



King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa  
www.sciencedirect.com



ORIGINAL ARTICLE

# Assessment of biological activity and UPLC–MS based chromatographic profiling of ethanolic extract of *Ochradenus arabicus*



M. Ajmal Ali <sup>a,\*</sup>, M. Abul Farah <sup>b,1</sup>, Fahad M. Al-Hemaid <sup>a</sup>,  
Faisal M. Abou-Tarboush <sup>b</sup>, Khaled M. Al-Anazi <sup>b</sup>, S.M. Wabaidur <sup>c</sup>,  
Z.A. Alothman <sup>c</sup>, Joongku Lee <sup>d</sup>

<sup>a</sup> Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

<sup>b</sup> Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

<sup>c</sup> Advanced Materials Research Chair, Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

<sup>d</sup> Department of Environment and Forest Resources, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

Received 22 November 2014; revised 23 January 2015; accepted 7 February 2015

Available online 14 February 2015

## KEYWORDS

*Ochradenus arabicus*;  
Cytotoxicity;  
ROS;  
Oxidative stress;  
UPLC–MS

**Abstract** Natural products from wild and medicinal plants, either in the form of crude extracts or pure compounds provide unlimited opportunities for new drug leads owing to the unmatched availability of chemical diversity. In the present study, the cytotoxic potential of crude ethanolic extract of *Ochradenus arabicus* was analyzed by MTT cell viability assay in MCF-7 adenocarcinoma breast cancer cells. We further investigated its effect against oxidative stress induced by anticancer drug doxorubicin. In addition, Ultra Performance Liquid Chromatography–Mass Spectrometry (UPLC–MS) based chromatographic profiling of crude extract of *O. arabicus* was performed. The MTT assay data showed that the extract is moderately toxic to the MCF-7 cells. However, its treatment alone does not induce oxidative stress while doxorubicin increases the level of oxidative stress in MCF-7 cells. Whereas, simultaneous treatment of plant extract and doxorubicin significantly ( $p < 0.05$ ) decreased the level of intracellular reactive oxygen species (ROS) and lipid peroxidation while an increase in the reduced glutathione and superoxide dismutase activity was observed in time and dose dependent manner. Hence, our finding confirmed cytotoxic and

\* Corresponding author. Tel.: +966 11 46 75734.

E-mail address: [alimohammad@ksu.edu.sa](mailto:alimohammad@ksu.edu.sa) (M.A. Ali).

<sup>1</sup> The first and second authors provided an equal contribution to this paper.

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

antioxidant potential of crude extract of *O. arabicus* in MCF-7 cells. However, further investigations on *O. arabicus* as a potential chemotherapeutic agent are needed. The analysis of bioactive compounds present in the plant extracts involving the applications of common phytochemical screening assays such as chromatographic techniques is discussed.

© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

Cancer (a leading cause of death worldwide) is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. Breast cancer ranks second as a cause of cancer death in women (Stewart and Wild, 2014; American Cancer Society, 2014). There has long been standing interest in the identification of natural products for the treatment of various diseases for thousands of years. Natural products possess immense pharmacological significance in the development of drugs (Dixon et al., 2007; Baker et al., 2007; Harvey, 2008) including cancer (Graham et al., 2000; Figueroa-Hernández et al., 2005; Madhuri and Pandey, 2009; Tan et al., 2011; Newman and Cragg, 2012), and were discovered through plant bioprospecting (Mann, 2002). Chemotherapy and radiotherapy are highly effective methods of cancer treatment but these methods exert severe side effects (Qi et al., 2010). Hence, the identification of novel natural products that possess better effectiveness against cancer, but less harmful effects has become desirable (Lachenmayer et al., 2010), and therefore, natural products are continuously being explored worldwide.

A body of clinical and experimental evidence suggests that oxidative stress is implicated in the onset and progression of many health problems (Nakabeppu et al., 2004; Hybertson et al., 2011). The imbalance between antioxidant defense and oxidant production in cells may affect signaling pathways for biologic processes and disrupt cellular function. Reactive oxygen species (ROS) are generated as by-products of cellular metabolism which are responsible for the alteration of macromolecules (Shieh et al., 2010). Dietary factors and natural antioxidants that reduce the impact of ROS can protect DNA damage and thus reduce the risk of cancers (Ke et al., 2013). The floral elements of unique arid plant biodiversity of Saudi Arabia are being practiced in folk medicine since ancient times (Rahman et al., 2004). Plants that grow under harsh desert stress conditions produced a high concentration of secondary metabolites that impart a wide range of pharmacological effects (Harlev et al., 2012).

Earlier phytochemical investigations on the members of Resedaceae such as *Caylusea*, *Reseda* and *Ochradenus* reveals kaempferol, quercetin, isorhamnetin and luteolin as a major flavonoid glycosides (Barakat et al., 1991; Cristea et al., 2003; Moiteiro et al., 2008; Marques et al., 2009; Berrehal et al., 2010; Villela et al., 2011). The use of extracts of members of the genus *Ochradenus* in folk medicine (Nawash and Al-Horani, 2011), antibacterial (Abutbul et al., 2005), anti-malarial (Sathiyamoorthy et al., 1999) and anticancer activity (Thoppil et al., 2013) has previously been reported. *Ochradenus arabicus* Chaudhary, Hillc. & A.G.Mill., is a shrub, distributed in desert regions of the Middle East i.e. Saudi Arabia, Yemen, Oman and UAE (Chaudhary, 1999). The biological activity and phytochemical screening of *O. arabicus*

is lacking. Hence, in continuation of our efforts to study the wild plants from desert regions, the present study aims to unravel the cytotoxic and antioxidant potential against MCF-7 adenocarcinoma breast cancer cells *in vitro* and chromatographic profiling of ethanolic extract of *O. arabicus*.

