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EVALUATION OF BIOLOGICAL POTENTIAL AND PHYSICO-CHEMICAL PROPERTIES OF *ACRIDOCARPUS ORIENTALIS* (MALPIGHIACEAE)

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Abstract

Acridocarpus orientalis, an arid land plant and famous in Arabian region for medicinal values, has been investigated for its biological and ecological role in the current study. The methanol (JAOMF) extract of stem and leaves of *A. orientalis* along with various fractions (*n*-hexane; JAOHF, chloroform; JAOCF, ethyl acetate; JAOEF, *n*-butanol; JAOBF, and aqueous; JAOWF) were tested for allelopathic, cytotoxic, anti-fungal, antioxidant and enzyme inhibition activities. In case of allelopathic potential, the higher concentration (100 mg) of dried leaves and stem were significantly suppressing the lettuce seed germination as compared to control. Among various fractions, *n*-butanol and chloroform have inhibited the radical and hypocotyl growths of lettuce seeds in a dose-dependent manner. The fractions showed a week growth inhibitory effects against *Chitomium globosum*, *Fusarium oxysporum*, and *Aspergillus niger*. Total flavonoid contents were significantly higher in ethyl acetate (279.85 µg/g of dry extract) and *n*-butanol (142.22 µg/g of dry extract) fractions of stem while *n*-hexane (77.6 µg/g of dry extract) fraction of leaves showed higher flavonoid contents. Total phenolic contents were found higher in the ethyl acetate fraction of stem (16.8 mg/g of dry weight) followed by *n*-butanol (10.6 mg/g of dry weight). Aqueous fraction of leaves showed significantly higher anti-lipid peroxidation (60.6%) activity followed by chloroform (49.9%). Higher concentration of chloroform fraction of stem expressed higher cytotoxic effects for adenocarcinoma and hepatoma cancer cell lines as compared to other fractions.

Key words: *Acridocarpus orientalis*, Anticancer, Enzyme inhibition, Allelopathy, Antifungal, Anti-lipid peroxidation, and Antioxidant.

Introduction

Sultanate of Oman, an arid land, is bestowed with ample and diverse plant resources. The numbers of species reported from the Sultanate are 1204, including 1182 angiospermic plants (Ghazanfar, 1992). About 7% of the flora consists of endemic species that are distributed in Dhofar (57 sp.), central Oman (11 sp.), and northern mountains (25 sp.) (Miller & Nyberg, 1991). In Oman, the plants have been in use traditionally to treat several diseases, to prepare food, for construction purposes, as ornamentals, and as handricrafts (Ghazanfar & Al-Sabahi, 1993; Pickering & Patzelt, 2008; Winbow, 2008). From the Northern and Central Oman, around 56 species are reported in herbal medicines (Ghazanfar & Al-Sabahi, 1993). Keeping in mind the medicinal importance of Omani plants, *A. orientalis* was selected for the current study.

Acridocarpus orientalis A. Juss. (Arabic name *Qafas*) of the family Malpighiaceae grows in gravel wadis and on slopes in Oman at an altitude of 300–1500 m (Pickering & Patzelt, 2008). The leaves of *A. orientalis* are crushed, mixed with oil and applied to relieve swellings, muscle pains, and to treat arthritis (Pickering & Patzelt, 2008). Several species of *Acridocarpus* are traditionally employed as folk medicines all over the world since ancient times. Some species are reported to have many ecological advantages as well. *A. socotranus* is frequently used in the traditional medication of Yemen for the cure of headache and muscle pain (Mothana *et al*., 2009; Hammiche & Maiza, 2006; Hussain *et al*., 2014a). The aerial parts (leaves and bark) of *A. chloropterus* in Tanzania are reported to have anti-trypanosomal, anti-plasmodial and anti-leishmanial activities (Malebo *et al*., 2009). *A. orientalis* is reported mostly from the border areas of Oman and UAE, where it is normally used for the cure of muscle pain, paralysis and joint pain, in addition to treat the udder inflammation in cattle (Mothana *et al*., 2009; Hammiche & Maiza, 2006). The treatment of *A. orientalis* against anti-flammatory diseases can lead the way to cancer treatment (Ghazanfar. 1990).

The whole plant and various fractions of *A. orientalis* (Fig. 1) are yet to be studied for their role in ecology and bioactive potential in various human health issues. Therefore, in present study, we aimed to assess the possible allelopathic potential of the plant parts (leaves and stem). Furthermore, because it is used for medicinal purposes, we evaluated its proximate parameters (Moisture, ash, proteins, fiber, fats, carbohydrates, and energy value), allelopathic, antifungal, cytotoxic, antioxidant and enzyme inhibition activities.

