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Phytochemical screening, cytotoxic and antimicrobial activities of *Limonium socotranum* and *Peperomia blanda* extracts

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Abstract. *Limonium socotranum* and *Peperomia blanda* are used in ethnomedicine to treat several diseases, such as infection, cancer, and inflammation. *P. blanda* (family: Piperaceae) is from the genus *Peperomia*, and mostly found in Madagascar, Yemen, USA to South America, while *L. socotranum* (family: Plumbaginaceae) from the genus *Limonium* and this species is found only on Socotra Island, Yemen. These plants have attracted great interest in recent years because of their phytochemical contents. Consequently, the current study is aimed to investigate the phytochemical constituents, the cytotoxic and antimicrobial activities of *L. socotranum* (leaves and stem) and *P. blanda* extracts. Successive extraction had been performed which resulted in nine crude extracts. Phytochemical screening of the extracts was then conducted using qualitative chemical analysis. The antimicrobial activity of the plant extracts was determined using the well diffusion method against eleven selected pathogenic microbes and the minimum inhibitory concentrations (MIC) were measured. The cytotoxic activities of the plant extracts against MCF-7 and HepG2 cell lines were investigated using sulforhodamine B assay. It was noted that methanol leaves extract from *L. socotranum* exhibited higher antibacterial activity against *Micrococcus luteus* (MIC 15.6 µg/mL), *Staphylococcus aureus* (MIC 125 µg/mL) and *Pseudomonas aeruginosa* (MIC 125 µg/mL), than stem parts, while petroleum ether extract displayed stronger antifungal activity, with MIC of 125 µg/mL. On the other hand, petroleum ether extract of *P. blanda* was effective against Gram-positive bacteria and exhibited moderate antifungal activity. Petroleum ether extract of *P. blanda* displayed cytotoxic activity against MCF-7 cells, with an IC_{50} of 4.60 ± 0.02 µg/mL, while the methanol extracts showed higher activity against the HepG2 cell line, with an IC_{50} of 13.90 ± 0.14 µg/mL. Phytochemical findings confirmed the presence of flavonoids, alkaloids and terpenoids. The promising obtained results suggest the potential use of these plants in cancer and antimicrobial therapies.

INTRODUCTION

Natural products, in particular those from medicinal plants, are becoming essential in primary health care, especially in developing countries (Mutee *et al.*, 2010; Newman & Cragg, 2016). The use of herbal remedies is widespread among the populations of Yemen, yet only few species from Yemeni flora have been experimentally explored (Mothana *et al.*, 2010). *Limonium* which belongs to the family Plumbaginaceae has a genus of 120 flowering species which are commonly known as Sea Lavender, Statice, or Marsh-rosemary. *L. socotranum* (Vierh.) Radcl.-Sm is an endemic plant in the Socotra Island and

has been reported to be used by the local people in Yemen as a remedy for dysentery or as a gargle or mouthwash for ulcerations. In addition, *Limonium* species have been reported to be used as anti-inflammatory, antiviral, and antibacterial agents and against cervical cancer (Eren & Özata, 2014). *Peperomia* is the second largest species of the Piperaceae family. Only a few species from the family have been investigated for their chemical contents (dos S Junior *et al.*, 2013; Felippe *et al.*, 2008). *Peperomia* species have been recorded to have numerous uses as traditional medicine to treat skin diseases, burns, eye infections, and asthma and as antibiotics (Gutierrez *et al.*, 2016).

Cancer, one of the prime causes of morbidity and mortality, with about 14 million new cases and 8 million deaths in 2012, affects human populations worldwide (McGuire, 2016). For this reason, cancer chemotherapy has become a major area of research focus. On the other hand, bacterial infections account for a large share of the global burden of infectious ailments (WHO, 2014), which impact negatively on human health and the economy. Chemotherapy with antibiotic and antibacterial agents has been the main treatment approach of clinical infections but the effectiveness of the treatment is continually being threatened by the increasing antibiotic resistance in some organisms. In most countries, high incidence of antibiotic resistance is related to micro-organisms that are responsible for common health-care associated and community-acquired infections such as pneumonia and urinary tract infections (WHO, 2014). In addition, the high incidence of opportunistic fungal infections among cancer patients remains a challenge to clinicians.