## 2. Materials and methods

### 2.1. Plant material and preparation of crude extracts

The plant material of *O. arabicus* was collected from wild habitat during plant explorations in Wadi Hanifa, Riyadh (Saudi Arabia). The taxonomic identification was confirmed through consultation of Flora of Saudi Arabia (Chaudhary, 1999). The collected plant materials were rinsed thoroughly with tap water to remove extraneous contaminants and cut into small pieces, oven-dried at 50 °C until stability of dry weight was observed, and then grounded into powder form with an electric-grinder. Crude extract was prepared by macerating the powdered plant material (1000 g) in 95% ethanol at room temperature for one week. The OA (*O. arabicus*) extracts were then filtered and the volume of the filtrate was reduced using rotary evaporator at low temperature and pressure. The crude extract was weighed and stored at –20 °C until used.

### 2.2. Cell culture methods

The human breast adenocarcinoma cells (MCF-7) procured from ATCC (Rockville, MD, USA) were used in this study. The cells were cultured in a humid environment at 37 °C and 5% CO<sub>2</sub> in a cell culture medium called minimum essential medium (MEM, Invitrogen, USA) supplemented with 15% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Invitrogen, USA). At 85–90% confluence, cells were harvested using 0.25% trypsin/EDTA solution and sub-cultured into 6-well plate or 96-well plate according to the requirement of experiments.

### 2.3. Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay developed by Mosmann (1983) with modification was used to screen the cytotoxic activity. Briefly, the MCF-7 cells ( $1 \times 10^4$  cells/well) were grown for overnight in 96-well flat bottom culture plates. The crude ethanolic extract of *O. arabicus* was initially dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 100 mg/ml. Then, it was further diluted into 1.0 mg/ml by adding complete cell culture medium, and serially diluted 2-fold with same medium to obtain the working solutions of six concentrations i.e. 1.0 mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.12 mg/ml,

0.06 mg/ml and 0.03 mg/ml. The cells were then exposed to above concentrations of *O. arabicus* extract for 24 h. In addition, negative/vehicle control, and 50  $\mu$ M doxorubicin (Sigma Aldrich, St. Louis, MO, USA) as positive control were also used for comparison. After the completion of desired treatment, 10  $\mu$ l of MTT reagent (Invitrogen, USA) prepared in 5.0 mg/ml Phosphate Buffered Saline (PBS) was added to each well and further incubated for 3 h at 37 °C. Finally, medium with MTT solution was removed, and 200  $\mu$ l of DMSO (Sigma Aldrich, St. Louis, MO, USA) was added to each well and further incubated for 20 min. The optical density (OD) of each well was measured at 550 nm using a microplate reader (Synergy, BioTek, USA). The results were generated from three independent experiments. Each experiment was performed in triplicate. The percentage of cytotoxicity compared to the untreated cells was determined. Further, MTT assay was performed with six narrow ranges of concentrations (i.e. 800  $\mu$ g/ml, 700  $\mu$ g/ml, 600  $\mu$ g/ml, 500  $\mu$ g/ml, 400  $\mu$ g/ml and 300  $\mu$ g/ml) for the determination of IC<sub>50</sub> value (concentration at which 50% cell proliferation inhibited).

#### 2.4. Experimental design

To analyze the oxidative stress, MCF-7 cells were exposed to either OA extract (300  $\mu$ g/ml) or doxorubicin (50  $\mu$ M), including negative control for a period of 12 and 24 h. Further, to study the antioxidant potential, cells were treated with biologically safe concentrations (50, 100 and 150  $\mu$ g/ml) of extract before 1 h, then were subjected to receive doxorubicin (50  $\mu$ M) for 12 and 24 h. At the end of the exposure, reactive oxygen species (ROS) generation, lipid peroxidation (LPO), superoxide dismutase (SOD) and glutathione (GSH) levels were determined.

#### 2.5. Intracellular reactive oxygen species (ROS) measurement

The generation of intracellular ROS was monitored using 2,7-dichlorofluorescein diacetate dye (DCFH-DA) (Wang and Joseph, 1999). The DCFH-DA passively enters the cell where it reacts with ROS to form the highly fluorescent compound dichlorofluorescein (DCF). For the quantitative estimation of intracellular ROS by spectrofluorometry,  $1 \times 10^4$  cells per well were seeded in 96-well culture plates (black-bottomed) and allowed to grow for overnight in a CO<sub>2</sub> incubator at 37 °C. Then, cells were treated as mentioned above for 12 and 24 h at 37 °C. At the end of respective treatment period, cells were washed twice with PBS and then incubated with 20  $\mu$ M working solution of DCFH-DA in serum free medium at 37 °C for 30 min. The reaction mixture was discarded and replaced by 100  $\mu$ l of PBS in each well. The green fluorescence intensity was detected using a Synergy microplate reader (Bio-Tek, Winooski, VA, USA) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The values were averaged from multiple wells and expressed as percent of fluorescence intensity relative to the control wells.