Materials and Methods

Plant collection: The air dried plant material (8.2 Kg) of *A. orientalis* was collected from Al-Hamra, in Ad-Dakhiliyah region of the Sultanate of Oman (2012) and identified by Dr. Syed Abdullah Gilani (plant taxonomist) at the Department of Biological Sciences and Chemistry, University of Nizwa, Nizwa, Oman. The voucher specimen was deposited in the herbarium of the department. The plant material was shade dried and stored at room temperature for further analysis.

Fig. 1. *Acridocarpus orientalis* growing in natural habitat.

Extraction, fractionation and isolation: The powdered plant material of stem (4.1 kg) and leaves (3.3 kg) were initially macerated in methanol separately for one week (each in 4-5 L) with rigorous shaking daily. The combined methanol filtrates were evaporated under reduced pressure using rotary evaporator resulting greenish crude extract of stem (339.1 g) and leaves (300 g) respectively. The stem residue was suspended in 1L of distilled water and was then partitioned in *n*-hexane (11.8 g), chloroform (205.5 g), ethyl acetate (17.4 g), *n*-butanol (13.4 g), and aqueous (67.3 g) fractions. The same procedure was carried out for the crude extract of leaves to get *n*-hexane (40.9 g), chloroform (84.8 g), ethyl acetate (30 g), *n*-butanol (58.3 g), and aqueous (76.7 g) fractions.

Allelopathic activities: Allelopathic potential of the stem and leaves along with different fractions of *A. orientalis* was determined according to the earlier reported method (Khan *et al*., 2010; Hussain *et al*., 2014a,b).

Antifungal activity: Antifungal activity of the *A. orientalis* stem was determined using well-diffusion method (Hussain *et al*., 2014a,c; Anees *et al*., 2018) with some modifications. Three different fungal strains (*Chitomium globosum, Fusarium oxysporum,* and *Aspergillus niger*) were spread on plates, and then 2 mg/mL of each fraction was added. The plates were incubated for five days.

Proximate analysis: Moisture, proteins, fats, ash, fiber, carbohydrates, and energy value (Kcal/100g) (proximate parameters) of the stem and leaves were determined by using standard reported methods (Hussain *et al*., 2009; Pearson, 1976).

Anticancer bioassay: Three cancer cell lines; HepG2 (human hepatoma), HCT116 and HT29 (colorectal adenocarcinoma) were screened for cytotoxicity screening of the plant extracts (Hussain *et al*., 2014b; Mosmann, 1983). Stock solutions of doxorubicin and plant extracts (25, 500 and 1000 µg/ml) were made in dimethyl sulfoxide (DMSO) (analytical grade) and were always made fresh just prior to experiments.

Urease and α-glucosidase enzyme inhibition assays: Urease and α-glucosidase (E.C.3.2.1.20) enzyme inhibition assays were performed according to the previous published protocols (Hussain *et al*., 2014b; Oki *et al*., 1999; Leyama *et al*., 2011; Rehman *et al*., 2017). Thiourea and acarbose were used as the standard inhibitors with 95±1.50% and 72±2.00 % inhibitions for urease and α-glucosidase, respectively. The % inhibition was calculated as.

$$\% \text{ Inhibition} = 100 - (OD_{\text{test well}} / OD_{\text{control}}) \times 100$$

The IC_{50} values were then calculated by using EZ-Fit enzyme kinetics program (Perrella Scientific Inc., Amherst, MA, USA) (Amin *et al*., 2013). All analyses were performed in triplicates.

Total phenolic and total flavonoid contents: The total phenolic content was quantified using Folin-Ciocalteau reagent (Ulukanli *et al*., 2010) and total flavonoids were determined with some modification in the reported assay (Dixit & Kar, 2009; Hussain *et al*., 2014b; Ahmed *et al*., 2018).

Anti-lipid peroxidation: Anti-lipid peroxidation assay was established by the TBARS (Thiobarbituric Acid Reactive Substance) method (Chang *et al*., 2009; Rehman *et al*., 2016). BHA (butylated hydroxyanisole) was used as standard inhibitors in anti-lipid peroxidation assay.

Antioxidant activity: DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging assay was carried out using previous protocol with slight modification (Adom *et al*., 2003; Hussain *et al*., 2014a,b; Khan *et al*., 2018).

Statistical analysis: All experiments were performed in triplicates. The means of the reading was presented with ± standard error (SE). All the differences were considered significant at $p<0.05$ using DMRT test by using statistical analysis software (version 9.1).

Results and Discussion

Proximate analysis: Proximate analysis of stem and leaves of *A. orientalis* showed high dry matter (91.11–96.22%), fiber (15.89–27.82%), energy value (354.77–371.41 Kcal/100g) and carbohydrate (70.34–84.73%) contents while low moisture (4.56–8.97%), ash (4.32–7.41%), fat (0.99–4.21%), nitrogen (0.93–1.41%), and protein (5.87–8.87%) contents were found (Table 1). Higher percentages of dry matter, fiber, carbohydrates and energy values were found in the stem than the leaves while the moisture, ash, fat, nitrogen, and protein contents were higher in the leaves than in the stem (Table 1).