This situation demands a constant search for new anticancer and antibacterial agents to curb cancer, infection and antibiotic resistance. Therefore, screening of plants for their medicinal values remains an important area in scientific research. Over the period from the 1940s until 2014, 75% of approved small molecules were identified to be originated from natural products or derived directly from them (Newman & Cragg, 2016). Therefore, this study was to estimate the phytochemical contents of two selected plants, namely *Limonium socotranum* (Vierh.) Radcl.-Sm and *Peperomia blanda* (Jacq.) Kunth and to evaluate the cytotoxic and antimicrobial activities.

MATERIALS AND METHODS

Plant material and extraction

The studied plants were harvested from the Diksam Plateau, Socotra Island, Yemen in March 2013 with approval numbers 120025 and 120026 from the General Directorate of plant protection, Yemen. These plants samples were identified by Dr. Abdul Wali Al-Khulaidi, Department of Botany, Taiz University, Yemen. The voucher specimen numbers of *Limonium socotranum* and *Peperomia blanda*, were LSLS/2013/95 and PPLS/2013/90/1, respectively. The plants were deposited in the Faculty of Pharmacy, Sana'a University, Yemen. Plant samples (leaves and stems) were oven-dried for seven days at 30°C, after which the dried samples were milled and extracted successively starting with petroleum ether (pet. ether) followed by dichloromethane (DCM) then methanol by maceration for 72 hours each. The extracts were then filtered and concentrated under reduced pressure using a rotatory evaporator to obtain crude extracts to be used for further biological screening. All solvents used were of analytical grade (Merck).

Phytochemical analysis of plant crude extracts

Qualitative screening of different phyto-chemicals compounds present in the selected plants extracts was carried out using chemical methods (Harborne, 1998; Kaur & Arora, 2009) described as the followings;

Determination of terpenoids (Salkowski test)

Each plant extract (0.5 g) was mixed with 2 mL of chloroform, followed by a few drops of concentrated sulfuric acid. A reddish-brown colour of the interface between chloroform and sulfuric acid layers suggested the occurrence of terpenoids.

Determination of alkaloids

i. Mayer's test: A few drops of Mayer's reagent were added to 2–3 mL of the extracts, and the presence of a creamy precipitate indicated the occurrence of alkaloids.

ii. Wagner's test: Drops of Wagner's reagent were mixed with 2–3 mL extracts, and the development of a reddish-brown colour showed the presence of alkaloids.

Flavonoid test

The transient appearance of a yellow colour when a small amount of the extract solution was mixed with ammonia solution (5 mL) and concentrated sulfuric acid (1 mL) indicated the presence of flavonoids.

Determination of phenolic compound (ferric chloride test)

The presence of phenolic compounds was suggested by the development of a dark green colour after an aqueous solution of the plant extract was mixed with a few drops of neutral 5% ferric chloride solution.

Determination of tannins

Different tested plant extracts (0.5 g) were heated in a water bath with 10 mL of water and filtered. Development of a brownish-green to blue-black colour after the addition of drops of 0.1% ferric chloride solution suggested the presence of tannins.

Determination of saponins

The plant extracts were shaken vigorously with water. Development of a persistent foam indicated the presence of saponins.

Determination of glycoside (Keller killiani test)

The plant extracts (0.5 g) were diluted with distilled water (5 mL). Then, glacial acetic acid (2 mL) and 1% ferric chloride solution (1 mL) were added to the extract solution, followed by addition of a few drops of concentrated sulfuric acid. A violet ring seen beneath a brown ring or a greenish ring formed above the brown ring affirmed the presence of glycosides.

Determination of Steroid test (Liebermann Burchard test)

The tested plant extracts (0.5 g) were mixed with acetic anhydride (2 mL), and concentrated sulfuric acid (1–2 drops) was added slowly alongside the edges of the test tubes. A change in the array of colours (purple, pink and progression through to a light green then very dark green) suggested the presence of steroids.