#### 2.6. Lipid peroxidation assay

The level of membrane lipid peroxidation was estimated by measuring the formation of malondialdehyde using the method of Ohkawa et al. (1979). Malondialdehyde (MDA) is

one of the products of membrane lipid peroxidation. Briefly, MCF-7 cells at a final density of approximately  $1 \times 10^5$  in a 25 cm<sup>2</sup> culture flask were grown for overnight and exposed to OA extract and doxorubicin either alone or in combination as indicated above for 12 and 24 h. At the end of exposure, the cells were washed twice with cold phosphate-buffered solution (PBS) and collected by centrifugation. 200  $\mu$ l of cell suspension was mixed with 1 ml of chromogen mixture containing 25 mM thiobarbituric acid, 0.5% of sodium dodecyl sulfate (w/v) and 5% acetic acid (v/v), pH 3.5. The sample mixture was incubated in boiling water for 30 min. Then it was cooled at room temperature and centrifuged at 2500 rpm for 5 min. The absorbance of the supernatant was read at 534 nm against blank and MDA standard. Lipid peroxidation was presented as relative percent of control.

#### 2.7. Glutathione (GSH) content

The GSH content was estimated by calorimetric method as described earlier by Beutler et al. (1963) using a commercial kit (Biodiagnostic) following the instructions provided in the kit. Briefly, after the respective exposure, MCF-7 cells were scrapped and collected by centrifugation. The cell pellet was homogenized in a cold buffer (50 mM potassium phosphate, pH 7.5 containing 2 mM EDTA). The samples were then centrifuged at 4000 rpm for 15 min at 4 °C and supernatant was collected. Then, 100  $\mu$ l of supernatant was mixed with 500  $\mu$ l of trichloroacetic acid (TCA, 500 mM) and kept for 5 min at room temperature. The above mixture was centrifuged at 3000 rpm for 15 min and 500  $\mu$ l of supernatant was mixed with 1 ml buffer and 100  $\mu$ l of 5,5'-dithionitrobenzoic acid (DTNB, 1 mM) and incubated for 10 min at 37 °C in water bath with shaking. The absorbance of yellow color developed was read at 405 nm using multiplate reader (Synergy, BioTek, USA).

#### 2.8. Measurement of superoxide dismutase (SOD) level

Superoxide dismutase activity was estimated employing a colorimetric method described by Nishikimi et al. (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulfate mediated reduction of nitroblue tetrazolium dye. Briefly, after the respective exposure, MCF-7 cells were scrapped and collected by centrifugation. The cell pellets were lysed in cell lysis buffer containing 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM Na<sub>2</sub>EDTA, and 1% Triton X-100. The cells were centrifuged at 12,000 rpm for 10 min at 4 °C. Then, 100  $\mu$ l of supernatant was mixed with 1 ml of the assay mixture that contained 50 mM phosphate buffer, 1 mM nitroblue tetrazolium, 1 mM reduced NADH. Finally, the reaction was initiated by adding 100  $\mu$ l phenazine methosulfate (0.1 mM) and increase in absorbance was measured at 560 nm for 5 min. The results were presented as relative percent of control.

#### 2.9. UPLC–MS based chromatographic profiling

Ultra Performance Liquid Chromatography–Mass Spectrometry (UPLC–MS) is an advanced technique for plant metabolite profiling. It allows the identification and quantification of a large range of common plant metabolites in a single chromatogram. Therefore, to explore the types of metabolites that could be present; the OA extract was screened through

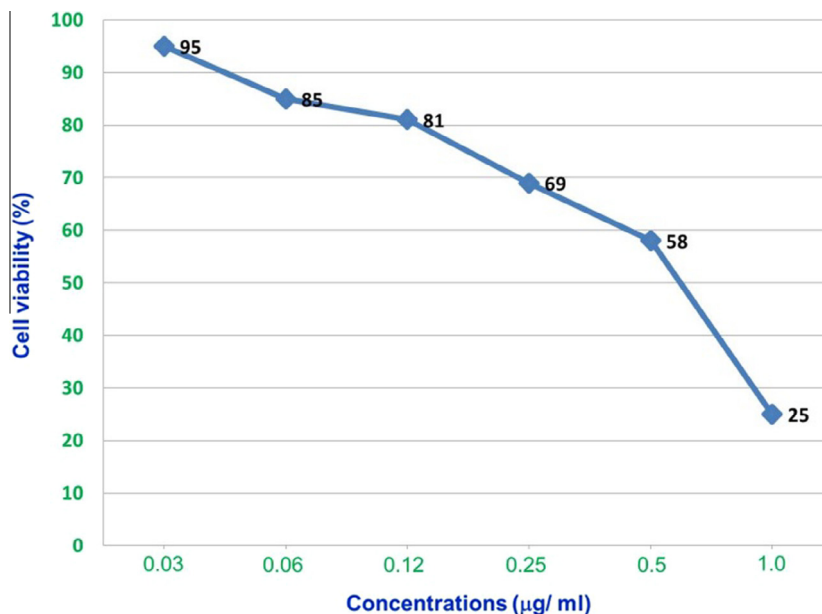
Acquity UPLC system fitted with an electrospray ionization (ESI) interface (Waters, Manchester, UK). The screening was performed in both positive and negative ESI mode. The Software Masslynx 4.1 was used for data acquisition.

