Pak. J. Bot., 46(6): 2027-2034, 2014.

PHYTOCHEMICAL AND BIOLOGICAL ASSESSMENT OF MEDICINALLY IMPORTANT PLANT *OCHRADENUS ARABICUS*

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Abstract

Jabal Al-Akhdar (Oman) is one of diverse floral region of Arabian Peninsula. *Ochradenus arabicus,* is an important medicinal plant to local people of the area. However, little is known about its potential role in biological activities against various emerging ailments. The collected plant samples were extracted with methanol and fractionated into *n*-hexane (JOAH), ethyl acetate (JOAE), chloroform (JOAC), *n*-butanol (JOAB) and water (JOAAQ). Various concentrations of these fractions were tested for their antimicrobial, anticancer, antioxidant, antidiabetic, phenolics, flavonoids, allopathic and nutrition quality properties. The results showed that fruits and leaves of *O. arabicus* have higher levels of carbohydrate, crude fats, fibres, proteins, moisture, ash and energy values. In phytotoxic activities, JOAAQ inhibited the lettuce seed germination and growth. The anticancer activities of fractions showed that JOAE, JOAB and JOAAQ are potent to reduce the cancer cell viability of HT29, HCT116, HepG2 and MCF-7 lines with a concentration of 1000 µg/ml. JOAB showed a meagre activity of 12% in α-Glucosidase inhibition assay. The total phenolic and flavonoid contents were significantly higher in JOAE, which also resulted in higher DPPH radical scavenging activity as compared to other fractions and control. JOAE also exhibited higher antibacterial and antifungal activities. The results of current findings suggest that *O. arabicus* is a potential medicinal plants, which could be subjected to advance column chromatography for lead compounds using a bioassay guided approach.

Key words: *Ochradenus arabicus*, antioxidants, anticancer, proximate parameters, antimicrobial

Introduction

Sultanate of Oman, being oil rich country, bestowed with a variety of medicinal plant resources. In non-timber forest products, Omani Frankincense is internationally famous and has been used in fragrance and medicine. The Omani flora comprised of 1174 plant species. Few of them are endemic to the area (Nadeem *et al*., 2012; Vincken *et al.,* 2007; Miller *et al.,* 1984). The use of medicinal herbs in northern and central Oman is still common today. Plants known for their curative effects are widely used against various diseases ranging from common cold and fever to diabetes. Even with the establishment of conventional hospitals, traditional medicine is still largely practiced. Jabal Al-Akhdar, located in the interior part of Oman, is a local epi-center of endemic plants of Oman and some other countries of Arabian Peninsula.

Ochradenus arabicus, belongs to genus *Ochradenus,* grows in limestone rocky ground, sandy arid places (Nadeem *et al*., 2012), desert and arid regions of Saudi Arabia, Oman, United Arab Emirates and Yemen. Because of its medicinal importance, it is used locally for curing different ailments (Vincken *et al.,* 2007). Its leaves are deciduous which suggests morphological adaptations to the water-deficient environment. Its flower is polygamous and simplification is seen in the structure. Only one species *O. baccatus* Del. is widely spread while most of the other species are restricted as endemic in the Arabian Peninsula especially in Oman and the horn of Africa (Miller, 1984).

O. arabicus has been recently studied for its effective propagation and conservation through tissues culture techniques (Nadeem *et al.,* 2012; Khan *et al*., 2012). Its molecular markers have also been studied for its loping its identification (Vincken *et al.,* 2007). There are numerous reports of bioactive compounds isolation and characterization from the genus. In case of its phytochemical evaluation, Barakat *et al*., (1991) isolated quercetin-3-*O*-β-glucosyl(1-2)-α-rhamnoside-7-*O*-α-rhamnoside, quercetin glycosides, quercetin-3-*O*-p-coumaryl(1-6)-β-glucosyl(1-6)-β-glucoside-7-*O*-α-rhamnoside, quercetin-3-gentiobioside, isoquercitrin, and other known kaempferol glycosides, afzelin and astragalin (Barakat *et al*., 1991). A little information is known about the potential biological activities of *O. arabicus*. Since, the plant is medicinally important for local people, therefore, in present study we carried out various screening experiments to understand its potential role and biological function. This is the first comprehensive study on this plant. We aimed to evaluate the potential of *Ochradenus arabicus* role as antimicrobial, anticancer, antioxidant, antidiabetic, phenolics, flavonoids, allopathic and nutrition quality properties.

