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Medicinal plants from Saudi Arabia and Indonesia: *In vitro* cytotoxicity evaluation on Vero and HEp-2 cells

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Many types of naturally growing plants are used traditionally for the treatment of different types of cancers and infectious diseases. In this report, the cytotoxic activity of 30 medicinal plants, commonly used in folk medicine in Saudi Arabia and Indonesia, was evaluated *in vitro* using Vero and HEp-2 cell lines. Plants were randomly chosen and harvested from different districts of both countries based on ethnobotanical information and subsequently extracted by methanol. Serial two-fold dilutions of each extract, starting from the concentration of 1000 µg/ml, were incubated with Vero and HEp-2 cells for 72 h. The cytotoxic effect of different extracts was *in vitro* characterized by identification of cellular alterations microscopically and cellular viability colormetrically. The plant extracts were classified according to the minimal toxic concentration and 50% cytotoxicity concentration indexes into three groups: highly cytotoxic (8 to 31 µg/ml), moderately cyototoxic (32 to 499 µg/ml) and low cyototoxic (500 to 1000 µg/ml). The results showed that the extracts of *Juniperus phoenicea* and *Calotropis procera* were highly cytotoxic on both cell lines to the minimal concentration of 1 µg/ml and may be well-considered as potential candidates for anticancer research. Two more extracts (*Datura inoxia* and *Citrullus colocynthis*) produced significant cytotoxicity to the minimum concentration of 16 µg/ml, with selective powerful activity of *C. colocynthis* on HEp-2 cells. The other extracts showed lower degrees of cytotoxicity and may be utilized for testing as antiviral agents using cell culture models.

Key words: *Calotropis procera*, cell titer blue assay, cytotoxic activity, *Juniperus phoenicea*, medicinal plants, methanolic extracts.

INTRODUCTION

Medicinal plants have emerged as a promising source of novel therapeutic agents due to their higher structural diversity and potency as compared to standard synthetic chemistry. In general, medicinal plants are cheap, easy to obtain and widely used in many countries such as China, India, Indonesia and a number of Middle East countries to treat various infectious diseases (Guo et al., 2006; Mukhtar et al., 2008). The World Health Organization has reported that about 80% of the world population depends on traditional medicine for their primary health care

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(Farnsworth et al., 1985; Gurib-Fakim, 2006). According to the estimate of Robbers et al. (1996), over 25% of modern medicines that are commonly used worldwide contain compounds extracted from medicinal plants. Medicinal plants provide the source of extremely useful active compounds that are difficult to produce commercially by synthetic methods. They also supply basic compounds that can be modified slightly to render them more active, effective and less toxic (Robbers et al., 1996).

Previous studies have shown that active compounds extracted from medicinal plants have considerable inhibitory effect on cancer cells (Moogkarndia et al., 2004; Takara et al., 2005; Shoeb, 2006; Nawab et al., 2011), bacteria (Tsibangu et al., 2002; Rangasamya et al., 2007; Akroum et al., 2009; Marzouk et al., 2012) and many viruses such as herpes simplex, human immunodeficiency, hepatitis B and influenza viruses (Rajbhandari et al., 2001; Mukhtar et al., 2008; Devi and Manoharan, 2009), beside their potential as antioxidants (Gupta et al., 2006; Krishnaiah et al., 2011). The use of medicinal plant extracts for cancer prevention and treatment was extensively studied (Mukhtar et al., 2008; Mehta et al., 2010). Extracts of medicinal plants are believed to contain a wide spectrum of polyphenolic, flavonoids, alkaloids, terpenoids and saponin compounds, which might have therapeutic properties and hinder cancer formation (Dai and Mumper, 2010). The isolation of the alkaloid active compounds vinblastine and vincristine from *Charanthus roseus*, has introduced a new era for the use of plant materials as anticancer agents (Shoeb, 2006).

In a recent record, about 60% of the currently used anticancer drugs have been isolated from natural products; mostly of plant origin (Nawab et al., 2011). Saudi Arabia and Indonesia are considered among the richest regions with plant diversity and harbors very important genetic resources of medicinal plants in the Arabian Peninsula and Southern Asia, respectively. More than 2,250 species of medicinal plants were estimated to be used for medical purposes in local communities of Saudi Arabia (Rahman et al., 2004). In Indonesia, the use of medicinal plants in traditional medicine was practiced long time ago using what is called 'Jamu', which is a combination of various medicinal plant preparations. A minimum of 151 medicinal plant species that belong to 57 families have been utilized as substantial materials of Jamu (Riswan and Roemantyo, 2002). Nevertheless, only few of the medicinal plants in both countries have been evaluated for their potential as anticancer agents (Amin and Mousa, 2007). Therefore, it is very interesting to explore more about the great probability of medicinal plants from Saudi Arabia and Indonesia for the treatment of cancer diseases. In this present report, based on ethnobotanical approach, we have evaluated *in vitro* the cytotoxic activity of 30 medicinal plants, commonly used by traditional healers in both countries, using Vero and HEp-2 cell lines.