Determination of coumarin

Filter paper test: A test tube containing the extract (0.5 g) and covered with filter paper soaked with dilute sodium hydroxide was heated in a hot water bath. After some time, a yellowish-green fluorescence developed on the filter paper.

Determination of anthraquinone (Borntrager's test)

The plant extract (100 mg) was mixed with 10 mL of benzene and filtered. The filtrate solution was then mixed with 10% ammonia solution (5 mL). Development of a pink, red or violet colour in the ammonia (lower) phase indicated the presence of anthroquinone.

Determination of reducing sugar (Fehling's test)

The plant extract (0.1 g) was dissolved in water using a water bath. Then, 2 mL of extract solution was mixed with 1 mL of Fehling's reagent A and B and heated for 10 min. The development of a brick-red precipitate in reagent A indicated the presence of reducing sugar.

Determination of ascorbic acid

Sample solution (0.1 g) was diluted with water then mixed with drops of sodium bicarbonate and ferrous sulphate to develop a deep violet colour that would disappear after addition of 5 mL dilute sulfuric acid to indicate the presence of ascorbic acid.

Crude extract preparation for biological assay

Crude extracts dissolved in 0.1% dimethyl sulfoxide (DMSO) at different concentra-

tions (15.63, 31.25, 62.5, 125, 250, 500, 1000 µg/mL) were prepared for each assay.

Bacterial and Fungal strains

Bacterial and fungal stock cultures were preserved on Muller Hinton agar and potato dextrose agar, respectively, and kept at 4°C. For determination of antimicrobial activity, eleven microorganisms were used: five Gram-positive strains, specifically *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (B145), *Micrococcus luteus* (ATCC 9341), *Enterococcus hirae* (ATCC 10541) and *Staphylococcus epidermidis* (a clinical isolate); four Gram-negative strains, specifically *Escherichia coli* (a clinical isolate), *Pseudomonas aeruginosa* (ATCC27853), *Klebsiella pneumoniae* (ATCC 2513) and *Salmonella ebony* (ATCC 6017); and two fungal strains which were *Candida albicans* (C2213) and *Aspergillus niger* (A121). All bacteria strains were obtained from ATCC and Microbiology Laboratory, Medical Faculty, Universiti Putra Malaysia, and all fungi strains were obtained from the Institute for Medical Research (IMR), Kuala Lumpur.

Antimicrobial assays

The antimicrobial assays were carried out using the well diffusion method (Javed *et al.*, 2014; Nair & Chanda, 2005). The antimicrobial activities were evaluated by measuring the inhibition zone diameter after incubating the plates at 37°C for 24 hours for bacteria and at 25°C for 48 hours for fungi. The minimal inhibitory concentrations (MICs) of each plant extracts were determined using the broth microdilution assay. Two-fold increment in concentration of the plant extracts ranging from 7.812 to 1000 µg/mL was used for the assay (Ndi *et al.*, 2007; Wayne, 2002). Gentamicin (20 µg/mL), ampicillin (10 µg/mL), nystatin (200 units/mL) and ketoconazole (50 µg/mL) were used as positive controls, and dimethyl sulfoxide (DMSO) was used as the negative control. To each sample was added 20 µL of an aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC, 5 mg/mL), and the mixture was incubated at 37°C for 1 hour. The appearance of a pink colour indicated the presence of growth; therefore, the MIC value was determined from the lowest concentration that remained colourless (Moulari *et al.*, 2006).

Determination of cytotoxic activity using the Sulforhodamine B (SRB) assay

The cytotoxic properties of the plant crude extracts were assessed using the SRB assay following the method reported by Vichai and Kirtikara (2006) against two human cell lines, namely the breast adenocarcinoma cell line, MCF-7, and the human liver cancer cell line, HepG2. Doxorubicin was chosen as a positive control. The plant extracts and the positive control prepared for the assay in concentrations at 0, 5, 12.5, 25 and 50 µg/mL. The treated cells were incubated for 48 hours. Percent cell viability was determined as follows:

Cell viability = [O.D. (treated cells) / O.D. (control cells)] x 100 %

Where O.D. is the optical density measured at a wavelength of 570 nm. The IC_{50} values (the concentration of the sample needed to inhibit cell growth by 50%) were also calculated.