Materials and Methods

Study area, sample collection and identification: Jabal Al-Akhdar is one of the diverse floral regions of the Oman, hosting large varieties of vascular plants. *Ochradenus arabicus* was collected during their growing season from Jabal Al-Akhdar in June 2012 and identified by plant taxonomist. After collection, the plant samples were

immediately washed with water, dried under shadow, chopped into the small pieces and then crushed using a stainless steel blender and passed through a 2 mm sieve. The voucher specimen has already been deposited in the Herbarium of the Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman.

Extraction and fractionation: The powdered plant material (15.4 kg) was initially extracted with methanol (28 L) three times for two months at room temperature. The combined methanol extract was concentrated under reduced pressure to get the residue (427.4 g) called methanolic extract (JOAM), followed by the fractionation on the basis of increasing polarity of organic solvents into *n*-hexane (JOAH), chloroform (JOAC), ethyl acetate (JOAE), *n*-butanol (JOAB) and aqueous (JOAAQ). To assess the bioactivities of different sub-fractions, various concentrations were made either in DMSO or water.

Anticancer activities: Four cancer cell lines colorectal adenocarcinoma (HT29), colorectal adenocarcinoma (HCT116); breast cancer cell line (MCF-7) and Human hepatoma derived cell line (HepG2) were used for the screening of cytotoxicity of the extracts obtained from medicinal plants. These cell lines were purchased from ATCC, Manassas, VA, USA. The cell lines were cultured in Advanced DMEM with 10% NBCS (inactivated) and 5mM l-glutamine, and then grown at 37ºC in a humid atmosphere with 5% CO_2 in air. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay developed by Mosmann, 1983, was used with minor modifications for the screening of medicinal plant extracts for cytotoxic activity.

Enzyme α-glucosidase assay: Enzyme inhibition assay (α-glucosidase, E.C.3.2.1.20) was performed according to the modified Oki method (1999). The inhibition was measured spectrophotometrically at pH 6.9 and at 37°C using 0.7 mM p-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 200 m units/ml enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. Acrabose was used as a positive control. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with the spectrophotometer (Molecular Devices, USA).

Total phenolic contents: The total phenolic content was quantified using Folin-Ciocalteau reagent as described by Singleton & Slinkard. (1977). Briefly, about 20 µl of extract or the gallic acid (positive standard) was diluted with autoclaved distilled water. The mixture was added with 2 N Folin-Ciocalteau reagents. The mixture vigorously vertexed and incubated for 10 minutes. A Na_2CO_3 (5%) was added and vertexed. The reaction mixture was incubated for 30 min at 25ºC. The upper supernatant layer was read at 765 nm. The curve for standard was prepared using 25-1000 µg/mL of gallic acid. The total phenolic was shown as gallic acid equivalents (µg/mg of extract). The experiment was repeated three times.

Determination of total flavonoids: Total flavonoid content was determined after some modification in the assay of Dixit and Kar (2009). Briefly, plant extract (50 µL) was mixed with autoclaved distilled water (50 µL) and $NaNO_2$ (5%; 100 µL). This was followed by addition of aluminum chloride (10%; 150 µL). The reaction mixture was vertexed vigorously and incubated for 15 min. 1 M NaOH (100 µL) was added to the eppendorf tube. The absorbance was read at 510 nm. Quercetin (25-1000 mg/L) was used as standard. The data was presented as µg equivalents to quercetin of the plant extract.

Antioxidant activity: The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging potential was assessed according to the methods of Adom *et al*., (2003). The DPPH solution (0.1 mM DPPH, 50 µL) was prepared in methanol and mixed with plant extract (20 to 1000 µg/mL; 50 µL). The reaction mixture was kept at room temperature for 1 hr in dark. The absorbance of the reaction was then measured at 490 nm. Blank (without plant extract) was used as negative control. Ascorbic acid and BHT (butyl hydroxyl toluene) were used as positive controls. The antioxidant activity was calculated by using the following formula:

$$\text{Percentage (\%)} = \frac{\text{absorbance of control} - \text{Absorbance of samples}}{\text{Absorbance of the control}} \times 100.$$