MATERIALS AND METHODS

Plants selection and collection

An assortment of 30 medicinal plants was chosen for the cytotoxicity testing based on: first, previous literature reviews and second, ethnobotanical information. The selected plants that belong to different family groups were collected from different districts of the Kingdom of Saudi Arabia (KSA) and Indonesia (IND) (Table 1).

Preparation of plant extracts

Plant samples were dried at room temperature and finely ground with a hammer mill. For *Aloe vera,* the leaves were cut and the gel was squeezed out in a sterile container. Each 20 g of powdered plant material or gel was extracted by maceration overnight with 200 ml methanol at room temperature. After filtration, methanol was evaporated under reduced pressure till complete dryness and the crude extract was dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 50 mg/ml. All crude extracts (stocks) were kept at -20°C for cytotoxicity testing procedures.

Cell culture

African green monkey kidney cells (Vero) and human larynx cancer cells (HEp-2) were kindly provided by Virology Research Group (VRG), College of Science, King Saud University, Saudi Arabia. Cells were cultured in Dulbecco's modified eagle's medium (D-MEM) supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml), and amphotericin B (0.25 µg/ml) (Sigma, St. Louis, MO). Cells treated with medicinal plant extracts were kept in maintenance medium containing 1% FBS, L-glutamine and antibiotics. All cells were incubated at 37°C with 5% CO_2.

In vitro evaluation of the cytotoxicity of plant extracts

Microscopic examination for morphological alterations

Monolayer cultures of Vero and HEp-2 cells (80 to 90% confluence) were prepared in 96 well plates. After removal of culture medium, cells were washed twice with phosphate buffered saline. Two-fold serial dilutions of the crude plant extracts were prepared in maintenance medium starting from the concentration of 1000 µg/ml, and added to cells in triplicates. Wells that received methanol 70% were served as positive controls and those that received maintenance media only were served as negative controls. All cultures were kept at 37°C in CO_2 incubator for 72 h with daily observation for morphological changes. Cellular alterations were recognized in the form of cell rounding, granulation, vacuolation, degeneration and lysis, as well as detachment of the monolayer. The minimal toxic concentration (MTC) was identified as the least concentration of the plant extract preparation that induce toxic effect(s) on culture cells as detected microscopically after 72 h of incubation.

Cell viability assay

In this assay, the number of viable cells was determined colorimetrically in 96-well plates. In independent set of experiment, following the incubation of confluent monolayer cultures of cells with two-fold dilution series of plant extracts for 72 h, 20 µl of CellTiter-Blue (CTB) reagent (Promega, Madison, WI) were added to each well. After 4 h of incubation at 37°C, the optical density (OD) was measured in all plate wells using ELx880 microplate reader (BioTek, Winooski, VT) with wavelength 570 nm. The cell viability was measured in each well using

Table 1. List of medicinal plants utilized in the current study.