### 2.10. Statistical analysis

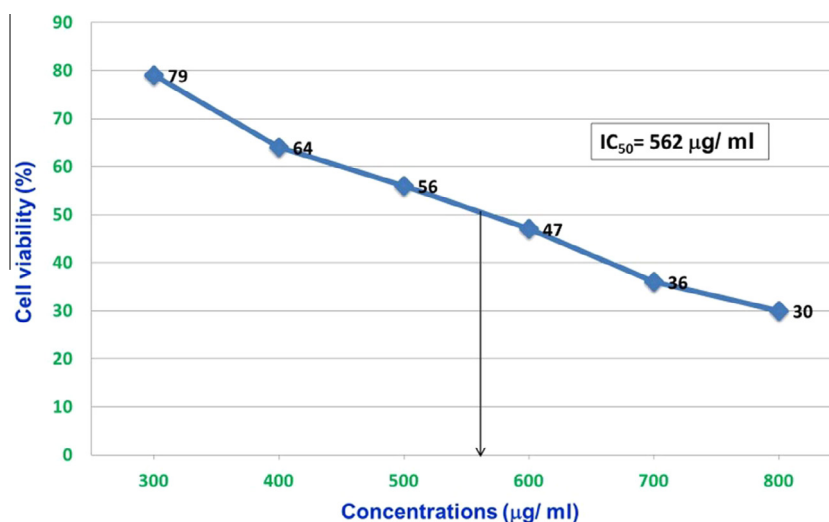
All experiments were carried out with three replicates and values are presented as mean  $\pm$  standard error of mean (SEM). Microsoft Office Excel was used for calculation and plotting of mean and standard deviation estimates in the graphs. The data were statistically analyzed by Student's *t*-test applying a significance level of  $p < 0.05$ .

### 3. Results and discussion

In the present study, an initial screening of OA extract was performed against human breast adenocarcinoma cells. The percentage viability of cells was 25% at a maximum concentration of 1 mg/ml, while the lowest concentration (0.03 mg/ml) induced 15% inhibition of cell proliferation (Fig. 1). Based on these results, the OA extracts were subjected to  $IC_{50}$  determination by applying narrow range of concentrations. The relative number of viable cells as a percentage of control was calculated, considering the absorbance at 550 nm for the control as 100%. The  $IC_{50}$  value was graphically obtained by plotting the percentage growth inhibition against the



**Figure 1** Inhibition of MCF-7 cell proliferation by crude ethanolic extract of *Ochradenus arabicus*. Cells were treated with indicated concentrations of extract for 24 h, and cell viability was determined by the MTT assay.



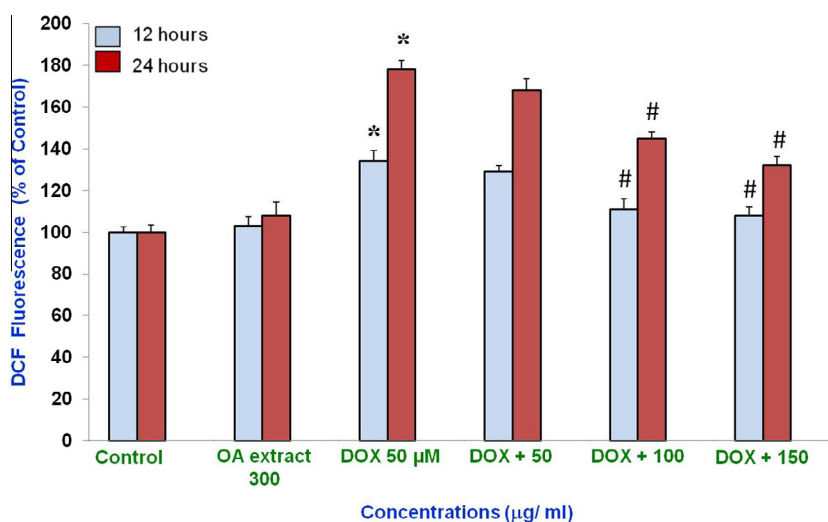
**Figure 2** Inhibition of MCF-7 cell proliferation by crude ethanolic extract of *Ochradenus arabicus*. Cells were treated with indicated concentrations of extract for 24 h, and cell viability was determined by the MTT assay. The  $IC_{50}$  value was estimated at 562  $\mu\text{g/ml}$  (indicated by arrow).

corresponding different concentrations of the test compound used. The OA extracts showed cytotoxicity at IC<sub>50</sub> value of approximately 562 µg/ml (Fig. 2), while, doxorubicin showed 35% growth inhibition at 50 µM concentrations (data not shown).

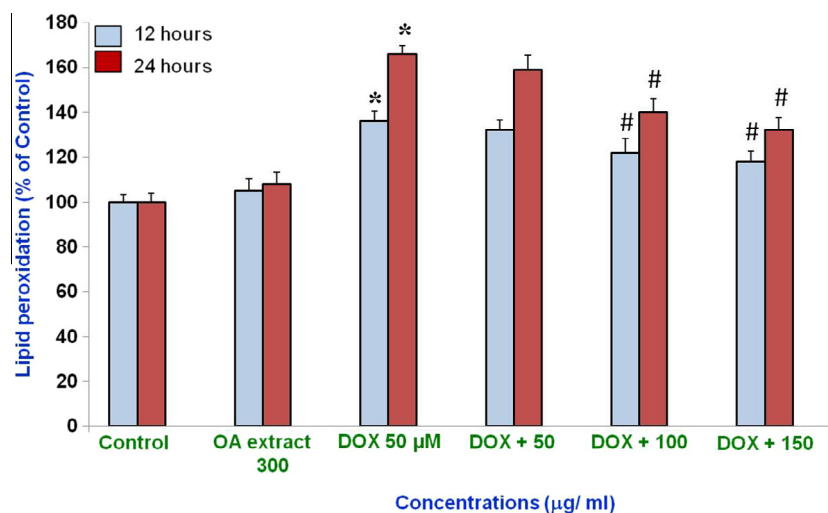
The MCF-7 cells were treated with either doxorubicin or OA extract alone or in combination for 12 and 24 h respectively and intracellular ROS generation was quantitatively measured by HDCF-DA assay using spectrofluorometry. The results (Fig. 3) revealed that OA extract alone was insignificant in generation of ROS while doxorubicin treatment significantly increased the level of ROS up to 1.34 and 1.78-fold in 12 and 24 h duration respectively. On the contrary, the simultaneous treatment reduced significantly ( $p < 0.05$ ) the level of ROS at 100 and 150 µg/ml. The lowest concentration of OA extract

