties such as anticancer, antitumor, anti-apoptotic, anti-inflammatory and antimicrobial. The phenolic content of FCE, FSE, and ETE latex extracts exerted a potent cytotoxic activity on breast MCF-7, liver HepG2 and acute myeloid leukemia HL-60 cancer cell lines, respectively. Further, the FCE and ETE latex extracts showed a moderate cytotoxic activity against colon HCT116 and lung A549 cancer cell lines, respectively. Therefore, they could be optional sources for anticancer compounds. On the whole, it is interesting to note that the phenolic content of FCE, FSE, and ETE latex extracts possessed chemotherapeutic characters.

Authors' contributions

AMA, MBH, WHS, and SAM had the original idea for the study and carried out the design. SAM, MMA and ASF designed the experiments. AMA, MBH, WHS and MMA performed the experiments, analyzed the data and writing the manuscript. All authors read, revised and approved the final draft manuscript.Acknowledgments

The authors gratefully acknowledge to National Research Centre, Cairo, Egypt, for providing the facilitations and equipment throughout the study. The authors declared that they did not have any funding source to support their study.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101199.

References

Abdel Hafez, O.M., Amin, K.M., Abdel-Latif, N.A., Mohamed, T.K., Ahmed, E.Y., Maher, T., 2009. Synthesis and antitumor activity of some new xanthotoxin derivatives. Eur. J. Med. Chem. 44, 2967–2974.
Abuajah, C.I., Ogbonna, A.C., Osuji, C.M., 2015. Functional components and medicinal properties of food a review. J. Food Sci. Technol. 52, 2522–2529.
Al-matani, S.K., Al-Wahaibi, R.N.S., Hossain, M.A., 2015. In vitro evaluation of the total phenolic and flavonoid contents and the antimicrobial and cytotoxicity activities of crude fruit extracts with different polarities from *Ficus sycomorus*. Pac. Sci. Rev.: Nat. Sci. Eng. 17, 103–108.
Amessis-Ouchemoukh, N., Ouchemoukh, S., Mezianta, N., Idiria, Y., Hernanzc, D., Stincod, C.M., Rodríguez-Pulido, F.J., Heredia, F.J., Madania, K., Luis, J., 2017. Bioactive metabolites involved in the antioxidant, anticancer and anticalpain activities of *Ficus carica* L., *Ceratonia siliqua* L. and *Quercusilex* L. extracts. Ind. Crops Prod. 95, 6–17.
Ao, C., Li, A., Elzaawely, A., Xuan, T.D., Tawata, S., 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa L* fil extract. Food Control 19, 940–948.
Ashok, D.C., Prachu, B.M., Umesh, J.U., Manohar, P.V., 2011. Antibacterial and antioxidant activity of plant latex. J. Pharm. Res. 4, 406–407.
Ashraf, A., Sarfraz, R.A., Rashid, M.A., Shahid, M., 2015. Antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant (*Euphorbia royleana*) from Pakistan. J. Food Drug Anal. 23, 109–115.
Ben Mohamed Maoulainine, L., Jelassi, A., Hassen, I., Ould Mohamed Salem Ould Boukhari, A., 2012. Antioxidant proprieties of methanolic and ethanolic extracts of *Euphorbia helioscopia*, (L.) aerial parts. Int. Food Res. J. 19, 1125–1130.