S/No	Scientific name	Family	Common name	Part of plant used	Selected traditional uses	Collection site
1	*Chamomilla recutita*	Asteraceae	Chamomile	Flower	Fever, inflammation, insomnia, ulcer, wound	Riyadh, KSA
2	*Dodonaea viscosa*	Sapindaceae	Hop bush	Leaves	Fever, gout, rheumatism, hemorrhoid, malaria	Tabuk, KSA
3	*Punica granatum*	Lytharaceae	Pomegranate	Peel	Diarrhea, cough, diuretic, vomiting, jaundice	Riyadh, KSA
4	*Psidium guajava*	Myrtaceae	Guava	Leaves	Diarrhea, dysentery, stomach, fever	Riyadh, KSA
5	*Hibiscus sabdariffa*	Malvaceae	Roselle	Flower	Diuretic, sedative, stomach, fever, liver	Riyadh, KSA
6	*Mentha piperita*	Lamiaceae	Peppermint	Leaves	Bronchitis, analgesic, colds, sore throat	Madinah, KSA
7	*Mentha longifolia*	Lamiaceae	Mint	Leaves	Bronchitis, carminative, analgesic, colds	Madinah, KSA
8	*Capparis spinosa*	Capparaceae	Caper bush	Leaves	Diuretic, astringent, tonic, stomach, headache	Tabuk, KSA
9	*Capparis cartilaginea*	Capparaceae	Caper	Leaves	Rheumatism, diabetes, antihelmintic	Tabuk, KSA
10	*Calotropis procera*	Asclepiadaceae	Sodom apple	Leaves	Tumors, liver, skin diseases, diabetes	Riyadh, KSA
11	*Juniperus phoenicea*	Cuppriscaea	Juniper	Leaves	Diarrhea, rheumatic, bronchitis, arthritis	Tabuk, KSA
12	*Citrullus colocynthis*	Cucurbitaceae	Bitter apple	Fruit	Jaundice, tumors, constipation, fever, scabie	Riyadh, KSA
13	*Foeniculum vulgare*	Apiaceae	Fennel	Leaves	Carminative, digestive, gastrointestinal	Riyadh, KSA
14	*Ziziphus spina-christi*	Rhamnaceae	Christ's Thorn Jujube	Leaves	Fever, pain, dandruff, inflammatory, wounds	Riyadh, KSA
15	*Senna indica*	Caesalpinaceae	Cassia	Leaves	Laxative, thypoid, cholera, jaundice, gout	Riyadh, KSA
16	*Salvia verbenaca*	Lamiaceae	Wild Sage	Leaves	Wounds, astringent, diuretic, antiseptic	Riyadh, KSA
17	*Lawsonia inermis*	Lythraceae	Henna	Powder	Diuretic, constipating, inflammations, cough	Riyadh, KSA
18	*Aloe vera*	Xanthorroeaceae	Aloe	Gel	Burning, skin irritations, tonic, shampoos	Riyadh, KSA
19	*Datura inoxia*	Solanaceae	Thorn-apple	Leaves	Anodyne, pain relief, wounds, hemorrhoids	Serpong, IND
20	*Curcuma xanthorrhiza*	Zingiberaceae	Temulawak	Rhizome	Skin inflammations, indigestion, constipation	Serpong, IND
21	*Curcuma zedoaria*	Zingiberaceae	Zedoary	Rhizome	Vomiting, stomach ulcers, menstrual	Serpong, IND
22	*Curcuma longa*	Zingiberaceae	Turmeric	Rhizome	Diarrhea, stomach ulcers, skin diseases	Serpong, IND
23	*Zingiber officinale*	Zingiberaceae	Ginger	Rhizome	Digestive disorder, arthritis, vomiting	Serpong, IND
24	*Andrographis paniculata*	Acanthaceae	Andrographis	Leaves	Leprosy, scabies, anti-inflammatory, diuretic	Serpong, IND
25	*Anredera cordifolia*	Bacellaceae	Madeira Vine	Leaves	Skin, gout, hypertension, inflammation	Serpong, IND
26	*Typhonium flagelliforme*	Araceae	Rodent Taro	Root	Expectorant for cough, pulmonary ailments	Serpong, IND
27	*Phyllanthus niruri*	Phyllanthaceae	Meniran	All parts	Diabetes, fever, cough, diarrhea, dysentery	Serpong, IND
28	*Allium sativum*	Alliaceae	Garlic	Bulb	Cough, colds, dysentery, hypertension	Serpong, IND
29	*Coriandrum sativum*	Apiaceae	Coriander	Fruit	Diabetes, ulcers, diuretic, liver, skin	Serpong, IND
30	*Momordica charanthia*	Cucurbitaceae	Bitter Melon	Fruit	Stomach, diabetes, anthelmintic, jaundice	Serpong, IND

KSA = Kingdom of Saudi Arabia; IND = Indonesia.

well using the following formula:

$$\text{Cell viability} = \frac{\text{OD (assay well)} - \text{OD (positive control)}}{\text{OD (cell control)} - \text{OD (positive control)}} \times 100$$

Table 2. Cytotoxic activity of plant extracts against Vero and Hep-2 Cells by microscopical examination.

Scientific name	Vero (Concentration, µg/ml)								HEp-2 (Concentration, µg/ml)							
	1000	500	250	125	64	32	16	8	1000	500	250	125	64	32	16	8
Chamomilla recutita	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Dodonaea viscosa	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
Punica granatum	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Psidium guajava	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-
Hibiscus sabdariffa	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Mentha piperita	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Mentha longifolia	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Capparis spinosa	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Capparis cartilaginea	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-
Calotropis procera	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Juniperus phoenicea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrullus colocynthis	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	-
Foeniculum vulgare	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Zizipus spina-christia	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Senna indica	+	+	+	+	-	-	-	-	+	+	+	-	-	-	-	-
Salvia verbenaca	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-
Lawsonia inermis	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Aloe vera	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-
Datura inoxia	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-
Curcuma xanthorrhiza	+	+	+	-	-	-	-	-	+	+	+	+	+	-	-	-
Curcuma zedoaria	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-
Curcuma longa	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
Zingiber officinale	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Andrographis paniculata	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
Anredera cordifolia	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-
Typhonium flagelliforme	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Phyllanthus niruri	+	+	+	+	-	-	-	-	+	+	+	+	+	-	-	-
Allium sativum	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-
Coriandrum sativum	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Momordica charanthia	+	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-

+indicates obvious morphological alterations; -indicates no cellular changes.

The 50% cytotoxicity concentration (CC_{50}) was calculated as the concentration of the plant extract that induced reduction in cell viability to 50%.

