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Research Article

Phytochemical, antimicrobial and antioxidant properties of *Launaea nudicaulis* and *Farsetia hamiltonii*

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ABSTRACT: Plant extracts could be beneficial for biocontrol of macro-organisms and micro-organisms which attack plants and it could be also beneficial for protection or treatment of some diseases in animals and human. In the current study, two plants from the arid areas in the Northern Regions in Saudi Arabia, namely, *Farsetia hamiltonii* and *Launaea nudicaulis* were screened for their phytochemical, antimicrobial and antioxidant properties. GC-MS screening of methanol extract of flowers of *F. hamiltonii* revealed the presence of triterpen compound (Betulins, Lanosterin), and quercterin. The methanol extract of *L. nudicaulis* revealed the presence of dihydro-isosteviol-methylester and flavone-4'-OH,5-OH,7-di-O-glucoside. Dihydrotorulosol and Urs-12-ene-3-one were detected in two extracts, where chloroform extract of *Launaea* were rich in alkaloid compounds, aspidofractinin, and tocopherol. The antioxidant activity showed that the chloroform extract of *L. nudicaulis* had higher antioxidant activity (IC50=1.6±0.1g/L; EC50=2.15±0.3g/L), followed by methanol extract of *F. hamiltonii* (IC50=3.6±0.1g/L; EC50=4.1±0.1g/L) and methanol extract of *L. nudicaulis* (IC50=7.1±0.2g/L;EC50=7.85±0.4g/L). The microbiological investigation revealed that methanol extract of *F. hamitonii* and chloroform extract of *Launaea nudicaulis* showed no antimicrobial activity against all tested microorganisms. While the methanol extract of *L. nudica* exhibited average antibacterial activity against *Staphylococcus epidermidis* ATCC 12228 and weak antibacterial activity against *S. aureus* ATCC 25923 and *Escherichia coli* ATCC 35218.

KEY WORDS: Antimicrobial, antioxidant, *Farsetia hamiltonii, Launaea nudicaulis*, phytochemical components

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INTRODUCTION

In nature, plants have developed several ways for protecting themselves against macro-organisms (animals, insects,..) or microorganisms (parasites, fungi,..). One such way is production of secondary metabolites that have biological control properties and some of these metabolites could possess bioactive benefits for animals and humans. These phytochemicals could be promising sources for drugs and remedies (Abdallah, 2011). Actually, based on the botanical literature which described the usage of numerous plants in medicine since antiquity, it can be considered that medicinal plants are the backbone of ancient and modern pharmacopoeia. Man first discovered the healing properties of plants and tried to use them as food. Later, based on their effects on his body he started collecting them for medicinal use. The medical knowledge and experience were passed from generation to generation. Such knowledge has been recorded by some great civilisations and sometimes mixed with myth and magic. Some of these applications are still used in developing countries (Samulesson, 1999). Accordingly, it is important to investigate and study medicinal plants based on ethnopharmacological knowledge.

The genus *Launaea* belongs to the family Asteraceae and comprises about 40 species. Many of the species of the genus *Launaea* are used in medicine for the treatment of skin diseases, tumours and dysentery (El-Bassuony and Abdel-Hamid, 2006). The studies on the genus with some species reveal the presence of specific phytochemicals which provides the plant, its medicinal properties (Sarg *et al.*, 1982; Saleh *et al.*, 1998).

Genus *Farsetia* is a medicinal plant belonging to family Cruciferae, having more than 20 species, *Farsetia ageptia* is known to be used by the native Bedouins as anti-diabetic and antispasmodic and it is used to relieve rheumatic pains and also consumed as a cooling medi-

cine after pounding (Mitchell-Olds *et al.,* 2005). *F. hamiltonii* Royle (Brassicaceae) is a desert medicinal plant and is commonly used to treat various diseases by inhabitants of the Cholistan desert. The plant is an annual shrub and often woody at base. The plant length is about 10-40 cm with densely appressed white hairs. The leaves are oblong linear and fruit is little longer, narrowed at either end. The root is thick woody and flower is white or pink colour, petals slightly longer than sepals and flowering season is March to September (Buolos, 1983). The ethanol extract of the aerial parts of *F. aegyptia* was investigated *in vitro* for its cytotoxic properties against MCF-7, HepG2 and HCT116 cell lines, which recorded IC_{50} = 17.90, 12.60 and 4.65 µg/ml, respectively, compared to Doxorubicin (positive control) which showed *in vitro* cytotoxic effect of IC_{50} = 2.97, 4.57 and 3.64 µg/ml, respectively, The phenolic-rich fraction of the ethanol extract, the fractioned parts led to the isolation of new flavonoid Known as kampferol 7-8 diglucoside, which showed high cytotoxic activity against MCF-7 and Hela cell lines (El-Sharkawy *et al.,* 2013).