The use of traditional medicines derived from aerial parts of plants are increasing and getting popularity throughout the developed and developing world (Jia & Zhang, 2005). Since the leaves of the selected plant showed high composition of moisture, ash, crude fat, nitrogen and protein contents, it could be considered more promising source for medicinal purposes. Previous results of Malebo *et al*., (2009) on the leaves of *A. orientalis* showed good anti-plasmodial, anti-trypanosomal and anti-leishmanial activities.

Table 1. Proximate analyses of stem and leaves of *A. orientalis*

Analysis	Stem (%) ± SD	Leaves (%) ± SD
Moisture	4.56 ± 0.13[b]	8.97 ± 0.14[a]
Dry matter	96.22 ± 0.12[a]	91.11 ± 0.14[a]
Ash	4.32 ± 0.01[b]	7.41 ± 0.02[a]
Fat	0.99 ± 0.02[b]	4.21 ± 0.01[a]
Nitrogen	0.93 ± 0.02[b]	1.41 ± 0.07[a]
Protein content	5.87 ± 0.02[b]	8.87 ± 0.04[a]
Fiber	27.82 ± 0.02[a]	15.89 ± 0.01[b]
Carbohydrate	84.73 ± 0.08[a]	70.34 ± 0.52[b]
Energy value (kcal/100g)	371.41 ± 0.04[a]	354.77 ± 0.09[b]

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.

Table 2. Effects of dried leaves and stem on the growth of lettuce seeds. The values of roots (R) and hypocotyls (H) for plant represent growth percentage and SD.

Plant name	Stem		Leaves	
	R (%)	H (%)	R (%)	H (%)
A. orientalis (200 mg)	57 ± 0.84	113 ± 1.23	27 ± 1.01	67 ± 0.94

Table 3. Allelopathic effects of various fractions of stem of *A. orientalis* on the growth of roots (R) and hypocotyls (H). The growth of roots and hypocotyls are shown in percentages.

Extract	100 ppm		500 ppm		1000 ppm	
	R (%)	H (%)	R (%)	H (%)	R (%)	H (%)
JAOHF	111	80	97	69	73	60
JAOCF	66	39	32	8	6.4	13.4
JAOBF	64	61	42	66	63	55
JAOWF	105	81	64	84	56	66

Allelopathic effects: Allelopathy plays the potential role in natural and agricultural ecosystems. The use of natural products and synthetic chemicals is in practice to improve the production of agricultural ecosystems through various means. One of the possibilities is to study allelopathic potentials of the plant species and to select the most bioactive ones for chemical analyses (Fujii *et al*., 2003; 2004).

Bioassay results of the tested plant displayed promising inhibitory effect on the development of lettuce seeds. When 20 and 200 mg dried leaves of *A. orientalis* were screened against lettuce seeds, promising inhibitory effects were determined on root and hypocotyl developments of lettuce seeds. However, at the lower concentration (200 mg), the dried stem of *A. orientalis* (57%) showed stronger inhibitory effects against the root growth of lettuce seeds (Table 2). The higher concentration of 200 mg dried leaves of *A. orientalis* (27%) showed significant inhibitory effects on root growth of lettuce seeds. In case of hypocotyl growth, *A. orientalis* showed stimulatory effects on lettuce seeds. However, 200 mg dried leaves of all the plants inhibited the hypocotyl growth of lettuce seeds. The leaf part was more inhibitive to lettuce seed growth as compared to stem and control.

Since the leaf or leaves portion presented more allelopathic effects, therefore, the various fractions (*n*-hexane, $CHCl_3$, *n*-BuOH and aqueous) of stem were studied using a concentration of 100, 500 and 1000 ppm. At the concentration of 100 ppm, *n*-butanol and chloroform extracts showed higher inhibitory effects (34–36%; Table 3) as compared to aqueous and *n*-hexane extracts, where stimulatory effects were observed on the root growths of lettuce seeds (Table 3; Fig. 2). In case of hypocotyl growth, 100 ppm concentrations exhibited allelopathic effects, in which, the chloroform extracts had higher inhibition (61%). With an increasing concentration, inhibitory effect of the extracts was also increased. At higher concentrations (500 ppm and 1000 ppm), all the extracts inhibited the growth of the radical and hypocotyls of lettuce seeds. From the results, it was observed that the chloroform extract showed higher inhibition of lettuce seeds growth as compared to *n*-hexane, *n*-BuOH and aqueous fractions.