Antimicrobial activities: Fungal pathogens viz. *Aspergillus niger*, *Fusarium oxysporum*, *Chaetomium globosum*, *Candida albicans, Neolentinus adherence* and *Alternaria alternate* and bacterial pathogens viz. *Staphylococcus aureus* and *Escherichia coli* were procured from Leibniz-Institute DSMZ (Braunschweig, Germany). Antifungal assay was performed using well-diffusion method (Anon., 2002a). Fungal growth was assessed against individual concentrations of extracts. The potato dextrose agar (PDA) and nutrient agar (NA) were autoclaved at 121ºC for 15 min in autoclaved. Petri dishes were added with 25 ml of sterile PDA medium and left for solidification for 30 min. The fungal and bacterial cells were grown on the PDA plates to serve as master plate and later as negative control. Two wells in each PDA/NA plates were made by using sterile Weller. The extracts were dissolved in autoclaved double distilled water. Various concentrations (500 and 1000 ppm) were prepared. The wells were loaded with different extract concentrations and fungal pads are transferred into the plates to assess the effect of fungal growth and zone of inhibition. The plates were incubated for 5 days at 27ºC. ZIC was recorded in millimetres and repeated twice. Three replications were maintained for each experiment.

Proximate analysis: By using the standard methods of the Association of the Analytical Chemists, moisture, ash, and crude fiber (on dry basis) were carried out (Hussain *et al*., 2013; Hussain *et al.,* 2009). The determination of proteins in terms of nitrogen was done by micro Kjeldahl method (Pearson & Hall., 1975). The nitrogen value was converted to protein by multiplying

to a factor of 6.25. The lipid content of the samples was done using Soxhlet type of the direct solvent extraction method. The solvent used was petroleum ether (boiling range 40-60ºC) (Hussain *et al.,* 2009). The crude fibre was also determined by the method described previously (Hussain *et al.,* 2013). The energy values (kcal/100 g) were determined by multiplying the values of carbohydrates, lipids and proteins by a factor of 4, 9, and 4 respectively, and taking the sum expressed in kilocalories (Hussain *et al.,* 2010; Hussain *et al.,* 2013). The total carbohydrates were determined by difference method [100 - (proteins + fats + moisture + ash in percentage) (Hussain *et al.,* 2010). All the proximate values were reported in percentage.

Allelopathic activities: Allelopathic effects of the sub-fractions were assessed by the method of Khan *et al*., (2010). Lettuce seed (*Lectuca sativa*) was utilized as an indicator species to know the effective concentration. Three different concentrations of 100, 500 and 1000 ppm of each sub-fraction were prepared by dissolving it in 5% DMSO. A filter paper (27 mm ø, Type Roshi Kaisha, Ltd, Tokyo) was placed in a petri dish. The dilutions were subjected on the filter paper and thus allowed to spread over it. Fifteen lettuce seeds were placed on it and the dishes were sealed and packed for incubation for 72 hours at room temperature. For each sub-fraction, mean, SD variance and standard error were calculated to determine the inhibition pattern at various concentration levels (Khan *et al*., 2009).

Statistical analysis: The experiment was replicated three times. The means of the reading was presented with ± standard error. Differences were considered significant at $p<0.05$ using DMRT test by using statistical analysis software (version 9.1).

Results and Discussion

Nutritional and proximate composition of *Ochradenus arabicus*: In proximate parameters, we assessed the moisture, ash, crude fat, crude fiber, carbohydrates and energy values of the leaves and fruits of *O. arabicus.* According to results, the moisture content was found to be highest in fruits (9.28%) than leaves (3.81%) of *O. arabicus*. The moisture content of the leaves was found lower than *N. sauvis* (8.44%) reported by Hussain *et al*., (2011). The results of ash content with standard deviation are presented in Table 1. Ash contents of fruits and leaves of *O. arabicus* were found to have 11.09 and 3.48% respectively. In comparison, ash content of fruit was found higher than the *N. sauvis* (7.91%) and lower than *Datura alba* (18.80%), *Phlomis cashmeriana* (17.66%) and *Calotropis procera* (17.62%), while ash values of fruit was also in good agreement with *Dalbergia sisso* (12.33%), *Phlomis bracteosa* (10.83%), and slightly lower than *Avera javanica* (14.23%) (Hussain *et al.,* 2010; Hussain *et al.,* 2013; Hussain *et al.,* 2011).

Fruits of *Ochradenus arabicus* were found to be highest in its crude fat content (3.97%) showing close similarity with *Rhiza stricta* (3.98%) and *Dalbergia sisso* (3.35%) (Hussain *et al.,* 2010). However, leaves contain crude fat (2.58%) lower to that of *Avera javanica* having 1.15% fat (Hussain *et al.,* 2011). Crude fiber of leaves (*Ochradenus arabicus*) were found to have value of 26.87%, followed by the fruits (17.66%) (Table 1). In comparison with other medicinal plants leaves showed close resemblance with *Aerva javanica* (29.18%) and *Calotropis procera* (29.49%) (Hussain *et al.,* 2010). A high intake of dietary fiber improves glycemic control, decreases hyper insulinemia, and lowers plasma lipid concentrations (Chandalia *et al*., 2000).