The present study was undertaken to explore the antioxidant and antimicrobial effects of these plants. Also, the photychemical study of the plants was performed to discuss the importance of the bioactive compounds as an antioxidant and/or antimicrobial agents.

MATERIALS AND METHODS

Plant Material

The aerial part of *Farsetia hamiltonii* (*F. stylosa)* and *Launaea nudicaulis* were collected from Eastern desert, Egypt, during the spring season, of 2015. The plant samples were authenticated by plant taxonomy specialist at Botany Department, Northern Border University. The plant materials were air-dried in the shade and ground to a fine powder for phytochemical and biological investigation**.**

Preparation of plant extracts

Each dried powdered plant material was filled separately in the thimble and extracted using 80% methanol with Soxhlet extractor, the extracted solution was distilled and evaporated. Briefly, plant powder was transferred to a cellulose thimble in an extraction chamber, placed above the collecting flask beside the reflux condenser. The solvent (Methanol) was then added to the flask, and heated under reflux until certain level of condensed solvent had accumulated in the thimble and then siphoned in the flask. This procedure was repeated about 10 times within about 72 hours for each tested sample. At the end of the extraction process, the solvent was removed by rotary evaporator. The same procedure was performed with fresh flowers of *Launaea nudicaulis* using chloroform instead of methanol.

Phytochemical screening:

The phytochemical constituents of methanol and chloroform extracts were screened and characterised as mentioned in (Earnsworth *et al.,*1979 and Bruneton, 1999).

GC-MS analysis

The extracts and active fractions were analysed by GC-MS as reported by (Adams, 1995). The GC-MS was analysed using Shimadzu GC-MS-QP 2010 gas chromatography-mass spectrometer equipped with capillary column DB-5-MS Agilent (30 m x 0.25 mm) and film thickness 0.25 µm. Helium was adjusted at pressure 81.90 K Pa which is used as a carrier gas, with a flow of 1.33 ml/min. The temperature in the injector was 250 °C, while the temperature of the oven progressed from 60 to 240 °C to 3 C/min. The mode of ionisation was the electronic impact at 70 eV. After that, each fraction was co-injected with a homologous series of linear hydrocarbons under the same experimental conditions in order to calculate the retention index (RI) of sample constituents using Van Den Dool and Kratz equation. The tested compounds were identified by analysing and comparing the mass spectra with a database of Wiley 7 libraries and also by comparing the RI with those of the literature (Araujo *et al.,* 2005; Morteza-Semnani *et al.,* 2007; Chaichana *et al.,* 2009).

Microorganisms

Six referenced pathogenic bacterial strains representing Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 10876) and Gram-negative bacteria (*Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 35218); additionally, two referenced fungal strains (*Candida albicans* ATCC 14053 and *Aspergillus niger* ATCC 6275) were also used to investigate the possible antimicrobial activity of the plant extracts. The bacterial and fungal strains were obtained from the Department of Pharmaceutics, Unaizah College of Pharmacy; and from the Department of Laboratory Sciences, College of Science and Arts at Al-Rass, Qassim University, KSA.

Antimicrobial testing

The antimicrobial activity of *Farsetia hamiltonii* and *Launaea nudicaulis* extracts were estimated by disc diffusion method as previously described (Abdallah *et al.,* 2017). The test microorganisms were sub-cultured in Mueller-Hinton agar for up to 24 hours for bacteria or subcultured in Sabouraud Dextrose Broth for up to 48 hours for fungi, these subcultures were considered as working samples.The working bacterial samples were adjusted to be equivalent to 0.5 McFarland (Approximately 1-2 × 10^8 CFU/ml), while the sub-cultured fungi in broth were used