RESULTS

In the present study, the cytotoxic activity of thirty (30) medicinal plants collected from Saudi Arabia and Indonesia and also representing twenty two (22) plant families (Table 1) was evaluated *in vitro* according to their effect on cell morphology (microscopic examination) and the metabolic reduction of CTB reagent (colorimetric assay) Vero and HEp-2 cells. The cytotoxic effect of plant extracts was classified into three groups; highly cytotoxic (8 to 31 µg/ml), moderate cyototoxic (32 to 499 µg/ml) and low cyototoxic (500 to 1000 µg/ml). This classification was consistent with both MTC and CC_{50} indexes for the majority of plant extracts. Vero cells, 3 plant extracts (10%) were highly cytotoxic, 22 (73.3%) were moderately cytotoxic, and 5 (16.7%) were low cytotoxic as indicated by MTC index. Whereas using CC_{50} index, 2 plant extracts (6.7%) were highly cytotoxic, 19 (63.3%) were moderately cytotoxic, and 9 (30%) were low cytotoxic. On the other hand, the plant extracts were distinguished in HEp-2 cells as: 4 (13.3%) highly cytotoxic, 21 (70%) moderately cytotoxic and 5 (16.7%) low cytotoxic using MTC index; and 2 (6.7%) highly cytotoxic, 23 (76.7%) moderate cytotoxic and 5 (16.7%) low cytotoxic using CC_{50} index (Figure 1).

Out of the eight medicinal plant species used in the study, one viz, *Juniperus phoenicea* belongs to Gymnospermae while the rest belong to Angiospermae. The extracts of *J. phoenicea* and *Calotropis procera* exhibited a substantial degree of cytotoxicity Vero and HEp-2 cells. Both extracts were capable to induce distinct morphological alterations in cell culture, as evaluated by microscopical examination (Table 2 and Figure 2), and effective inhibition of cell viability, as measured by CTB assay (Table 3 and Figure 4), down to the concentration of 8 µg/ml. Further evaluation of lower concentrations of the two extracts outlined that the cytotoxic activity can extend down to the concentration of 1 µg/ml (data not shown). Two other extracts; *Datura inoxia* and *Citrullus colocynthis*, were included in the highly cytotoxic group albeit their effect is lower than *J. phoenicea* and *C. procera* and is not well-consistent in microscopical examination and CTB assay (Tables 2 and 3 and Figures 3 and 4). The extract of *Datura inoxia* was

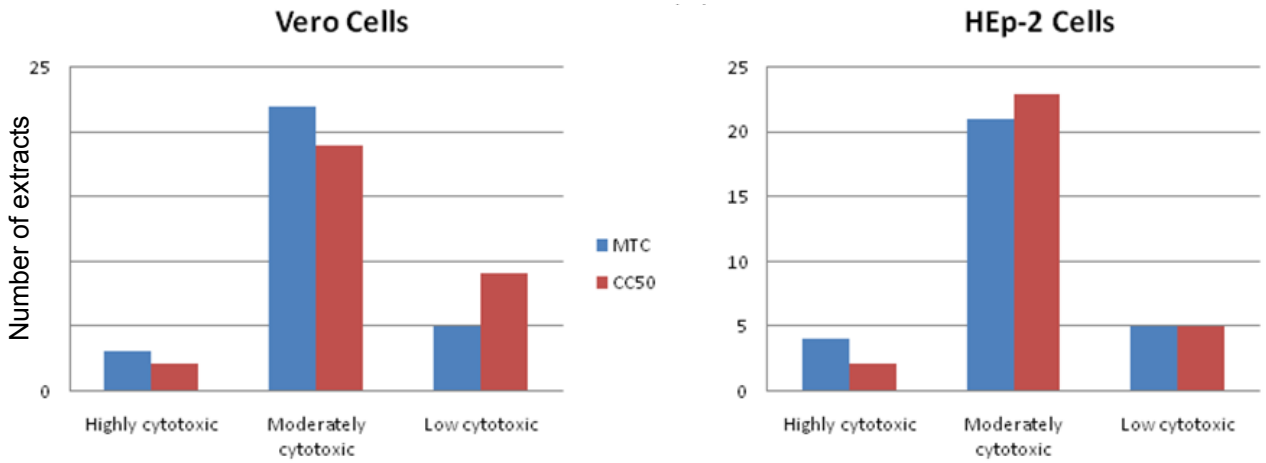

Figure 1. Cytotoxicity of the plant extracts Vero and Hep-2 cells. The different medicinal plants are classified into three categories according to their effect on culture cells as indicated by MTC and CC_{50} indexes; highly cytotoxic (8 to 32 µg/ml), moderately cyototoxic (32 to 499 µg/ml) and low cyototoxic (500 to 1000 µg/ml).

Table 3. The limit of cytotoxic activity of plant extracts Vero and HEp-2 cells as indicated by MTC and CC_{50}.