The protein content of the medicinal plants was calculated on the basis of the available nitrogen using Kjeldahl method and was observed in the range of 5.86-12.77% with fruits having the highest value (12.77%), followed by leaves (5.86%) (Table 1). The fruits of *O. arabicus* showed close value to *Phlomis bracteosa* (10.61%) and *Phlomis cashmeriana* (9.51%) belong to the family Labiateae (Hussain *et al.,* 2010). The carbohydrate content of analyzed samples revealed that leaveshad highest amount of carbohydrates (84.25%), followed by the decreasing order of fruits (63.19%) (Table 1). The contribution of the carbohydrates to the energy in a food ration recommended (Anon., 1990; 2002b) is from 55 to 75%. The carbohydrates of fruits fall in the acceptable range set by WHO. Thus, only fruits can be used as a source of energy contribution in a food ration.

According to the results of the energy calculations, based on the carbohydrates, fats, and protein content, the highest value was found in the leaves of *O. arabicus* (383.73 kcal/100 g), while the fruits were found to contain the lowest energy value (339.63 kcal/100g) (Table 1). The energy value of the both parts (fruits and leaves) was found comparatively higher to the reported values of some Nigerian leafy vegetables (248.8-307.1 kcal/100g) (Isong *et al*., 1999). some Ghanian green leafy vegetables like *Corchorus tridens* (283.1 kcal/100g), sweet potato leaves (288.3 kcal/100g) (Asibey & Tavie, 1999), *Calotropis procera* (312.41 kcal/100g) and *Datura alba* (308.10 Kcal/100g) reported by Hussain *et al*., (2011).

Table 1. Proximate analysis of different parts (fruits and leaves) of *O. arabicus.*

O. arabicus	Moisture	Ash	C. Fats	Proteins	C. Fibers	CHO	E. Value
Fruits	9.28 ± 0.14a	11.09 ± 0.05a	3.97 ± 0.01a	12.77 ± 0.13a	17.66 ± 0.15b	63.19 ± 0.42b	339.63 ± 0.81b
Leaves	3.81 ± 0.15b	3.48 ± 0.03b	2.58 ± 0.14b	5.86 ± 0.01b	26.87 ± 0.21a	84.25 ± 0.54a	383.73 ± 0.68a

CHO = Carbohydrates, C. Fats = Crude fats, C. Fiber = Crude fiber, E. Value = Energy value; the different letter (s) in each column shows values are significantly different ($p<0.05$) as evaluated by the DMRT. ± shows the standard deviation of mean values of three replicates

Allelopathic activities: Allelopathy is the effect of a plant on the growth and development of another plant species by secretion of chemical constituents (Fujii *et al.,* 2003). Studying this phenomenon is considered to be one of the promising remedial measures for problems confronted to obtaining sustainable agriculture (Inderjit & Duke, 2003; Azirak & Karaman, 2008; Khan *et al*., 2009). In cropping system, the biological control of weeds is essential to enhance crop productivity. Such methods have been highly appreciated and practiced to obtained higher yields from present crops with little application of synthetic fertilizers and herbicides (Khan *et al*., 2010).

In the present study, the allelopathic effect of each fraction (JOAH, JOAE, JOAC, JOAB and JOAAQ) of *O. arabicus* was evaluated by using three concentrations gradient. The results of the assay revealed that aqueous fraction at 1000 ppm have shown significant inhibitory effect towards the growth of root and hypocotyl of lettuce seeds, while at 1000 and 500 ppm, both the aqueous and crude extract of methanol inhibited moderate activity on the root growth and germination of lettuce seeds respectively. In case of shoot growth and germination of lettuce seeds, aqueous fraction revealed the highest activity at 1000 ppm and moderate activity at 500 ppm (Fig. 1). The effective concentration needed to cause 50% inhibition of lettuce seed increased from high polar (methanol) to low polar (*n*-hexane) sub fractions (Fig. 1).