Caliskan, O., AytekinPolat, A., 2011. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the Eastern Mediterranean region of Turkey. Sci. Hortic. (Amst.) 128, 473–478.
Caxito, M.L., Victório, C.P., Costa, H.B., Romão, W., Kuster, R.M., Gattass, C.R., 2017. Anti-proliferative activity of extracts of *Euphorbia tirucalli* L (*Euphorbiaceae*) from three regions of Brazil. Trop. J. Pharmaceut. Res. 16, 1013–1020.
Feng, Y.C., Li, W.L., He, F., Kong, T., Huang, X., Gao, Z., Lu, N., Li, H., 2015. Aqueous two-phase system as an effective tool for purification of phenolic compounds from fig fruits (*Ficus carica* L. Sep). Sci. Technol. 50, 1785–1793.
Ganeshpurkar, A., Saluja, A.K., 2017. The pharmacological potential of rutin. Saudi Pharmaceut. J. 25, 149–164.
Garrait, G., Jarrige, J.F., Blanquet, S., Beyssac, E., Cardot, J.M., 2006. Gastrointestinal absorption and urinary excretion of trans-cinnamic and p-coumaric acids in rats. J. Agric. Food Chem. 54, 2944–2950.
Jaganathan, S.K., Mandal, M., 2009. Anti-proliferative effects of honey and of its polyphenols: a review. J. Biomed. Biotechnol. https://doi.org/10.1155/2009/830616.
Jahan, N., Rehman, K.U., Ali, S., Bhatti, I.A., 2011. Antioxidant activity of gemmo therapeutically treated indiginous medicinal plants. Asian J. Chem. 23, 3461–3470.
Jasmine, R., Manikandan, K., karthikeyan, 2015. Evaluating the antioxidant and anticancer property of *ficus carica* fruit. Afr. J. Biotechnol. 14, 634–641.
Jiang, Y., Kusama, K., Satoh, K., Takayama, F., Watanabe, S., Sakagami, H., 2000. Induction of cytotoxicity by chlorogenic acid in human oral tumor cell lines. Phytomedicine 7, 483–491.
Kaur, P., Arora, R., Gill, N.S., 2013. Review on oxygen heterocycles. Indo. Am. J. Pharm. Res. 3 (11).
Khanbabaee, K., Ree, T.V., 2001. Tannins: classification and definition. Nat. Prod. Rep. 18, 641–649.
Kim, J., Park, E.J., 2002. Cytotoxic anticancer candidates from natural resources. Current medicinal chemistry. Anti-cancer Agents 2, 485–537.
Kim, K.H., Tsao, R., Yang, R., Cui, S.W., 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chem. 95, 466–473.
Kok, S.H., Gambari, R., Chui, C.H., Yuen, M.C., Lin, E., Wong, R.S., Lau, F.Y., Cheng, G.Y., Lam, W.S., Chan, S.H., Lam, K.H., Cheng, C.H., Lai, P.B., Yu, M.W., Cheung, F., Tang, J.C., Chan, A.S., 2008. Synthesis and anticancer activity of benzothiazole containing phthalimide on human carcinoma cell lines. Bioorg. Med. Chem. 16, 3626–3631.
Kumar, A.P., Garcia, G.E., Ghosh, R., Rajnarayanan, R.V., Alworth, W.L., Slaga, T.J., 2003. 4-Hydroxy-3- methoxybenzoic acid methyl ester: a curcumin derivative targets Akt/NF kappa B cell survival signaling pathway: potential for prostate cancer management. Neoplasia 5, 255–266.
Lakhanpal, P., Rai, D.K., 2007. Quercetin: a versatile flavonoid. Internet J. Med. Update 2, 22–37.
Lansky, E.P., Paavilainen, H.M., Pawlus, A.D., Newman, R.A., 2008. Ficus spp. (fig): ethnobotany and potential as anticancer and anti-inflammatory agents. J. Ethnopharmacol. 119, 195–213.
Ma, L., Chen, H., Dong, P., Lu, X., 2013. Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom Inonotus obliquus. Food Chem. 139,