Scientific name	Vero		HEp-2	
	MTC*	CC_{50}*	MTC	CC_{50}
Chamomilla recutita	1000	>1000	1000	>1000
Dodonaea viscosa	250	382.77	125	266.85
Punica granatum	250	667.46	250	369.26
Psidium guajava	250	402.86	500	402.68
Hibiscus sabdariffa	250	500.89	250	445.65
Mentha piperita	500	724.95	250	337.91
Mentha longifolia	500	>1000	500	>1000
Capparis spinosa	1000	>1000	500	>1000
Capparis cartilaginea	64	182.15	32	72.16
Calotropis procera	8	5.96	8	4.9
Juniperus phoenicea	8	6.63	8	29.79
Citrullus colocynthis	64	109.39	16	37.78
Foeniculum vulgare	125	589.58	125	546.51
Zizipus spina-christia	250	452.66	250	403.2
Senna indica	125	204.69	250	452.49
Salvia verbenaca	64	73.65	64	114.36
Lawsonia inermis	250	308.11	250	334.28
Aloe vera	250	598.7	125	250.95
Datura inoxia	16	54.98	16	110.22
Curcuma xanthorrhiza	250	374.84	64	92.61
Curcuma zedoaria	64	75.33	125	282.69
Curcuma longa	32	45.88	32	50.13
Zingiber officinale	125	218.31	125	264.96
Andrographis paniculata	125	569.18	64	125.57
Anredera cordifolia	64	77.99	64	112.62
Typhonium flagelliforme	250	729.23	250	274.65
Phyllanthus niruri	125	426.17	64	120.73
Allium sativum	250	324.86	250	442.66
Coriandrum sativum	500	585.24	500	813.63
Momordica charanthia	250	667.39	125	297.27

*Concentrations are expressed as µg/ml. MTC: Minimum toxic concentration. CC_{50}: 50% cytotoxic concentration.

effective on both cell lines down to the concentration of 16 µg/ml microscopically and 54.98, 110.22 µg/ml as determined by CTB assay on Vero and HEp-2 cells, respectively. In contrary, *C. colocynthis* extract was much potent on HEp-2 cells (MTC: 16 µg/ml; CC_{50}: 37.78) than on Vero cells (MTC: 64 and 109.39 µg/ml).

DISCUSSION

Medicinal plants remain a major source for the development of new anticancer drugs (Fouchea et al., 2008). Recent scientific research has shown that many plants used in traditional medicine are potentially toxic, allergic, mutagenic, and/or carcinogenic (Ahmad et al., 2006; Akintonwa et al., 2009). Therefore, *in vitro* cytotoxic evaluation programs are important to obtain effective anticancer agents that have certain desirable properties such as: little or no toxic effects on normal cells; high efficacy on multiple sites; capability of oral consumption; known mechanism of action; low cost, and acceptance by human population (Aziz et al., 2003). Cytotoxicity testing of medicinal plants is not only important to evaluate and validate the safety of medicinal plants for traditional use, but also provides guidance in the search for new active compounds.

In the present study, the cytotoxicity of 30 plant extracts was evaluated Vero and HEp-2 cells indicating variable degrees of cellular degeneration. The extracts of *J. phoenicea* and *C. procera* showed the most powerful cytotoxicity in both cell lines (Figure 2). Comparable results were previously obtained by testing the cytotoxic activity of the essential oils of *J. phoenicea* leaves and berries on a variety of cancer cell lines derived from brain (U251), lung (H460), liver (HepG2), breast (MCF7) and cervical (Hela) tissues (El-Sawi et al., 2007). Ethanolic root extracts of wild *C. procera* also showed, marked *in*