Fujii *et al*., (2004) also showed similar results. According to his reports, increased concentrations of leaf extracts (*Sapindus emarginatus, Terminalia tomentasa*, and *Vitex negundoi*) supressed the growth of some vegetable species. However, this was also observed that reduced concentrations of such extract can also stimulate the germination and growth of seeds (Khan *et al*., 2009). The inhibitory effect suggests that the extract may comprise of allelopathic compound which are affecting the germination potential of seeds. The reason for an inhibitory and stimulatory effect of methanol on the germination is due to the presence of different levels of following allelo-chemicals in the extracts of *O. arabicus* rich in secondary metabolites including: alkaloids, glycosides, coumarins, flavonoids, and steroids etc.

DPPH radical scavenging activity: Antioxidant analysis was performed to evaluate the free radical scavenging properties of six different extracts viz. methanol (JOAM), n-hexane (JOAH), chloroform (JOAM), ethyl acetate (JOAE), n-butanol (JOAB) and aqueous (JOAAQ) of *O. arabicus* using ascorbic acid as standard antioxidant. The results are presented in Fig. 2. Among extracts, the ethyl acetate fraction showed significantly higher activity as a most promising antioxidant activity followed by methanol and butanol, respectively. DPPH radical scavenging activity of the JOAAQ extracts was significantly lower as compared to other extracts and control. Ascorbic acid being a potent antioxidant and radical scavenger has an important role in metabolism. The JOAE showed a similar level of DPPH radical scavenging in comparison to ascorbic acid.

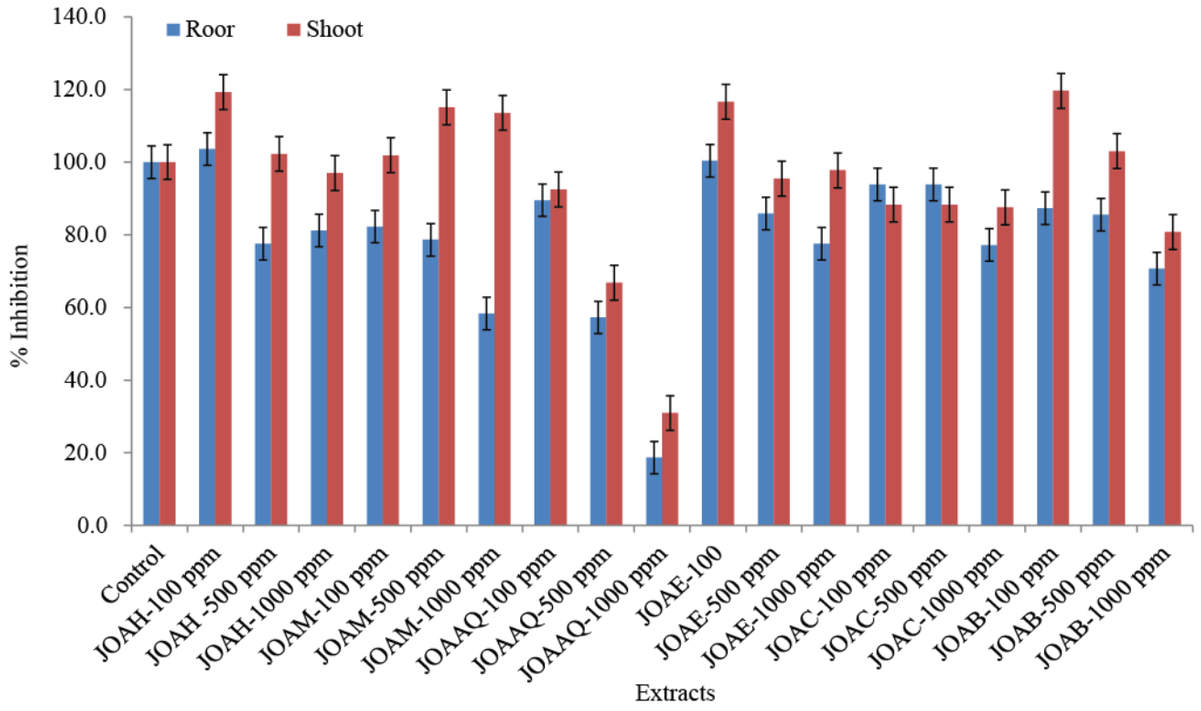

Fig. 1. Effect of various extracts of JOA on the root and shoot of lettuce seeds. The error bars shows the standard deviation of the mean values. The different letter (s) shows that values of each extract are significantly different (p>0.05) in comparison with control as evaluated by DMRT.