(50 µg/ml) was found not to be effective. However, the exposure of OA extract significantly prevented the ROS induced by doxorubicin in MCF-7 cells. The potential of OA extract on doxorubicin induced lipid peroxidation in MCF-7 cells is summarized in Fig. 4. As shown in figure, exposure of 50 µM doxorubicin resulted in a significant ( $p < 0.05$ ) increase in membrane lipid peroxidation of 1.33 and 1.66-fold at 12 and 24 h respectively, as compared to the control cells. While, OA extract alone did not induce lipid peroxidation. Whereas, co-exposure of OA extract significantly reduced the lipid peroxidation specifically at higher duration and concentration.

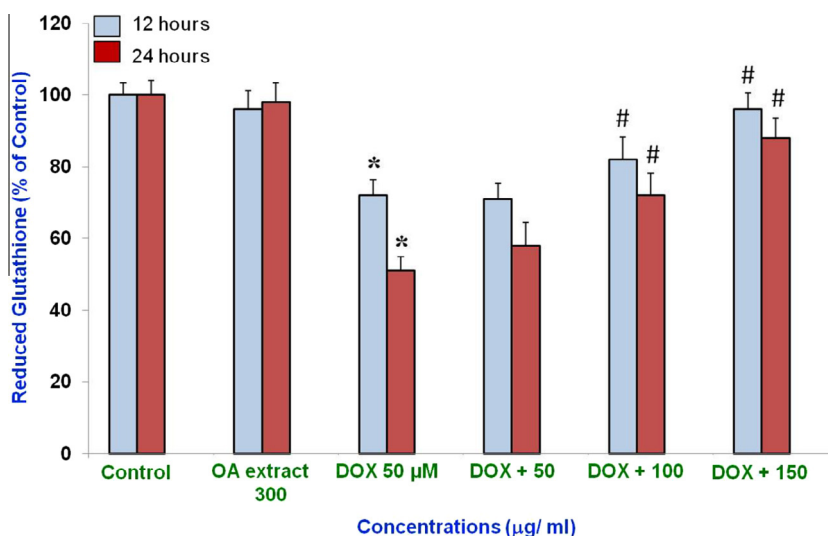
The effect of OA extract on the depletion of glutathione induced by doxorubicin exposure is presented in Fig. 5. The results clearly indicated that treatment with doxorubicin at 50 µM reduced the glutathione level up to 28% and 49% after



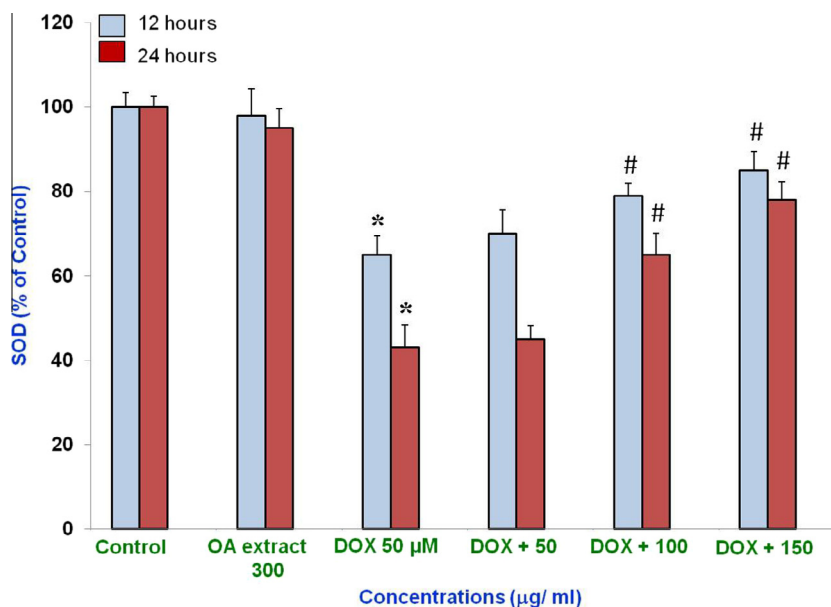
**Figure 3** Percentage of DCF fluorescence for detection of ROS in MCF-7 cells after exposure of *Ochradenus arabicus* extract and doxorubicin either alone or in combination for 12 h and 24 h. Each value represents the mean  $\pm$  SE of three experiments ( $P < 0.05$ ). \*indicates significant from Control, #indicates significant protective effects from doxorubicin.



**Figure 4** Levels of lipid peroxides in MCF-7 cells after exposure of *Ochradenus arabicus* extract and doxorubicin either alone or in combination for 12 h and 24 h. Each value represents the mean  $\pm$  SE of three experiments ( $P < 0.05$ ). \*indicates significant from Control, #indicates significant protective effects from doxorubicin.