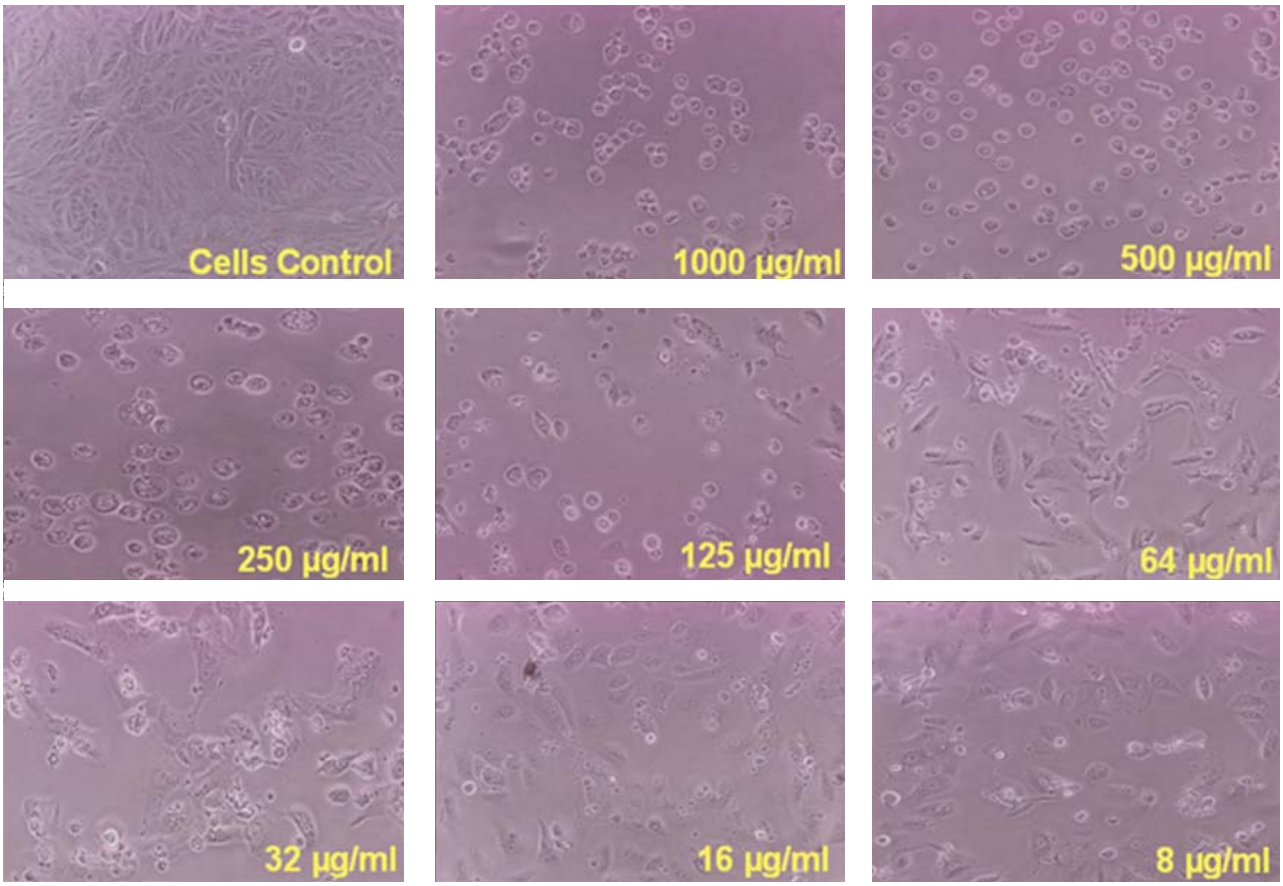

Figure 2. A tracking record of the morphological changes Vero cells after treatment with *J. phoenicea* extract (representation for the effect of highly cytotoxic group of plant extracts on both cell lines).Various concentrations of the extract, ranging from 1000 to 8 µg/ml, were incubated with Vero cells for 72 h. Cellular alterations were recorded and photographs were captured using inverted microscope at 100 magnification (Eclipse TS100, Nikon, Japan).

vitro cytotoxicity against cancer cells of oral (KB) and nervous (SNB-78) origin at three different concentrations; 10, 30 and 100 µg/ml (Bhagat et al., 2010). Moreover, the results obtained by Murti et al. (2012) demonstrated that leaves of *C. procera* extracted by N-butanol possess strong cytotoxic activity against HEp-2 cells with a mean CC_{50} value of 3.7 µg /ml.

In this regard, it is worthy to mention that the mean CC_{50} value of both extracts in our analysis were lower than 30 µg/ml (5.95 and 6.63 Vero cells, and 4.9 and 29.79 on HEp-2 cells for *J. phoenicea* and *C. procera*, respectively). According to the standards of U.S. National Cancer Institute (NCI), the CC_{50} value of a good anticancer candidate should be lower than 30 µg/ml to avoid unspecific effects (Suffness and Pezzuto, 1990). Therefore, these two extracts may be considered as promising candidates for further evaluation against different kinds of tumors both *in vitro* and *in vivo.* Although the CC_{50} values of *Datura inoxia* and *C. colocynthis* extracts are exceeding 30, their potent cytotoxic effect may be considered for further evaluation using other cell types. The same theory could be applied on certain extracts of the moderately cytotoxic group, which are capable to induce cytotoxicity down to a concentration of 32 µg/ml (microscopically) and 72 µg/ml (using CTB assay) like *Curcuma longa, Capparis cartilaginea, Salvia vertbenaca* and *Anredera cordifolia*.

Phytochemical analysis of *J. phoenicea* and *Calotropis procera* extracts indicated the existence of different potential compounds such as flavonoids, alkaloids, tannins, saponins and phenolics (Mossa et al., 1991; Qnais et al., 2005; Hayouni et al., 2007; Moronkola et al., 2011; Medini et al., 2013). Flavonoid and phenolic compounds are important groups of medicinal plant metabolites which have various biological activities in the control of cancer and other human diseases (Ghasemzadeh and Ghasemzadeh, 2011). Flavonoids are widely distributed natural products of medicinal plants with more than 2000 different active compounds of wide-spectrum potential (example flavonos, flavanones, flavonols, anthocyanidins and isoflanos) (Robbers et al., 1996). Whereas, certain phenolic compounds, like pyrogallol and catechol showed powerful antimicrobial activity in different study sets (Mason and Wasserman, 1987). Further experiments are