directly as working fungal samples. Different autoclaved agar media, Mueller-Hinton agar for bacteria or Sabouraud Dextrose Agar for fungi (20 ml) was poured to a sterile disposable Petri-dish (Size 100x15mm) and left until solidified. The working microbial samples were swapped over the surface of the prepared plates using sterile disposable cotton swap. 6 mm sterile filter paper discs (Prepared from filter paper No.1) were immersed in the reconstituted extracts at 400 mg/ml, the methanol extracts were reconstituted in 10% DMSO (Dimethyl sulfoxide), while the chloroform extract was reconstituted in chloroform. Whatman blank discs were immersed in reconstituted extracts, left for 5 minutes until saturated and then loaded on the agar plates, except for the chloroform discs which were left at room temperature until totally dried and then loaded on the agar plates. Antibiotic discs were also loaded on the plates, namely ; gentamicin 10 µg/disc (Oxoid, UK) for bacteria or clotrimazole 10 mg/ml (Canesten, Switzerland) for fungi to serve as positive controls, sterile discs saturated with 10% DMSO was also loaded on the plate to serve as negative control. All the seeded plates were incubated at 37°C for 18 hours for bacteria or for up to 48 hours for fungi. The test was repeated thrice and the mean inhibition zone±standard error of means was calculated.

Antioxidant testing

(i) DPPH scavenging activity

The DPPH assay is known to provide reliable information regarding the antioxidant capacity of specific extracts or compounds within a short time scale (Huey-Chun *et al.,* 2012). Hydrogen atoms or the ability of electrons donation of the plant extract and some pure compounds were recorded from the bleaching of a purple-coloured methanol solution of DPPH (Kubola *et al*., 2008). Briefly, 0.5 mL of each sample concentration was mixed with the same volume of DPPH methanol solution (0.04 g/L). The mixture was shaken vigorously and left for 30 min in darkness and at 25°C; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Abdellah *et al*., 2016).The percentage inhibition of activity was calculated as:

$$\% \text{ Inhibition} = \frac{(\text{A blank} - \text{A sample})}{\text{A blank}} \times 100$$

Quercetin and ascorbic acid were used as positive control, the concentration showing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the concentration of extract.

(ii) Reducing power: Iron (III) to Iron (II)

The reductive capacity of the extract was determined using ferric to ferrous iron reduction assay as determined spectrophotometrically from the formation of Perl's Prussian blue coloured complex (Dorman *et al*., 2003). Briefly, 1 mL of each sample was mixed with 2.5 mL of 0.2 mol/L phosphate buffer (pH= 7.0) and 2.5 mL of potassium hexacyanoferrate solution $K_3Fe(CN)_6$. After incubation for up to 30 minutes at 50°C, aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture and then 2.5 ml distilled water and 2.5 ml Fe CL_3 (0.5 mL, 0.1%) were added and mixed with 2.5 mL of this solution. The absorbance of the mix was measured with spectrophotometer at 700 nm. Ascorbic acid and Quercetin were also employed as standards for comparison. The EC_{50}, which is the effective concentration of the sample at which the absorbance is 0.5 was determined.

RESULTS AND DISCUSSION

The results of phytochemical investigations are presented in (Table 1), methanol extract of *Farsetia hamiltonii* exhibited presence of flavonoids, sterols, glycosides, tannins and saponins; methanol extract of *Launaea nudicaulis* showed presence of alkaloids, flavonoids, sterols, glycosides and saponins.; while, chloroform extract of *L. nudicaulis* revealed presence of alkaloids, flavonoids and saponins. The GC-MS analysis of plant extracts and fractions are shown (Table 2). There were ten major compounds in methanol extract and only five compounds in chloroform extract. The extraction of hydroalcohol using defatted plant powder showed interesting results with GC-MS, and the identified important compounds included flavones-glucoside and some indole alkaloids in methanol extracts, and spidofractinin in chloroform extract.

The compounds identified by GC-MS, belong to the different class of natural products such as terpenes, flavonoids and alkaloids, this was also confirmed by preliminary phytochemical analysis of the plant extracts which showed that the plants are rich in flavonoids, alkaloids, saponins and terpenes (Table 1).