In the current study, the dried leaves of *A. orientalis* at higher concentration strongly inhibited the root and hypocotyls growths of lettuce seeds as the chloroform fraction had allelochemical constituents. Our earlier allelopathic studies on Pakistani medicinal plants showed that 10 mg and 50 mg dried leaves of *Teucrium stocksianum* inhibited the root growth up to 18% and 46%, respectively (Gilani *et al*., 2010). Similarly, 10 mg and 50 mg dried leaves of *T. stocksianum* inhibited 1% and 8% growth of hypocotyls, respectively (Gilani *et al*., 2010). The present results also conformed the reports of Khan *et al*., (2009) & 2010), who suggested that the increased concentration of a specific medicinal plants fraction could inhibit the growth of lettuce seeds. The report also suggested that the allelopathic behavior of the plant was due to the growth conditions of the plant. This is relative to our results as *A. orientalis* is growing in arid environment, therefore, it might have abundance of allelochemical to enable the plants for increased competition for nutrients and water.

Anti-fungal activity: When *n*-hexane, chloroform, *n*-butanol, and aqueous fractions of *Acridocarpus orientalis* were tested at 2 mg/mL against *Chaetomium globosum*, *Fusarium oxysporum*, and *Aspergillus niger*, weaker growth inhibition (64.3–92.8 mm) was observed in some cases only (Table 4). Most of the fractions, however, stimulated the growth of the fungal strains.

***C. globosum*:** *n*-hexane and aqueous fractions of *A. orientalis* weakly inhibited the growth of *C. globosum* up to 9.1% while 109.1–136.4 % stimulation in fungal growth was observed in case of other fractions. ***F. oxysporum*:** All fractions of the plant stimulated the growth of *F. oxysporum* (107.1–185.7 %). ***A. niger*:** None of the plant fractions, in case of *A. niger*, inhibited the fungal progression, rather, all the fractions enhanced the growth of fungus ranging from 150 to 212.5 % (Table 4).

Chaetomium globosum is saprophytic ascomycetes and also one of the important biocontrol agents against late potato blight, *Phytophthora infestans* (Shanthiyaa *et al*., 2013), *Pythium ultimum* and soil borne and seed borne plant pathogens (Di Pietro *et al*., 1992). An aerobic filamentous fungus, *Aspergillus niger* grows on organic matter, in soil and litter, in compost and on decaying plant material, and was industrially exploited for the production of citric acid (Schuster *et al*., 2002).

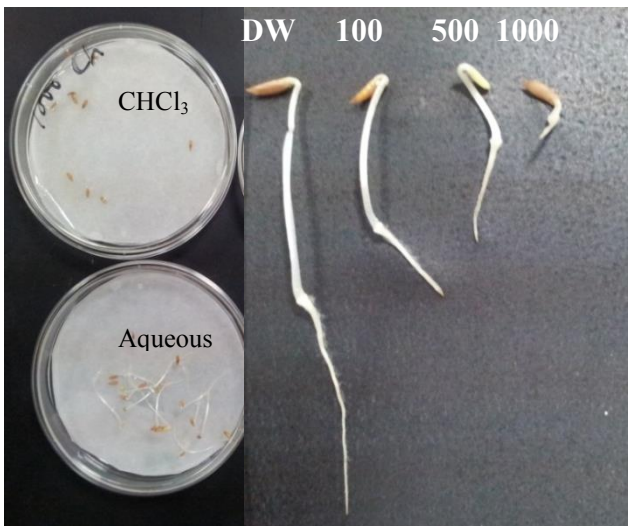

Fig. 2. Allelopathic effects of chloroform fraction of leaves of *A. orientalis*.

Table 4. Effect of various fractions of stem of *A. orientalis* on the growth of fungal strains.

Extract	Growth (mm)	Area	Growth (%)
	Chaetomium globosum		
Control	11 ± 0.23	102 ± 0.23	100 ± 1.1
JAOHF	10 ± 0.84	78.5 ± 0.34	90.9 ± 1.3
JAOCF	15 ± 0.14	176.6 ± 0.65	136.4 ± 1.9
JAOBF	12 ± 0.86	113.04 ± 0.84	109.1 ± 1.67
JAOWF	12 ± 0.04	113.04± 0.87	109.1 ± 3.3
	Fusarium oxysporum		
Control	14± 0.23	153.9 ± 0.84	100 ± 3.42
JAOHF	15± 0.31	176.6 ± 1.32	107.1 ± 2.34
JAOCF	26± 0.56	530.6 ± 3.45	185.7 ± 2.39
JAOBF	18± 0.78	254.3 ± 9.4	128.5 ± 4.2
JAOWF	21± 0.34	346.2 ± 4.43	150 ± 1.2
	Aspergillus niger		
Control	8.0 ± 0.23	50.2 ± 2.56	100 ± 8.30
JAOHF	17 ± 0.04	226.9± 0.43	212.5 ± 32
JAOCF	17 ± 0.44	226.9 ± 0.09	212.5 ± 0.84
JAOBF	14 ± 0.67	153.9 ± 1.67	175 ± 2.1
JAOWF	12 ± 0.23	113.04 ± 1.23	150 ± 2.3