Statistical analyses

Data were presented as means ± standard deviation (SD). Statistical analysis was carried out by one-way analysis of variance (ANOVA) to analyse the statistical significant differences of each result through SPSS 20.0 package (IBM Corporation, USA), followed by Student's *t*-test; p values < 0.05 were considered to indicate statistically significant differences.

RESULTS

The stems and leaves of *L. socotranum* were subjected to extraction separately, whereas the stems and leaves of *P. blanda* were subjected to extraction together. The extractions were performed successively using three types of solvents with different polarities starting with the non-polar solvent petroleum ether followed by DCM and finally

Table 1. Phytochemical analysis of the investigated plant extracts

Chemical constituent	*P. blanda* (Leaves and stem)			*L. socotranum* (Leaves and flower)			*L. socotranum* (Stem)		
	PE	DCM	MEOH	PE	DCM	MEOH	PE	DCM	MEOH
Terpenoids	+	+	–	+	+	-	+	–	–
Alkaloids	+	+	+	–	–	–	–	–	–
Coumarins	–	–	+	–	–	–	–	–	–
Flavonoids	–	+	+	+	–	+	–	–	+
Tannins	–	–	+	–	–	+	–	–	+
Saponins	–	–	+	–	–	–	–	–	–
Phenol	–	–	+	–	–	+	–	–	+
Glycoside	+	–	–	–	–	+	+	–	–
Steroid	+	+	+	+	–	–	+	+	-
Anthraquinone	–	–	+	–	–	+	–	–	+
Reducing sugar	–	–	+	+	–	–	–	–	+
Ascorbic acid	+	+	+	–	–	+	–	+	+

PE: petroleum ether; DCM: dichloromethane; MeOH: methanol. (+) present; (–) absent.

Table 2. The percentage yield (w/w) of crude extracts and the IC_{50} values of the investigated plants against MCF-7 and HepG2 cells.

Plant species	Dry Weight (Powder)	Extract	Yield (% w/w) of crude extract	IC_{50} value (µg/mL)	
				MCF-7	HepG2
P. blanda (Leaves and stem)	50 g	PE	4.37%	4.60±0.02	24.39±0.49
		DCM	4.7%	10.70±0.10	20.85±1.67
		MeOH	13.23%	8.11±0.05	13.06±1.06
L. socotranum (Leaves and flower)	80 g	PE	1.23%	19.65±0.10	9.97±0.79
		DCM	1.45%	17.60±0.13	20.62±0.56
		MeOH	2.65%	8.70±0.08	13.90±0.14
L. socotranum (Stem)	60 g	PE	0.55%	14.57±1.90	16.97±0.54
		DCM	0.25%	21.8±1.30	11.15±0.98
		MeOH	7.5%	17.18±0.08	24.86±0.087
Doxorubicin				3.39±0.01	7.38±0.11

PE: petroleum ether; DCM: dichloromethane; MeOH: methanol.
* Values were represented mean±Standard deviation, values were significantly different at $P < 0.05$.

methanol to yield nine crude extracts. The percent yield of methanol extract of *P. blanda* (13.23%) was higher than DCM and Pet. ether extracts (4.70 and 4.30%, respectively). Similarly, the highest percent yield for both leaves and stems of *L. socotranum* (2.65% and 7.50%, respectively) was obtained in the methanol extract. The chemical analysis of the studied extracts demonstrated the presence of various active groups of constituents, such as flavonoids, terpenoids, tannins, anthraquinones and alkaloids as illustrated in Table 1.