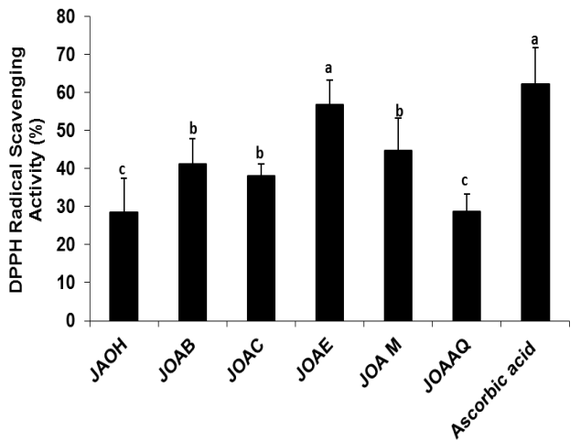

Fig. 2. DPPH radical scavenging activity of the different extracts of *O. arabicus*. The error bars shows the standard deviation of the mean values. The different letter (s) shows that values of each extract are significantly different (p>0.05) in comparison with positive control as evaluated by DMRT.

Crude extracts of medicinal plants are rich in phenolics are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Antioxidants have been detected in a number of agricultural and food products including cereals, fruits, vegetables and oil seeds (Adom *et al.,* 2003; Anon., 2002). Traditionally, synthetic antioxidants such as butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA), and ascorbic acid have been widely used as antioxidants in the food industry (Nawar & Lipids, 1996; Cai *et al*., 2004). In the present study, the radical scavenging effects were high in JOAE subfraction, suggesting the presence of bioactive metabolites which has oxidative stress mitigation properties.

Antimicrobial effects of extracts: Fungal pathogens viz. *A. niger*, *F. oxysporum*, *C. globosum*, *C. albicans, N. adherence* and *A. alternata* and bacterial pathogens *S. aureus* and *E. coli* are of wider concern for producing diseases in agricultural crops. *A. alternata* has been known to cause black mould disease in ripen tomato fruits (Pearson & Hall, 1975). *F. oxysporum* is a soil-borne fungus affecting the root and stem of cucumber plants (*Cucumis sativus* L.) (Vakalounakis & Chalkias, 2004). Additionally, Fusarium wilts disease has affected more than hundred species of crop plants and their yield (Vakalounakis & Chalkias, 2004). Similarly, other pathogenic fungi and bacteria are extremely potent to limit the growth and yield of crop plants, thus risking the food security. Plant extracts and their components have been known to exhibit biological activities, especially antifungal, antibacterial, and antioxidant (Ismail *et al*., 2012). Inhibiting their growth through naturally occurring metabolites is an environmental friendly strategy. In present study, we assessed the effects of various extracts of the *O. arabicus* on these noxious microbes and their growth.

The effects of *n*-hexane (JOAH), ethyl acetate (JOAE), chloroform (JOAC), butanol (JOAB) and water (JOAAQ) fractions on the growth of fungal (*F. oxysporum, C. albicans, A. niger, C. globosum, A. alternate* and *N. adherence*) and bacterial strains (*S. aureus* and *E. coli*) were determined using the highest concentrations of 2 mg/mL per well. Lower than this concentration, there were no growth inhibitory effects. According to the results, ethyl acetate extract was significantly potent to the growth of *A. niger, C. globosum,* and *A. alternata*as compared to other fungal and bacterial strains. Similarly, the effect of chloroform extract against *C. albicans* was also significantly inhibitory (Table 2). *n*-Hexane fractions inhibited moderately against *F. oxysporum* as compared to other extracts. Other extracts were found inactive against fungal pathogens and their growth. Antibacterial activity of the crude extracts and various sub fractions of *O. arabicus* was tested against gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli)* at 2 mg/mL concentration due to the least sensitivity. All the fractions (*n*-hexane, chloroform, n-butanol, ethyl acetate and aqueous) and crude extract of methanol were found inactive against *S. aureus* and *E. coli* (Table 2).

Anticancer activities of the extracts: The extracts were assessed for their anticancer activities using different colorectal adenocarcinoma (HT29 and HCT116), hepatoma derived cell (HepG2) and breast cancer cell (MCF-7) lines. Various low concentrations of the extracts were prepared and tested against the grown cell cultures. The screening results showed that JOAE, JOAB and JOAAQ were supressing the cancer cell growth while other extracts such as JOAH, JOAC and JOAM were inactive as compared to the control. The active fractions were subjected to concentration gradient effects of extracts. According to results of colorectal adenocarcinoma (HT29 and HCT116) cell lines treatments, 100 µg/mL of JOAE, 25 µg/mL of JOAB and 1 µg/mL of JOAAQ has reduced the cancer cell viability as compared to other extracts, their concentrations and control cells (Fig. 3).