The overall study showed that *n*-hexane growth enhancement of fungal strains was observed in most of the fractions. The strain F0142 (*Chaetomium globosum*), isolated from barnyard grass, attributed significant disease control efficacy against wheat leaf rust (*Puccinia recondita*) and rice blast (*Magnaporthe grisea*) (Park *et al*., 2005). However, growth stimulation of *C. globosum* by various fractions of *A. orientalis* may help to grow *C. globosum* quickly when the purpose is to use it as biocontrol agent against late potato blight, *Phytophthora infestans* (Shanthiyaa *et al*., 2013) and several other soil borne fungi (Di Pietro *et al*., 1992). The methanolic extract of *Adiantum capillus-veneris* of stem and leaves inhibited the growth of *Providencia, Proteus vulgaris, Vibrio cholerae, Klebsiella pneumoniae, Shigella, Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* (Hussain *et al*., 2014c). Ethyl acetate and chloroform fractions of *Nepeta. leavigata*; chloroform fraction of *Nepeta kurramensis*; chloroform, *n*-hexane and methanol extracts of *R. reniformis* exhibited significant inhibitory effects against fungal strains (Shinwari *et al*., 2013). Citric acid is used in soft drinks, fruit juices, desserts, jams, jellies etc, which is commercially exploited from *Aspergillus niger* (Schuster *et al*., 2002). Growth production of *A. niger* may be enhanced by the plant extracts of *A. orientalis* with careful treatment to avoid spore dust and one of the toxic allergins, ochratoxin (Schuster *et al*., 2002).

Anticancer activity: In cancer treatment, chemotherapy is the most effective method in which different drugs like carboplatin, cisplatin, doxorubicin, melphalan, cyclophosphamide, and gemcitabine are used (Black & Livingston, 1990). However, due to the development of various side effects, therapeutic efficacy of most of them is limited in the host and/or the acquired drug resistance by the cancer cells (Kartalou & Essigmann, 2001). To reduce the side effects and better remedy, many plant species are used with varying success (Roja & Rao, 2000). Higher plants containing medicinal drugs play a crucial role in the health care of human beings (Huang *et al*., 1992). Natural products, isolated from medicinal plants, having more than 50% modern drugs are in clinical use.

Different fractions of *A. orientalis* were tested for their anticancer activities using different cancer cell lines including colorectal adenocarcinoma (HT29 and HCT116) and human hepatoma (HepG2). Different concentrations of the extracts and pure compounds were prepared and screened against the grown cell cultures. The screening results exhibited that chloroform and *n*-hexane fractions were suppressing the cancer cell growth at 500 and 1000 µg/mL, while other fractions (EtOAc, aqueous, methanol and *n*-butanol) were inactive as compared to the control. In present findings of colorectal adenocarcinoma (HT29 and HCT116) cell lines treatments, chloroform and *n*-hexane fractions have reduced the cancer cell viability as compared to other extracts (Fig. 3) at a concentration of 500 and 1000 µg/mL.

In case of HepG2 cancer cells, only chloroform fraction was found to be active against growing cancer cells when compared with the other fractions. The concentration was increased to 1000 µg/mL in order to know the IC_{50} of the extract. The treatment of *n*-hexane and $CHCl_3$ prominently suppressed the cancer cell's viability of HT29 and HCT116 to less than 30 and 20% respectively. However, other fractions such as ethyl acetate, aqueous, methanol and n-butanol did not any effect on cancer cell viability.

Enzyme inhibition analysis: The disease (diabetes mellitus type II) has become a serious and common issue not only in developed countries but also in developing countries due to the changes in dietary habits and people's lifestyle (Khan *et al*., 2012). α-Glucosidase inhibitors are usually used to prevent type II diabetes (van de Laar *et al*., 2005; Heacock *et al*., 2005). Hence, natural α-glucosidase inhibitors from food supplements and medicinal plant sources have become an attractive therapeutic approach for curing post-prandial hyperglycemia (Murai *et al*., 2002; Chen *et al*., 2007).