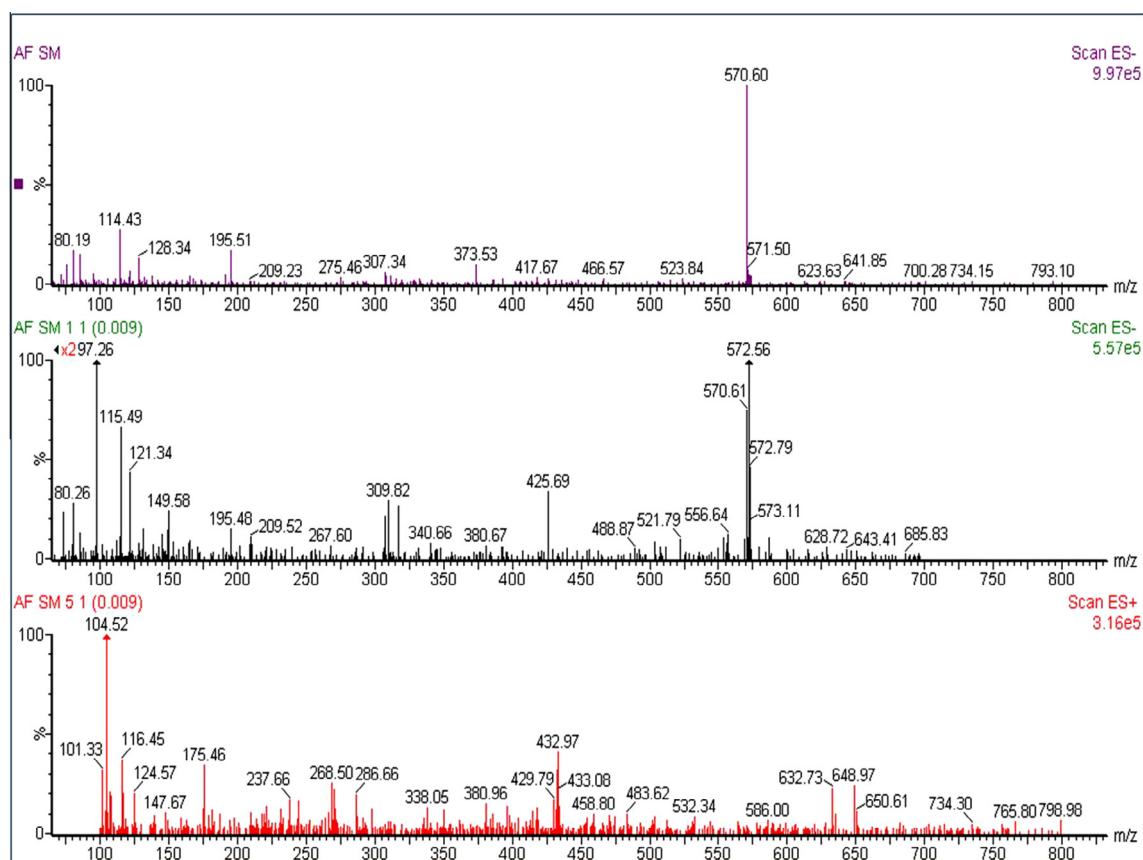
**Figure 5** Depletion in glutathione levels in MCF-7 cells after exposure of *Ochradenus arabicus* extract and doxorubicin either alone or in combination for 12 h and 24 h. Each value represents the mean  $\pm$  SE of three experiments ( $P < 0.05$ ). \* indicates significant from Control, # indicates significant protective effects from doxorubicin.



**Figure 6** Levels of SOD activity in MCF-7 cells after exposure of *Ochradenus arabicus* extract and doxorubicin either alone or in combination for 12 h and 24 h. Each value represents the mean  $\pm$  SE of three experiments ( $P < 0.05$ ). \* indicates significant from Control, # indicates significant protective effects from doxorubicin.

12 and 24 h, respectively in MCF-7 cells. While OA extract found to be ineffective in changing the level of glutathione in these cells. Eventually, the co-exposure of OA extract resulted in the significant recovery of glutathione level. The level of glutathione reached up to 96% and 88% at 100 and 150, 50 µg/ml concentration. The exposure of OA extract alone did not induce any changes in the activity of SOD in MCF-7 cells. While treatment with 50 µM doxorubicin significantly reduced the activity of SOD after 12 and 24 h, which were determined as 65% and 43% respectively, when compared with the control (Fig. 6). On the other hand, exposure of OA extract significantly attenuated the SOD activity that was found to increase up to 85% and 78% after 12 and 24 h, respectively.

Doxorubicin is an anthracycline and potent antitumor drug widely used in the clinic for the treatment of a broad spectrum of cancers (Buzdar et al., 1985; Singal and Iliskovic, 1998). However, this drug continues to pose serious concern such as generation of ROS resulting in oxidative stress and cellular changes. Overproduction of ROS can cause oxidative damage to biomolecules (lipids, proteins, DNA) (Uttara et al., 2009). In the present study, doxorubicin increased significantly ROS level which leads to the changes in oxidative stress biomarkers in MCF-7 cells. However, the simultaneous treatment of MCF-7 cells with different concentrations of OA extract decreased the ROS generation and MDA level which is a biomarker of lipid peroxidation. Moreover, the levels of the



**Figure 7** UPLC–MS spectrum obtained by screening of ethanolic extract of *Ochradenus arabicus* in –Ve and +Ve ESI mode.

antioxidant molecule, glutathione, and the activity of the antioxidant enzymes, superoxide dismutase, were elevated as compared to doxorubicin treatment alone. These findings confirmed the antioxidant potential of OA extract in MCF-7 cells that could be attributed to the presence of major flavonoids such as kaempferol, quercetin, isorhamnetin and luteolin (Barakat et al., 1991; Cristea et al., 2003; Moiteiro et al., 2008; Marques et al., 2009; Berrehal et al., 2010; Villela et al., 2011). The majority of natural antioxidants are polyphenols including flavonoids, which exhibit strong antioxidant activity by acting as free radical scavengers, hydrogen donors, singlet oxygen quenchers, and metal ion chelators, in addition to inducing gene expressions of antioxidant enzymes (Krinsky, 1992; Rice-Evans, 2001).