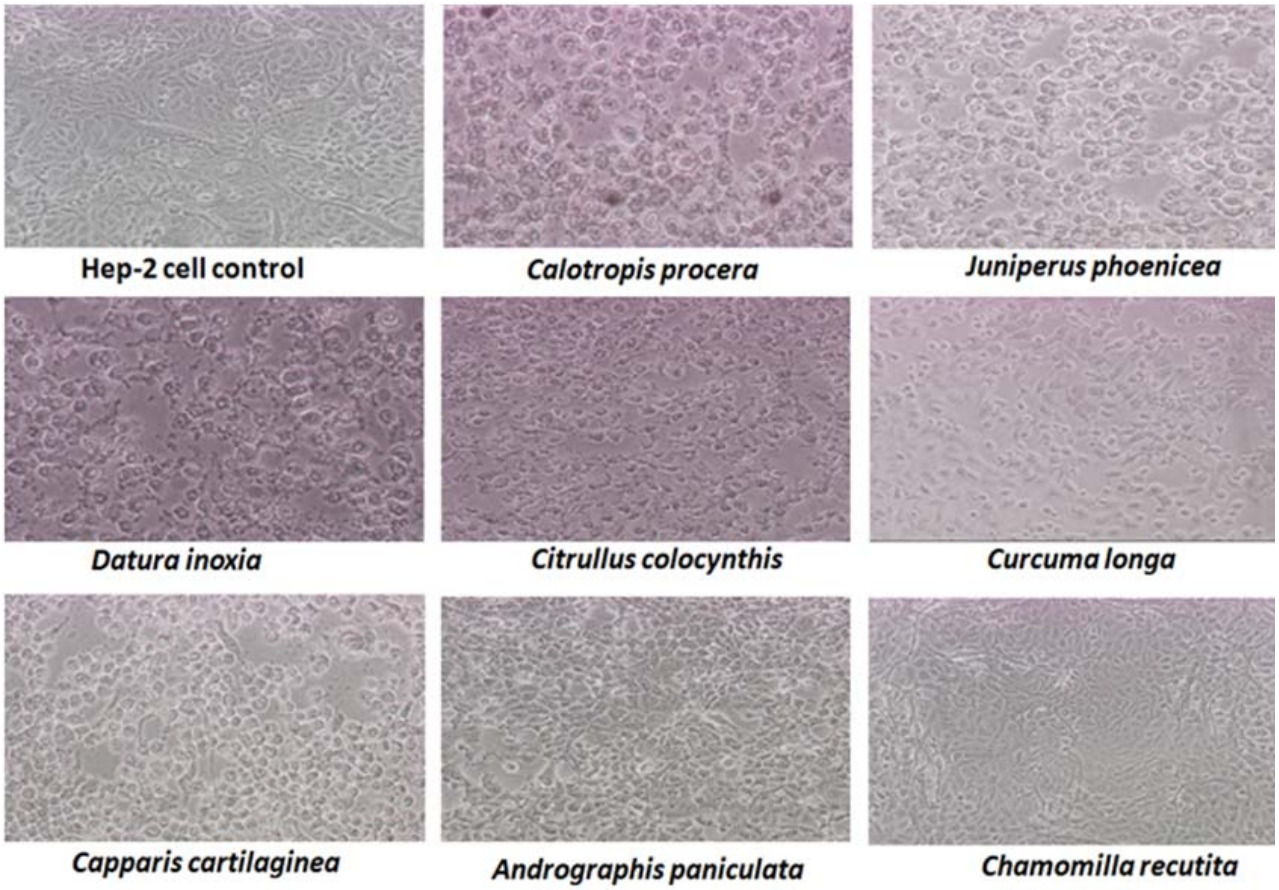

Figure 3. Morphological changes of Hep-2 cells after treatment with different plant extracts. Cells were incubated with the extracts of *C. procera, J. phoenicea, D. inoxia, C. colocynthis, C. longa, C. cartilaginea, A. paniculata* and *C. recutita* at a concentration of 64 µg/ml for 72 h. Cellular alterations were recorded in comparison to untreated cells and photographs were captured using inverted microscope at 100× magnification (Eclipse TS100, Nikon, Japan).

required to isolate pure active compounds from these plant extracts of high cytotoxicity and to determine their anticancer activities using different cancer cell lines and animal models.

A recent study demonstrated that *Datura inoxia* leaves, which are extracted using organic solvents like petroleum ether, are five times more toxic than aqueous extracts in mice (Kutaifa et al., 2012). The superior toxicity of organic extracts was proposed as regarded to the presence of active compounds such as flavonoids and essential oils that are soluble in organic solvents but not in water. However, other investigators claimed that aqueous extracts can induce apoptosis of cancer cells through activation of caspase-3 and -9, and suppression of vascular endothelial growth factor (VEGF) and tumor necrosis factor-alpha (TNF-α) (Pandey et al., 2011). The potent cytotoxic effect of methanolic extracts of *Datura inoxia* in cell culture in the current study further potentiate the concept of using organic solvents for such purpose. However, more comprehensive studies are necessary to justify the role of different active compounds of both types of extracts in combating cancer cells. On the other hand, the selective cytotoxicity of *C. colocynthis* on HEp-2 cells is an observation that was recorded before using the ethanolic extract at a concentration of 100 µg/ml (Afshari et al., 2005). This may suggest a degree of affinity toward epithelial cell carcinomas. Inclusion of several cell lines of different sources will justify this speculation empirically. The majority of the plant extracts tested in this study (18 out of 30) showed MTC and CC_{50} values that did extend below the concentration of 125 µg/ml (Tables 2 and 3). This level of cytotoxicity leaves considerable range of non-cytotoxic concentrations that enable testing of such extracts on both cell lines for potential antiviral activity. Vijayan et al. (2004) demonstrated that the replication of herpes simplex virus on Vero cells was completely inhibited by the extracts of *Hyparicum mysorense and Hypericum hookerianum* at concentrations of 100 and 50 µg/ml, respectively. Similarly, the extracts of *Hydroclathrus clathrus* and *Lobophora variegata* held up the infectivity of respiratory syncytial virus on HEp-2 cells at concentrations of 100 and 25 µg/ml, respectively