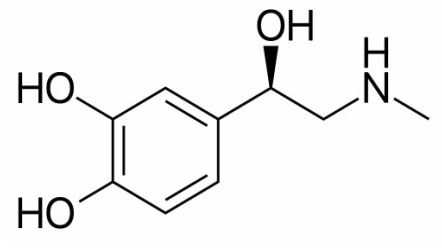
The dominant components present in methanolic extract of *Farsetia hamiltonii* were dihydrocoumarin, Betulins, Chrysene and Quercetin, whereas *Launaea nudicaulis* was found to be rich with flavonoid glucoside; kampferol-7-diglucoside was detected by GC-MS, Olean-12-en-28-al, cyclic 1,2-ethanediyl mercaptal, Urs-12-ene-3one, and dihydrotrosol phytol were detected in methanol extracts of both *L. nudicaulis* and *F. hamiltonii*. Chloroform extract of *L. nudicaulis* was very rich with volatile compounds, Andrographolide diterpen class, camphor and vitimen E were found enriched in flowers; and Epinephrine, endole

alkaloids were also major compounds detected. also. The chemical structure of some of these detected compounds are shown (Fig. 1).

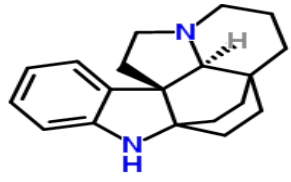
Table 1. Phytochemical screening for secondary metabolites

Launaea nudicaulis- chloroform Extract	*Launaea nudicaulis*- methanol Extract	*Farsetia hamiltonii*-methanol Extract	Compound
++	+	-	Alkaloids
++	+++	+++	Flavonoids
-	++	++	Sterols
-	++	++	Glycosides
-	-	+	Tannins
+	+++	++	Saponins

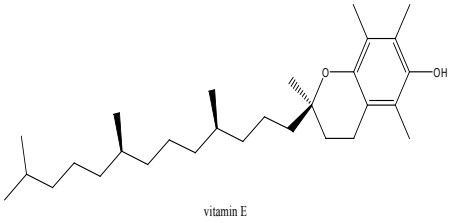
Plant monographs and pharmacopoeia describe only the physicochemical characteristic features of the materials. In recent years, the use of modern methods for the identification and the quantification of bioactive principles with standards are useful for the standardisation of medicinal plants and their formulations, as has been emphasised by the World Health Organization. GC and LC with different detection techniques are relatively expensive and can be used to identify the phytoconstituents that are largely present in herbal medicines and formulations (Eisenhauer *et al.,* 2009). Phytol diterpene is widely used as antimicrobial, antioxidant, antitumor, anticancer, antiarthritic, immunestimulatory, antidiabetic, chemopreventive, pesticidal, and has sunscreen properties (Dr. Duke's, 2016).



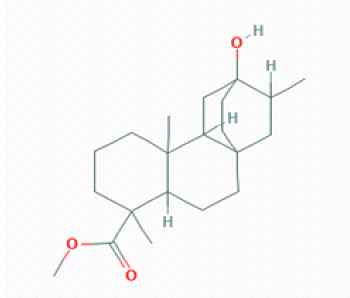
Epinephrin



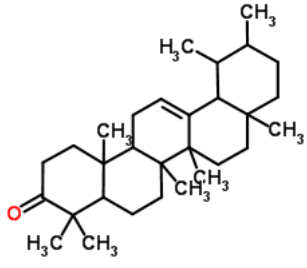
Aspidofractinin



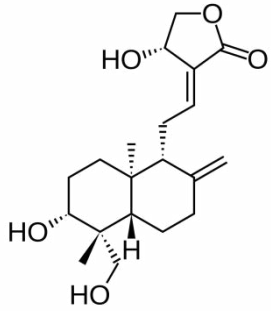
Tocopherol



Dihydro-isosteviol methyl ester



Urs-12-en-3-one



Andrographolide

Fig. 1. Structure of some detected compounds.