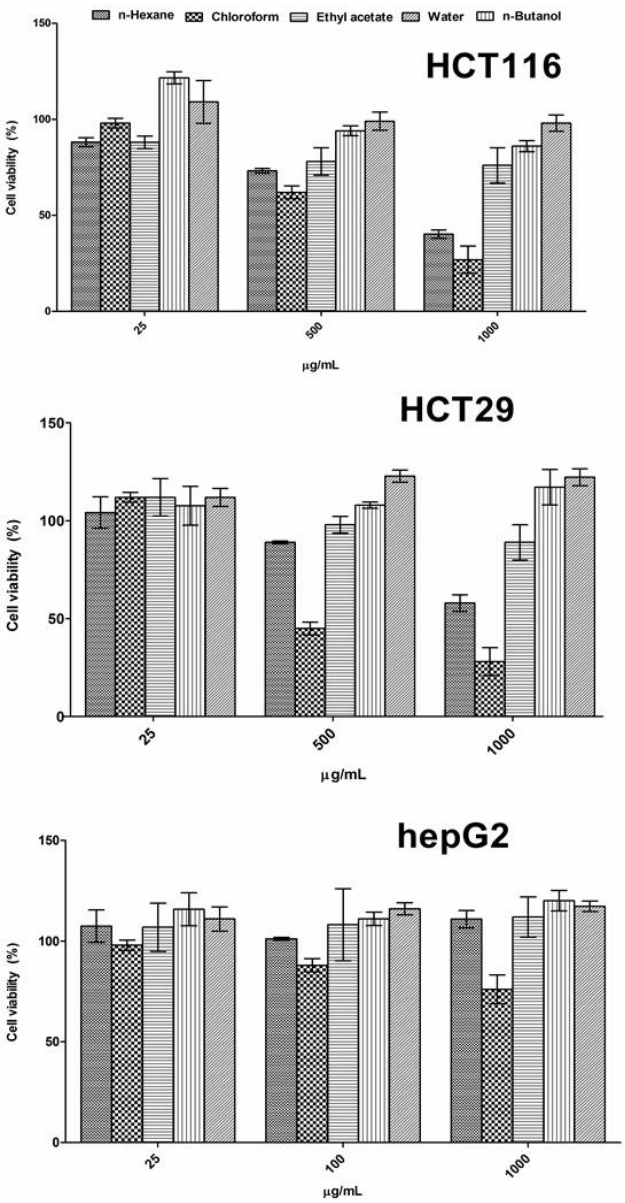

Fig. 3. Anticancer activity of different extracts of stem of *A. orientalis* plant at different concentration (25, 100 and 1000 ppm).

Various fractions obtained from *A. orienatalis,* were tested for their inhibitory potential against two enzymes including urease enzyme and α- glucosidase enzyme. As shown in table 5, $JAOBF_{(S)}$ fraction exhibited promising inhibition (IC_{50} =150±2.50 µg/mL) against urease enzyme. Fractions $JAOEF_{(S)}$ (IC_{50} = 283±1.25 µg /mL) and $JAOBF_{(L)}$ (IC_{50} = 300±2.25 µg/mL) also showed moderate inhibitory potential against urease enzyme as compared to standard urease enzyme inhibitor (Thiourea).

In α- glucosidase enzyme inhibition assay, four fractions (JAOEF, JAOBF, JAOWF, JAOMF) of *A. orienatalis* obtained from stem of the plant exhibited significant inhibition with IC_{50} values 30±1.00, 52±2.50, 65±2.00, and 88±1.50 µg/ml), respectively. Aqououes fraction ($JAOWF_{(L)}$), obtained from leaf of the plant showed moderate inhibition with IC_{50} value of 155±2.50 µg/mL). Acarbose was used as standard inhibitor (Table 5).

These results provided important information about the fractions that possess active constituents, which are actually responsible for the enzyme inhibition. In future isolation and characterization of these active secondary metabolites from these fractions might be interesting to know actual compounds responsible for their activity and to understand their underlying mechanism of inhibition against these enzymes.

Total phenolic contents: The total phenolic contents were measured in both the stem and the leaves of *A. orientalis*. The results showed that the crude extract as well as the fractions of the stem (0.5–16.8 mg/g of dry wieght) had higher phenolic contents than the leaves (4.1–8.4 mg/g of dry wieght) (Fig. 4). Ethyl acetate (16.8 mg/g of dry wieght) and *n*-butanol (10.6 mg/g of dry wieght) fractions of the stem showed higher phenolic contents than the other stem fractions. In case of the leaves, the *n*-butanol (8.4 mg/g of dry wieght) and chloroform (4.8 mg/g of dry wieght) fractions had higher phenolic contents than the other fractions of the leaves. Recent study by Ksiksi & Hamza (2012) demonstrated that *A. orientalis* from Al Ain exhibited the highest polyphenolic content when compared with Omani species which further confirmed our results.

Table 5. Urease enzyme inhibition activity of various fractions of stem and leaves of *A. orienatalis*.