Table 2. Effect of different fractions of *Ochradenus arabicus* on the growth of plant pathogens (2 mg/mL).

Fraction	Microbial growth (mm)					
	JOAH	**JOAC**	**JOAE**	**JOAM**	**JOAAQ**	**JOAB**
Fusarium oxysporum	14 ± 0.23c	21.5 ± 0.4a	19.5 ± 0.1a	20.7 ± 0.2a	18.5 ± 0.6b	25.5 ± 0.8a
Candida albicans	16.8 ± 0.76b	8.5 ± 0.56d	14.7 ± 0.3c	13.0 ± 0.53c	13.1 ± 0.1c	12.5 ± 0.8d
Neolentinus adherence	18.7 ± 0.2a	18.5 ± 0.9b	16.5 ± 0.4b	19 ± 0.5a	25.5 ± 1.0a	19.5 ± 0.5b
Aspergillus niger	12.1 ± 0.2d	13.1 ± 0.23c	10.4 ± 0.6d	13.2 ± 0.7c	15.2 ± 1.2c	14.5 ± 0.7c
Chaetomium globosum	12.9 ± 0.9d	13.5 ± 0.5c	11.2 ± 0.7cd	13.0 ± 1.3c	14.7 ± 0.1c	14.6 ± 0.6c
Alternaria alternate	13.7 ± 0.33cd	14.5 ± 1.1c	12.4 ± 0.2cd	15.0 ± 0.4b	15.3 ± 0.6c	14.7 ± 0.3c
Staphylococcus aureus	NA	NA	NA	NA	NA	NA
Escherichia coli	NA	NA	NA	NA	NA	NA

NA = no activity (similar to control); The different letter (s) in each column shows values are significantly different (p<0.05) as evaluated by the DMRT. ± shows the standard deviation of mean values of three replicates

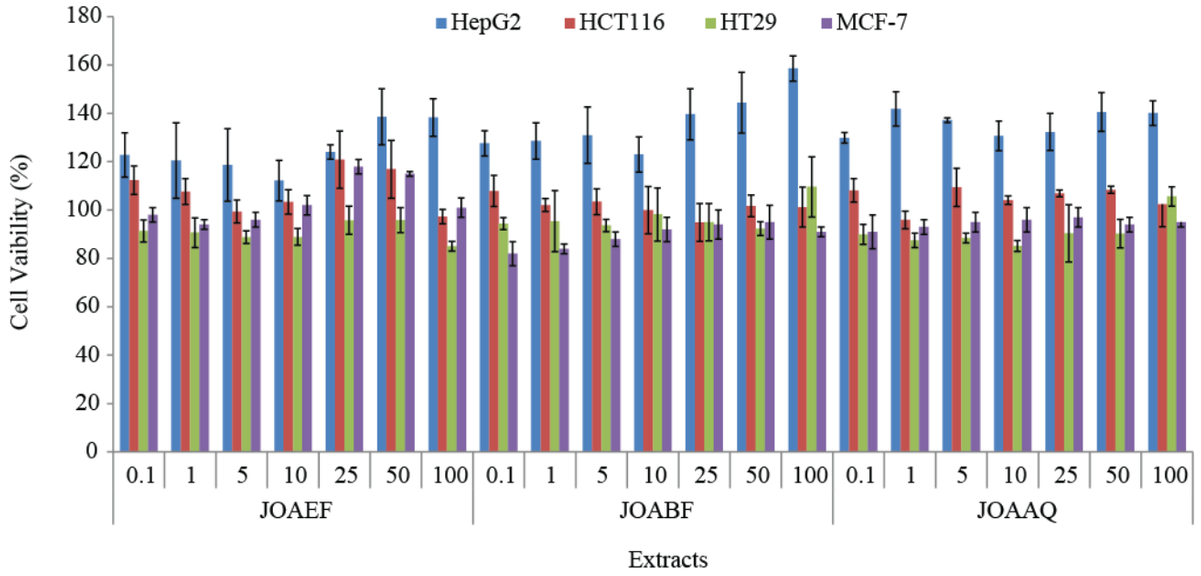

Fig. 3. Anticancer activities of various concentrations of the extracts against HT29, HCT116, HepG2 and MCF-7lines. The error bars shows the standard deviation of the mean values.