Table 2. The results of GC-MS analysis of plant extracts and fractions

Methanol Extract *Farsetia hamiltonii*	Methanol Extract *Launaea nudicaulis*	Chloroform Extract *Launaea nudicaulis*
Vanillin	Phytol	Phytol
Betulins		Lupan-3-ol-acetate
Urs-12-ene-3-one	Urs-12-ene-3-one	Epinepherin
Lanosterin	d-orandrostane	
Cyclolaudenol	1,2-Epoxycholestan-3-one	Aspidofractinin
Globulol	-	Globulol
3-O-Acetyl-delta-24-cycloartenol	Chrysene	Juniper camphor
Dihydrocoumarone	Flavone 4'-OH,5-OH,7-di-O-glucoside	Alpha-Tocopherol
Dihydrotorulosol	Dihydrotorulosol	Andrographolide
Qurectein	Olean-12-en-28-al, cyclic 1,2-ethanediyl mercaptal	
Chrysene	Delta- 5-Cholesten-3.beta.-ol	
Hexa -hydrothunbergol		
Neophytadiene	Dihydro-isosteviol methyl ester	
Coniferol		
Phytol		
Juniper camphor		

The antimicrobial investigation revealed that the fungal strains did not show any susceptibility against all the tested extracts. As well, all bacterial strains did not exhibit any susceptibility against the methanol extract of *Farsetia hamitonii*, and the chloroform extract of *Launaea nudicaulis.* While, some degree of antibacterial activity was detected with the methanol extract of *L. nudicaulis*, where three of six bacterial strains recorded varied zone of inhibitions and these bacteria were *Staphylococcus epidermidis* ATCC 12228 (10.5±0.5 mm), *S. aureus* ATCC 25923 (6.7±0.3mm) and *Escherichia coli* ATCC 35218 (6.5±0.5mm), respectively at concentration 400 mg/ml, compared to the antibiotic gentamicin 10µg/disc (Table 3). Also, It was observed that 10% DMSO is a good solvent for methanolic crude extracts and it has no effect on the bacterial growth. *Farsetia hamitonii* is a typical desert plant, with some medicinal applications, it is employed as antirheumatic and tonic (Hayat *et al.,* 2015). However, to the best of our knowledge, there is no adequate antimicrobial studies on this plant that has been reported till now and our findings suggest that methanol extract of this plant has no antimicrobial activity, although different species of *Farsetia* (*Farsetia aegyptia*) was reported to possess significant antimicrobial activity against *Klebsiella pneumonia* and *Aspergillus niger* (Atta *et al.,* 2013). The methanol extract of *Launaea nudicaulis* showed maximum inhibition against *S. epidermidis* (Gram-positive), weak inhibition against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Accordingly, this plant tends to be active against the Gram-positive bacteria rather than the Gram-negatives. Our results that showed a moderate antibacterial activity of *Launaea nudicaulis* against the Gram-positive bacteria agrees with (Nivas and Boominathan,

Table 3. The antimicrobial activity of the extracts

Tested Compound	Mean Zone of Growth Inhibition in millimiter (including the Disc Diameter 6mm)							
	Gram-Positive Bacteria				Gram-Negative Bacteria		Fungi	
	Sa	Se	Ef	Bc	Ec	Kp	An	Ca
Launaea nudicaulis Chloroform extract (400 mg/ml)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Launaea nudicaulis Methanol extract (400mg/ml)	6.7±0.3	10.5±0.5	6.0±0.0	6.0±0.0	6.5±0.5	6.0±0.0	6.0±0.0	6.0±0.0
Farsetia hamitonii Methanol extract (400mg/ml)	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Gentamicin (10 µg/disc)	29.5±0.5	23.0±1.0	13.5±0.5	25.0±0..0	22.5±0.5	14.0±0.0	N/A	N/A
Clotrimazole (10 mg/ml)	N/A	N/A	N/A	N/A	N/A	N/A	35.0±1.0	32.0±2.6
10% DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

*Disc diameter=6.0 mm, 6.0±0.0= No activity (the disc diameter), Zone of inhibition is the mean of three replicates ±standard deviation, N/A=Not applicable, Sa=*Staphylococcus aureus* ATCC 25923, Se=*Staphylococcus epidermidis* ATCC 12228, Ef=*Enterococcus faecalis* ATCC 29212, Bc=*Bacillus cereus* ATCC 10876, Ec=*Escherichia coli* ATCC 35218, Kp=*Klebsiella pneumoniae* ATCC 700603, An=*Aspergillus niger* ATCC 6275 and Ca=*Candida albicans* (Clinical isolate). DMSO= *Dimethyl sulfoxide*