Sample code (0.5 mg/ml)	Urease enzyme (%) inhibition	IC_{50} (µg/ml))	α- Glucosidase enzyme (%) inhibition	IC_{50} (µg/ml))
$JAOMF_{(S)}$	65 ± 1.500	345 ± 2.00	92 ± 2.50	88 ± 1.50
$JAOWF_{(S)}$	40 ± 1.00	-	91 ± 1.00	65 ± 2.00
$JAOBF_{(S)}$	80 ± 2.50	150 ± 2. 50	88 ± 1.00	52 ± 2.50
$JAOCF_{(S)}$	40 ± 1.00	-	NA	-
$JAOEF_{(S)}$	75 ± 2.50	283 ± 1.25	85 ± 2.50	30 ± 1.00
$JAOHF_{(S)}$	NA	-	52 ± 2.00	-
$JAOMF_{(L)}$	NA	-	55 ± 1.50	448 ± 2.00
$JAOWF_{(L)}$	56 ± 1.00	-	82 ± 1.00	155 ± 2.50
$JAOBF_{(L)}$	70 ± 2.00	300 ± 2.25	22 ± 1.50	-
$JAOCF_{(L)}$	NA	-	NA	-
$JAOEF_{(L)}$	35 ± 1.50	-	NA	-
$JAOHF_{(L)}$	35 ± 1.00	-	40 ± 1.00	-
Thiourea	95 ± 1.50	21 ± 1.50 (mM)	NA	-
Acarbose	-	-	72 ± 2.00	90 ± 2.50 (µM)

NA* = Not active

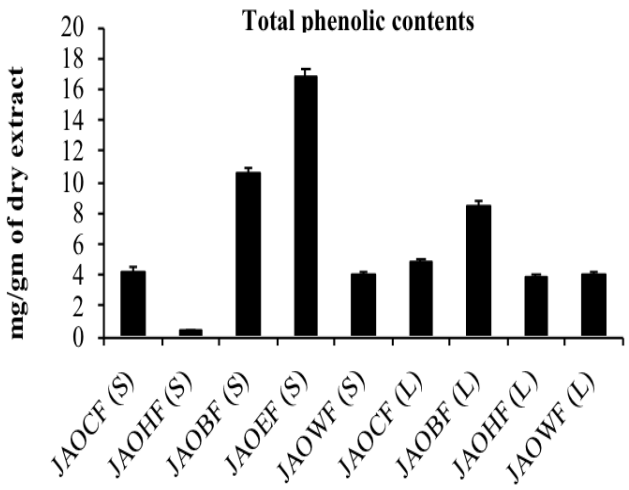

Fig. 4. Total phenolic contents in stem and leaves fractions of *A. orientalis* expressed in mg/g of dry extract. The values in the bar are presented with the standard deviation of mean values (three replications).

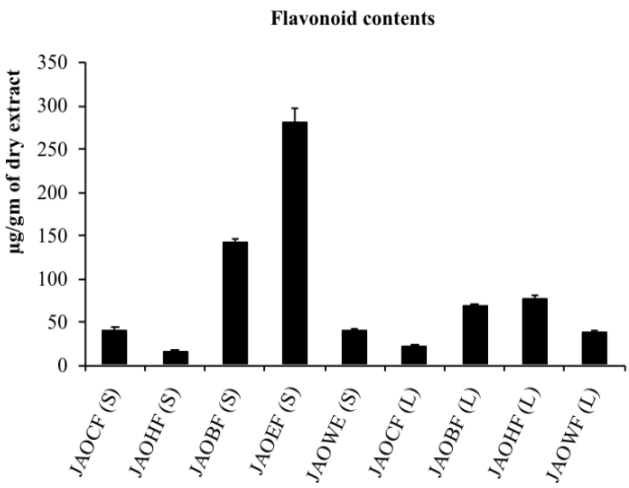

Fig. 5. Total flavonoid contents in stem and leaves fractions of *A. orientalis* expressed in µg/g of dry extract. The values in the bar are presented with the standard deviation of mean values (three replications).

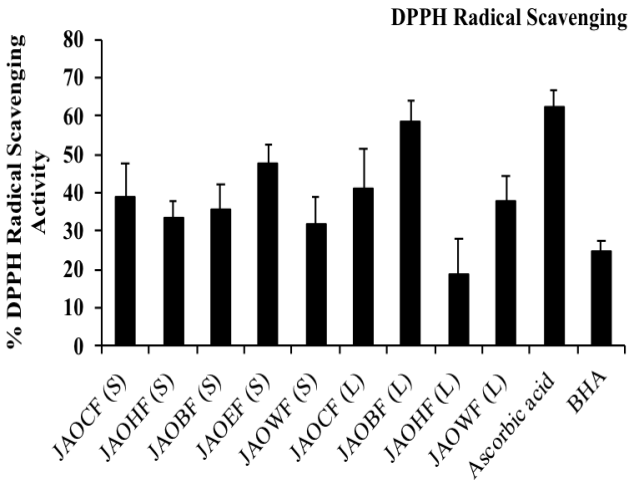

Fig. 6. DPPH radical scavenging activity of stem and leaves fractions of *A. orientalis* expressed in percentages. The values in the bar are presented with the standard deviation of mean values (three replications).