503–508.
Mann, J., 2002. Natural products in cancer chemotherapy: past, present and future. Nat. Rev. Canc. 2, 143–148.
Marrelli, M., Menichini, F., Statti, G.A., Bonesi, M., Duez, P., Menichini, F., Conforti, F., 2012. Changes in the phenolic and lipophilic composition: in the enzyme inhibition and anti-proliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation. Food Chem. Toxicol. 50, 726–733.
Meher, C.P., Sethy, S.P., Pochaiah, B., 2013. Structure and biological activities: steroid moieties. Res. J. Pharmaceut. Biol. Chem. Sci. 4, 253–272.
Michels, G., Watjen, W., Weber, N., Niering, P., Chovolou, Y., Kampkotter, A., Proksch, P., Kahl, R., 2006. Resveratrol induces apoptotic cell death in rat H4IIE hepatoma cells but necrosis in C6 glioma cells. Toxicology 225, 173–182.
Mohamed, S.A., Awad, M.A., El-Dengawy, E.F.A., Abdel-Mageed, H.M., El-Badry, M.O., Salah, H.A., Abdel-Aty, A.M., Fahmy, A.S., 2016. Total phenolic and flavonoid contents and antioxidant activities of sixteen commercial date cultivars grown in Saudi Arabia. RSC Adv. 6, 44814–44818.
Munro, B., Vuong, Q.V., Chalmers, A.C., Goldsmith, C.D., Bowyer, M.C., Scarlett, C.J., 2015. Phytochemical, antioxidant and anti-cancer properties of *Euphorbia tirucalli* methanolic and aqueous extracts. Antioxidants 4, 647–661.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Roya, R.N., Laskarb, S., Sen, S.K., 2006. Dibutyl phthalate, the bioactive compound produced by Streptomyces albidoflavus 321.2. Microbiol. Res. 161, 121–126.
Rukhsana, K., Varghese, Vijesh, Akhilesh, V.P., Jisha Krishnan, E.K., Priya Bhaskaran, K.P., Bindu, P.U., Sebastian, C.D., 2015. GC-MS determination of chemical components in the bioactive secretion of Anoplodesmus Saussur II (Humbert, 1865). Int. J. Pharm. Sci. Res. 6, 650–653.
Sandeep, A., Prakash, N.B., 2014. Synthesis, characterization and antifungal activity of quinazoline thione derivatives of 3, 4-dihydro-1(2h)-naphthalenone. Int. Res. J. Pharm. 5, 476–480.
Santos, A., Van Ree, R., 2011. Mimickers of allergy or relevant allergens? Int. Arch. Allergy Immunol. 155, 191–204.
Semaming, Y., Pannengpetch, P., Chattipakorn, S.C., Chattipakorn, N., 2015. Pharmacological properties of protocatechuic acid and its potential roles as complementary medicine. J. Evid. Based Complementary Altern. Med, ID 593902, 11. https://doi.org/10.1155/2015/593902.
Shamsabadipour, S., Zarei, S.M., Ghanadian, M., Ayatollahi, S.A., Rahimnejad, M.R., Saeedi, H., Aghaei, M., 2018. A new Taraxastane triterpene from *Euphorbia Denticulata* with cytotoxic activity against prostate cancer Cells. Iran. J. Pharm. Res. (IJPR) 17, 336–342.
Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.
Solomon, A., Golubowicz, S., Yablowicz, Z., Grossman, S., Bergman, M., Gottlieb, H.E., Altman, A., Kerem, Z., Flaishman, M.A., 2006. Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* L.). J. Agric. Food Chem. 54, 7717–7723.
Ullman, S.B., 1952. The inhibitory and necrosis-inducing effects of the latex of *Ficus carica* L. on transplanted and spontaneous tumors. Exp. Med. Surg. 10, 26–49.
Ullman, S.B., Clark, G.M., Roan, K.M., 1952. The effects of fraction R3 of the latex of *Ficus carica* L. on the tissue of mice bearing spontaneous mammary tumors. Exp. Med. Surg. 10, 287–305.
Upadhyay, R.K., 2011. Plant latex: a natural source of pharmaceuticals and pesticides: a review. Int. J. Green Pharm. 5, 169–180.
Veberic, R., Colaric, M., Stampar, F., 2008. Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. Food Chem. 106, 153–157.
Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D., 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem. 46, 4113–4117.
Waczuk, E.P., Kamdem, J.P., Abolaji, A.O., Meinerz, D.F., Bueno, D.C., Nascimento, T.K., Dorow, T.S., Boligon, A.A., Athayde, M.L., Rocha, J.B., Ávila, D.S., 2015. *Euphorbia tirucalli* aqueous extract induces cytotoxicity, genotoxicity and changes in antioxidant gene expression in human leukocytes. Toxicol. Res. 4, 739–748.
Wu, C., Sun, Z., Ye, Y., Han, X., Song, X., Liu, S., 2013. Psoralen inhibits bone metastasis of breast cancer in mice. Fitoterapia 91, 205–210.
Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.