2015) who reported that *L. nudica* showed average antibacterial activity against *Streptococcus pneumonia* (Gram-positive). The antibacterial activity of the methanol extract of *L. nudicaulis* is mainly attributed to some bioactive phytochemical compounds in the methanol extract. Some phytochemical classes reported as antimicrobial agents, such as flavonoids, terpenoids, alkaloids, tannins, saponins, glycosides, anthraquinones, among others (Omojate *et al.,* 2014). Although, chloroform extract of *L. nudicaulis* and methanol extract of *F. hamiltonii* possess some of the above mentioned phytochemical compounds, the extracts did not show any antimicrobial activity, hence it can be deduced that each phytochemical class are of diverse types with different bioactive effects, as analyzed the antioxidant results (Table 4), The methanol extract of *Launaea nudicaulis* showed average antibacterial activity and showed least antioxidant activity, while the other two plant extracts which exhibited higher antioxidant revealed no antimicrobial effects.

To confirm the traditional use of medicinal plants, chloroform and methanol extracts of *Launaea nudicaulis* and methanol extract of *Farsetia hamiltonii* were tested for the antioxidant activity (*in vitro*). In this study,the DPPH scavenging activity and the reducing power of Iron (III) to Iron (II) were employed and the results are shown in Table 4.

Table 4. Antioxidant activity of plant extracts by DPPH (IC_{50}) and reducing power (EC_{50})

Test	LC	TC	FC	AC	QC
IC_{50} (g/L)	1.6±0.1	7.1±0.2	3.6±0.1	0.031± 0.001	0.012± 0.002
EC_{50} (g/L)	2.15±0.3	7.85±0.4	4.1±0.1	0.095± 0.002	0.019± 0.003

LC: Chloroform extract of *Launaea nudicaulis*, TC: Methanol extract of *L. nudicaulis*, FC: Methanol extract of *Farsetia hamiltonii*, AC: Ascorbic acid, QC: Quercetin

In DPPH assay, the hydrogen donating ability of the extract was determined by converting the DPPH radical to non-radical by the reduction process. In FRAP assay, the reducing power are associated with the presence of compounds that exert their action by breaking the free radical chain by donating a hydrogen atom.

The results of this study showed that in general the tested extracts have a low antioxidant activity compared to that of Ascorbic acid and Quercetin. However, the chloroform extract of *Launaea nudicaulis* had relatively higher antioxidant activity compared to other tested extracts with an IC_{50} of 1.6±0.1g/L and an EC_{50} of 2.15±0.3g/L, followed by methanol extract of *Farsetia hamiltonii* with an IC_{50} of 3.6±0.1g/L and an EC_{50} of 4.1±0.1g/L. However, the methanol extract of *L. nudicaulis* had the lower antioxidant activity with an IC_{50} of 7.1±0.2g/L and an EC_{50} of 7.85±0.4g/L.

The antioxidant compounds are essential and play an important role as a health protecting factor and can reduce the risk for chronic diseases including cancer and heart disease (Prakash and Durgesh, 2010). Higher plants provide a rich source of natural antioxidants, such as tocophenols and polyphenols which are widely present in vegetables, fruits, seeds, spices, cereals and herbs (Abdalla and Roozen, 1999; Shahidi and Zhong, 2015). The Antioxidant potential can be monitored by different methods with varied mechanisms, such as reducing power, single electron transfer, hydrogen atom transfer, and metal chelation (Shahidi and Zhong, 2015).

In this study, the reducing power and DPPH scavenging assays were used, and the results showed that the tested extracts have a low antioxidant activity compared to that of Ascorbic acid and Quercetin, because they are in a form of crude and not pure. Furthermore, the chloroform extract of *Launaea nudicaulis* had a higher antioxidant activity, followed by methanol extract of *Farsetia hamiltonii* and methanol extract of *L. nudicaulis*. The chemical analysis of plant extracts showed that the chloroform extract of *L. nudicaulis* contains the alpha-tocopherol which is a major liposoluble antioxidant substance (Mallet *et al.,* 1994). Also, the methanol extract of *F. hamiltonii* contains quercetin that has a powerful antioxidant activity (Gordon *et al.,* 1998). A study in Algeria showed that the antioxidant activity of *F. hamiltonii* (methanolic extract) was not significant because it is poor in substances that have an antioxidant activity (Hayat *et al.,* 2014; Hayat *et al.,* 2015). In another study, the chromatographic purification showed that the methanol extract of *L. nudicaulis* contains substances which don't have antioxidant activity (Saleem *et al.,* 2012).

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