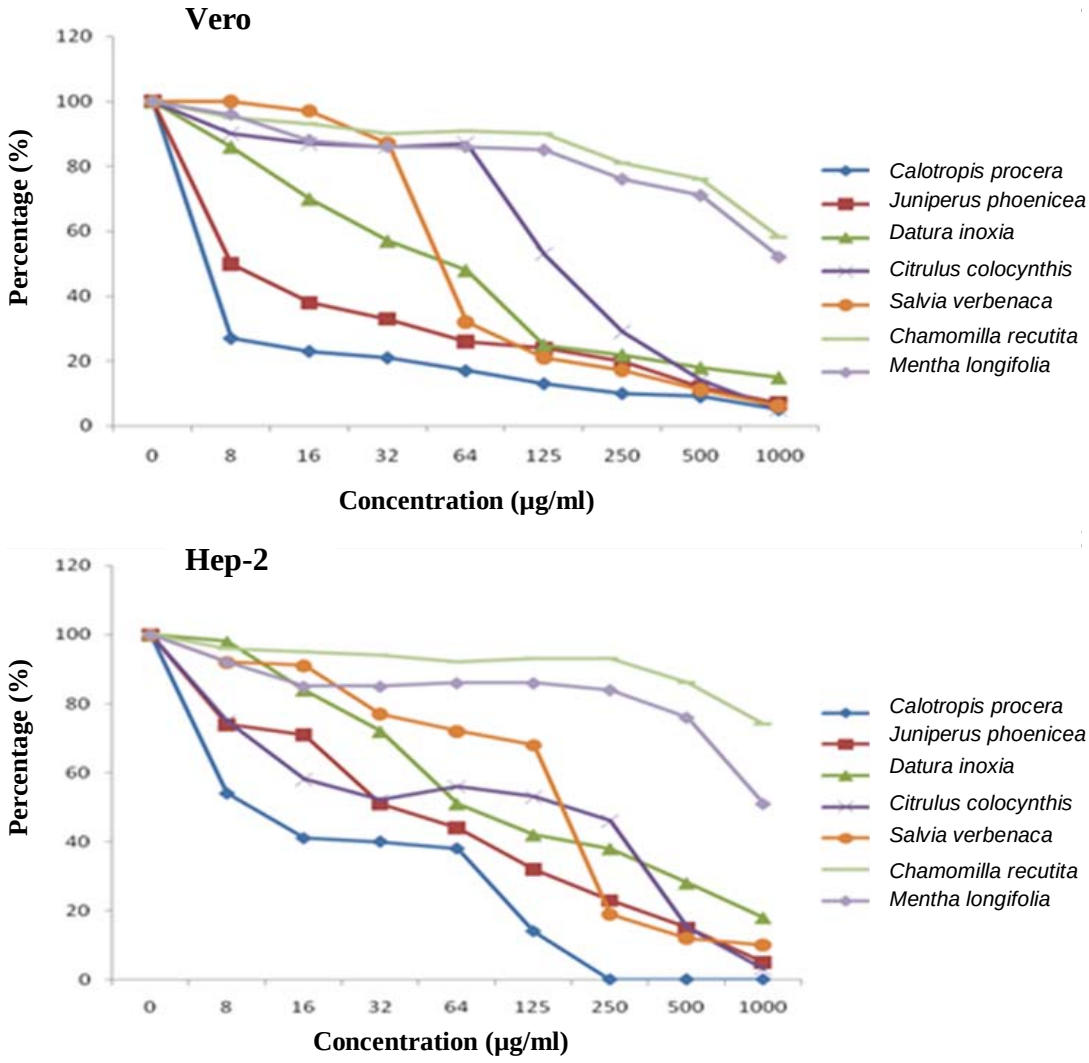

Figure 4. Cell viability plot of representative plant extracts as measured by CTB assay. Vero and Hep-2 cells were incubated for 72 h with extracts of *Calotropis procera, Juniperus phoenicea, Datura inoxia, Citrullus colocynthis, Salvia berbenaca, Chamomilla recutia* and *Menta longifolia.* Cell viability was determined for each extract at various concentrations ranging from 1000 to 8 µg/ml using cell titer blue assay.

(Wang et al., 2008).

Conclusion

The cytotoxic activity of 30 medicinal plants harvested from Saudi Arabia and Indonesia was evaluated Vero and HEp-2 cells. The extracts of *J. phoenicea* and *C. procera* leaves were highly cytotoxic on both cell lines even with minute concentrations, while the extracts of *D. inoxia* and *C. colocynthis* were cytotoxic down to the concentration of 16 µg/ml, with selective effect of the latter on HEp-2 cells. The majority of extracts showed cytotoxic activity of limited extent, which allows the use of both cell types as models for evaluating the potential effect of these plant extracts against viral infections.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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