In case of HepG2 cancer cells, none of the concentrations of JOAE, JOAB and JOAAQ were active to suppress the growing cancer cells. Most of the cellular activity and growth was higher than the control. However, when these extracts were applied to MCF-7 cell lines, the lower concentrations such as 1 µg/mL and 5 µg/mL of JOAE, JOAB, and JOAAQ has suppressed the cancer cells as compared to higher concentrations of the extracts (Fig. 3). To know the IC50 activity of the extract, the concentration was increased to 1000 µg/mL. The treatment of JOAE, JOAH, JOAC and JOAM significantly suppressed the cancer cell's viability of HT29 and HCT116 to 42, 15, 14 and 22% respectively. However, the JOAB and JOAAQ did not affect the cancer cell viability.

Medicinal plants or their secondary metabolites have been directly or indirectly playing an important role towards cancer (Wink *et al.,* 2005). Even though there are number of synthetic antitumor agents available, efforts are still on to search for effective naturally occurring anti-carcinogens that would prevent, slow or reverse cancer development (Newman *et al.,* 2003; Nipun *et al*,. 2011). Our results shows that *O. arabicus* has the potential to discover the potent anticancer agents. Further fractionation of the bioactive fraction can help us to find the lead anticancer compound.

α-Glucosidase activity: The α-glucosidase activity was evaluated for crude extracts and their sub fractions using p-nitrophenyl α-D glucopyranoside as substrate and acrabose as a positive standard (Kimra *et al.,* 2004; Chiasson, 2006). The results of the assay shows that only JOAB was 10% active to inhibit the activity as compared to acrabose while other extracts JOAC, JOAE, JOAH, JOAAQ, and JOAM were inactive to present any inhibitory effect during the reaction.

Total phenolic contents and total flavonoid contents: Total phenolic contents were assessed in all the crude methanolic extracts and its sub fractions. The total phenolic content was significantly higher (6.43 µg/g) in JOAE which was followed by JOAB (2.2 µg/g) and JOAC (2.3 µg/g) fractions (Fig. 4). While in case of JOAH, JOAM and JOAAQ, total phenolic content was significantly lower. The total flavonoid content was significantly higher in JOAE (79.21 µg/g) whilst JOAH, JOAM, JOAAQ, JOAB and JOAC have significantly lower flavonoids content (Fig. 4).

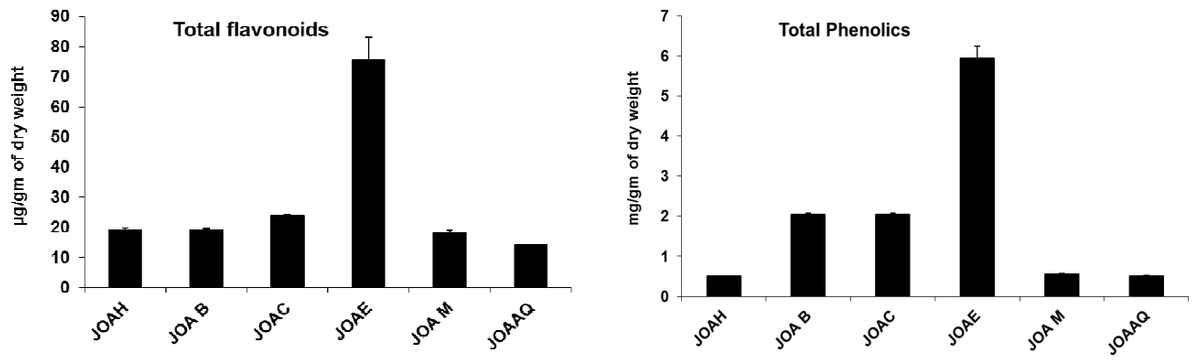

Fig. 4. Total phenolics and flavonoid contents in various extracts. The error bars shows the standard deviation of the mean values.

Previously, numerous flavonoids such asquercetin 3-O- β -glucosyl (1→2)- α -rhamnoside-7-O- α -rhamnoside and quercetin 3-O-p-coumaryl (1→6)- β -glucosyl (1→6)- β-glucoside-7-O-α-rhamnoside were isolated from the aerial parts of *O. baccatus* (Barkat *et al*., 1991; Shabana *et al*., 1990; Pinent *et al*., 2008). However, *O. arabicus* has not been subjected to such analysis, which could be interesting to search for bioactive secondary metabolites.

Acknowledgment

The authors would like to thank The Oman Research Council (TRC) for financial support through project entitled "Phytochemical Investigations, Proximate Analysis and Biological Activity of Some Selected Omani Medicinal Plants" (ORG/CBS/12/004).

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(Received for publication 8 June 2013)