The OA extract was diluted and filtered through a 0.22  $\mu\text{m}$  PVDF syringe filter (Membrane Solutions, Texas, USA) before being injected into the UPLC–MS system. The spectrum obtained by direct infusion (both in ESI +Ve and –Ve mode) of OA extract is shown in Fig. 7. The major peaks in the –Ve mode were observed at  $m/z$  ratio of 97.26, 115.49, 570.61 and 572.56 while in the +Ve mode a peak at  $m/z$  ratio of 104.52 was evident. Identification of the compounds could be done with the aid of ( $m/z$ ) ratio evaluation of the resulting data by searching against the spectral library.

So far the identification of candidate having anticancer potential from the arid floristic biodiversity of the Arabian gulf region in general is concern; the exhaustive survey of literature reveals that there are such limited documentation (Amin and Mousa, 2007; Mothana et al., 2009), and wild plant of Saudi Arabia in particular have previously been poorly explored

except some latest reports on some medicinal and wild plants (Almehdar et al., 2012; Elkady, 2013; Ali et al., 2014), and the perusal of literature also reveals that *O. arabicus* have not been previously included in any plant bioprospecting program for biological activity elsewhere. In Conclusion, for the first time we have shown that ethanolic extract of *O. arabicus* is moderately toxic to the MCF-7 cells and confirmed its antioxidant potential. However, further investigations on *O. arabicus* as a potential chemotherapeutic agent are needed.

#### Acknowledgments

Research supported by the King Saud University, Deanship of Scientific Research, College of Science, Research Center.

#### References

- Abutbul, S., Golan-Goldhirsh, A., Barazani, O., Ofir, R., Zilberg, D., 2005. Screening of desert plants for use against bacterial pathogens in fish. *Isr. J. Aquacult. Bamid.* 57, 71–80.
- Ali, M.A., Farah, M.A., Al-Hemaid, F.M.A., Abou-Tarboush, F.M., 2014. *In vitro* cytotoxicity screening of some wild plants extracts from Saudi Arabia on human breast adenocarcinoma cells. *Genet. Mol. Res.* 13 (2), 3981–3990.
- Almehdar, H., Abdallah, H.M., Osman, A.M., Abdel-Sattar, E.A., 2012. *In vitro* cytotoxic screening of selected Saudi medicinal plants. *J. Nat. Med.* 66, 406–412.
- American Cancer Society, 2014. *Cancer Facts & Figures 2014*. American Cancer Society, Atlanta.