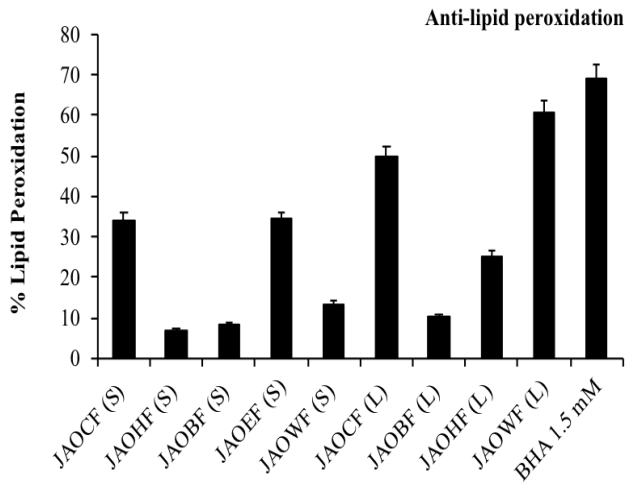

Fig. 7. Lipid peroxidation activity of stem and leaves fractions of *A. orientalis* expressed in percentages. The values in the bar are presented with the standard deviation of mean values (three replications).

The total flavonoid contents in the stem and leaves of *A. orientalis* showed that the stem (16.38–279.85 µg/g of dry wieght) had comparatively higher total flavonoid contents than the leaves (22.60–77.64 µg/g of dry wieght). The lowest flavonoid contents, however, were found in *n*-hexane fraction of the stem (16.38 µg/g of dry wieght). The ethyl acetate fraction (279.85 µg/g of dry wieght) of stem had higher flavonoid contents than the rest of the stem fractions. Similarly, *n*-hexane fraction of leaves showed higher flavonoid contents (77.64 µg/g of dry wieght) as compared to the other fractions of leaves (Fig. 5).

DPPH radical scavenging: DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activities of crude extracts and fractions of *A. orientalis* roots and stems were tested by evaluating their DPPH radical scavenging capacities. Both the stem (31.8–47.6%) and the leaves (18.8–44.7%) extracts and fractions showed mixed patterns of radical scavengig activities. Ethyl acetete fraction of the stem (47.6%) and n-butanol fraction of leaves (58.6%) showed higher radical scavenging activity than the other fractions (Fig. 6). The fractions in both the plant parts comparatively showed higher radical scavenging activities than BHA (24.9%), but lower than the ascorbic acid (62.3%). In our results, the highest antioxidant activity was found in the ethyl acetate fraction of *A. orientalis* stem. The results of *A. orientalis* may not be comparable with the results of other plants due to the chemotaxonomic and taxonomic differences at family, generic and species level. However, an earlier report showed that an ethyl acetate fraction of *Geranium wallichianum* had highest antioxidant activity as compared to the *n*-butanol and aqueous extracts (Ismail *et al*., 2009).

Anti-lipid peroxidation: When the stem and leaves were tested for anti-lipid peroxidation by Thiobarbituric Acid Reactive Substance (TBARS) Assay, the crude extract and fractions of leaves showed higher percentage of oxidative degradation of lipids as compared to the stem extract and respective fractions (Fig. 7). Aqueous and chloroform

fractions of leaves showed higher inhibition of 60.6% and 49.9%, respectively. In case of TBARS bioassay of stem, ethyl acetate (34.5%) and chloroform (34.2%) fractions showed higher percentages of lipid peroxidation as compared to the rest of the fractions (Fig. 7).

Conclusion

In the present study, the determination of proximate analysis, enzyme inhibition bioassays, antifungal and other biological activities of the different fractions of leaves and stem of *A. orientalis* are reported. The ethyl acetate fraction of *A. orientalis* stem showed significant amount of flavonoids, phenolic contents and also exhibited promising antioxidant, lipid peroxidation, anticancer, α-glucosidase and urease enzyme inhibition activities. In the present studies the ethyl acetate fraction of stem of *A. orientalis* grown in Oman contains a substantial amount of bioactive constituents, which might be responsible for its biological activities. *A. orientalis* is a commonly used traditional medicinal plant against inflammatory diseases that may have potential in cancer treatment. Furthermore, *In vitro* and *In vivo* anticancer studies are required to determine the potential of the active extracts of *A. orientalis* for therapeutic uses of this plant to prevent some chronic diseases.

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