- Amin, A., Mousa, M., 2007. Merits of anti-cancer plants from the Arabian Gulf Region. *Cancer Ther.* 5, 55–66.
- Baker, D.D., Chu, M., Oza, U., Rajgarhia, V., 2007. The value of natural products to future pharmaceutical discovery. *Nat. Prod. Rep.* 24 (6), 1225–1242.
- Barakat, H.H., El-Mousallamy, A.M.D., Souleman, A.M.A., Awadalla, S., 1991. Flavonoids of *Ochradenus baccatus*. *Phytochemistry* 30 (11), 3777–3779.
- Berrehal, D., Khalfallah, A., Kabouche, A., Kabouche, Z., Karioti, A., Bilia, A.R., 2010. Flavonoid glycosides from *Randonia africana* Coss. (Resedaceae). *Biochem. Syst. Ecol.* 38, 1007–1009.
- Beutler, E., Duron, O., Kelly, Bm., 1963. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61, 882–888.
- Buzdar, A.U., Marcus, C., Smith, T.L., Blumenschein, G.R., 1985. Early and delayed clinical cardiotoxicity of doxorubicin. *Cancer* 55 (12), 2761–2765.
- Chaudhary, S., 1999. Resedaceae. In: Chaudhary, S. (Ed.), *Flora of the Kingdom of Saudi Arabia*. Ministry of Agriculture and Water National Herbarium, National Agriculture and Water Research Center, Riyadh, Saudi Arabia, pp. 536–543.
- Cristea, D., Isabelle, B., Gérard, V., 2003. Identification and quantitative HPLC analysis of the main flavonoids present in weld (*Reseda luteola* L.). *Dyes Pigm.* 57, 267–272.
- Dixon, N., Wong, L.S., Geerlings, T.H., Micklefield, J., 2007. Cellular targets of natural products. *Nat. Prod. Rep.* 24, 1288–1310.
- Elkady, A.I., 2013. Crude alkaloid extract of *Rhazya stricta* inhibits cell growth and sensitizes human lung cancer cells to cisplatin through induction of apoptosis. *Genet. Mol. Biol.* 36, 12–21.
- Figueroa-Hernández, J.L., Sandoval, G.G., Ascencio, V.J., Figueroa-Espitia, J.L., Fernández Saavedra, G., 2005. Plant products with anticancer properties employed in the treatment of bowel cancer: literature review 1985 and 2004. *Proc. West. Pharmacol. Soc.* 48, 77–83.
- Graham, J.G., Quinn, M.L., Fabricant, D.S., Farnsworth, N.R., 2000. Plants used against cancer – an extension of the work of Jonathan Hartwell. *J. Ethnopharmacol.* 73, 347–377.
- Harlev, E., Nevo, E., Lansky, E.P., Lansky, S., Bishayee, A., 2012. Anticancer attributes of desert plants: a review. *Anticancer Drugs* 23, 255–271.
- Harvey, A.L., 2008. Natural products in drug discovery. *Drug Discov. Today* 13, 894–901.
- Hybertson, B.M., Gao, B., Bose, S.K., McCord, J.M., 2011. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol. Aspects Med.* 32 (4–6), 234–246.
- Ke, Y., Xu, X., Wu, S., Huang, J., Geng, Y., Misra, H., Li, Y., 2013. Protective effects of extracts from *Fructus rhodomyrti* against oxidative DNA damage in vitro and in vivo. *Oxid. Med. Cell. Longev.* 2013, 507407.
- Krinsky, N.I., 1992. Mechanism of action of biological antioxidants. *Proc. Soc. Exp. Biol. Med.* 200, 248–254.
- Lachenmayer, A., Alsinet, C., Chang, C.Y., Llovet, J.M., 2010. Molecular approaches to treatment of hepatocellular carcinoma. *Dig. Liver Dis.* 42 (Suppl. 3), S264–S272.
- Madhuri, S., Pandey, G., 2009. Some anticancer medicinal plants of foreign origin. *Curr. Sci.* 96, 779–783.
- Mann, J., 2002. Natural products in cancer chemotherapy: past, present and future. *Nat. Rev. Cancer* 2, 143–148.
- Marques, R., Sousa, M.M., Oliveira, M.C., Melo, M.J., 2009. Characterization of weld (*Reseda luteola* L.) and spurge flax (*Daphne gnidium* L.) by high-performance liquid chromatography-diode array detection-mass spectrometry in Arraiolos historical textiles. *J. Chromatogr. A* 1216 (9), 1395–1402.
- Moiteiro, C., Gaspar, H., Rodrigues, A., Lopes, J.F., Carnide, V., 2008. HPLC quantification of dye flavonoids in *Reseda luteola* L. from Portugal. *J. Sep. Sci.* 31, 3683–3687.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63.
- Mothana, R.A., Lindequist, U., Gruenert, R., Bednarski, P.J., 2009. Studies of the *in vitro* anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqatra. *BMC Complement. Altern. Med.* 9, 7.
- Nakabeppu, Y., Tsuchimoto, D., Furuichi, M., Sakumi, K., 2004. The defense mechanisms in mammalian cells against oxidative damage in nucleic acids and their involvement in the suppression of mutagenesis and cell death. *Free Radic. Res.* 38 (5), 423–429.
- Nawash, O.S., Al-Horani, A.S., 2011. The most important medicinal plants in Wadi Araba desert in South West Jordan: a review article. *Adv. Environ. Biol.* 5, 418–425.
- Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Prod.* 75, 311–335.
- Nishikimi, M., Appaji, N., Yagi, K., 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.* 46 (2), 849–854.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Annal. Biochem.* 95, 351–358.
- Qi, F., Li, A., Inagaki, Y., Gao, J., Li, J., Kokudo, N., Xiao-Kang, Li., Wei, T., 2010. Chinese herbal medicines as adjuvant treatment during chemo- or radio-therapy for cancer. *Biosci. Trends* 4 (6), 297–307.
- Rahman, M.A., Mossa, J.S., Al-Said, M.S., Al-Yahya, M.A., 2004. Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterapia* 75, 149–161.
- Rice-Evans, C., 2001. Flavonoid antioxidants. *Curr. Med. Chem.* 8, 797–807.
- Sathiyamoorthy, P., Lugasi-Evgi, H., Schlesinger, I., Kedar, J., Gopas, Y., Pollack, Golan-Goldhirsh, A., 1999. Screening for cytotoxic and antimalarial activities in desert plants of the Negev and Bedouin market plant products. *Pharm. Biol.* 37, 188–195.
- Shieh, P.C., Chen, Y.O., Kuo, D.H., Chen, F.A., Tsai, M.L., Chang, I.S., 2010. Induction of apoptosis by [8]-shogaol via reactive oxygen species generation, glutathione depletion, and caspase activation in human leukemia cells. *J. Agric. Food Chem.* 58, 3847–3854.
- Singal, P.K., Iliskovic, N., 1998. Doxorubicin-induced cardiomyopathy. *N. Engl. J.* 339 (13), 900–905.
- Stewart, B.W., Wild, C.P. (Eds.), 2014. *World Cancer Report 2014*. International Agency for Research on Cancer, Lyon, France.
- Tan, W., Lu, J., Huang, M., Li, Y., Chen, M., Wu, G., Gong, J., Zhong, Z., Xu, Z., Dang, Y., Guo, J., Chen, X., Wang, Y., 2011. Anti-cancer natural products isolated from Chinese medicinal herbs. *Chin. Med.* 6 (1), 27.
- Thoppil, R.J., Harlev, E., Mandal, A., Nevo, E., Bishayee, A., 2013. Antitumor activities of extracts from selected desert plants against HepG2 human hepatocellular carcinoma cells. *Pharm. Biol.* 51 (5), 668–674.
- Uttara, B., Singh, A.V., Zamboni, P., Mahajan, R.T., 2009. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr. Neuropharmacol.* 7, 65–74.
- Villela, A., van der Klift, E.J., Mattheussens, E.S., Derksen, G.C., Zuilhof, H., van Beek, T.A., 2011. Fast chromatographic separation for the quantification of the main flavone dyes in *Reseda luteola* (weld). *J. Chromatogr. A* 1218 (47), 8544–8550.
- Wang, H., Joseph, J.A., 1999. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic. Biol. Med.* 27 (5–6), 612–616.