The cytotoxic activities of different plants against MCF-7 and HepG2 cells were determined, as illustrated in Table 2. *P. blanda* crude extracts exhibited higher cytotoxic activity against MCF-7 than *L. socotranum*, with IC_{50} ranging from 4.60 to 10.70 µg/mL, and the lowest IC_{50} was recorded from pet. ether extract with an IC_{50} value of 4.60 ± 0.02 µg/mL. Whereas, *L.*

socotranum extracts illustrated cytotoxic activity against the MCF-7 cell line, with IC_{50} values ranging from 8.70 to 21.8 µg/mL, and the lowest IC_{50} value was recorded from the MeOH extract of *L. socotranum* leaves, with an IC_{50} value of 8.70 ± 0.08 µg/mL. Furthermore, petroleum ether extract of *L. socotranum* leaves exhibited the highest cytotoxic activity against HepG2 cells (IC_{50} value of 9.97 ± 0.79 µg/mL) compared to other extracts, including those of *P. blanda*. Results were comparable with the positive control doxorubicin, with IC_{50} values of 3.39 ± 0.01 and 7.73 ± 0.11 µg/mL against MCF-7 and HepG2 cells, respectively. In addition, the methanol extracts of both plants exhibited high activity against MCF-7 cells, with an IC_{50} value range of 8.11 to 17.18 µg/mL, and against HepG2 cells, with IC_{50} values in the range of 13.06 to 24.86 µg/mL, as summarized in Table 2.

Preliminary screening of the antibacterial activity of the nine crude extracts against the nine bacterial species is outlined in Table 3. DMSO was used as a negative control and solvent for the extracts. Ampicillin and gentamicin were used as antibacterial positive controls, whereas ketoconazole and nystatin were used as antifungal positive controls. The preliminary screening was conducted using a well diffusion assay. Extracts with zone of inhibitions ≥15 mm were considered to have high antimicrobial activity, and such crude extracts are considered as potential antimicrobials (Kuete, 2010).

The methanol extracts from both leaves and stems of *L. socotranum* exhibited antibacterial activity against Gram-positive and Gram-negative bacteria. However, the petroleum ether extract of *P. blanda* showed stronger activity against *E. hirae*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. Accordingly, the MIC value was determined for crude extracts that resulted in an inhibition zone ≥15 mm. According to the Kuete classification of antimicrobial activity (Dzoyem *et al.*, 2013), the MIC value of the petroleum ether extract of *P. blanda* was much stronger than that of the other extracts, with an MIC value of 62.5 µg/mL against *S. epidermis*, and the petroleum ether extract of *P. blanda* displayed moderate activity against *E. hirae*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, with MIC values between 125 and 250 µg/mL. The methanol extract of *L. socotranum* leaves exhibited the highest activity against *Micrococcus luteus*, with an MIC value of 15.6 µg/mL, and against *S. aureus* and *P. aeruginosa*, with MIC values of 125 µg/mL. In addition, the stem extracts showed strong activity against *S. epidermis*, *S. aureus* and *M. luteus*, with MIC values ranging from 62.5–125 µg/mL, as summarized in Table 4.

The strong antifungal activity of *L. socotranum* against the tested species *C. albicans* and *A. niger* was also noted. In general, among the investigated plant extracts tested for antifungal activity as summarized in Table 3, the pet. ether extract of *L. socotranum* leaves exhibited the highest potential activity against *C. albicans*, with an MIC value of 62.5 µg/mL, while the stem extract gave potential activity against *A. niger*, with an MIC value of 62.5 µg/mL, as shown in Table 4.

DISCUSSION

Peperomia blanda and *Limonium socotranum* can be found in the Socotra Archipelago (one of the islands of Yemen); this island contains more than 800 plant species. *Limonium socotranum* is one of 307 endemic plants that make the island a valuable natural wealth of new pharmacologically active plants (Miller & Morris, 2004). In this study, the *in vitro* antimicrobial and cytotoxic activities of these two plants were evaluated. From the literature data, the chemical composition of *Limonium* contains amino acids, vitamins, tannins, flavonoids, inorganic elements, polysaccharides, organic acids and alkaloids (Eren & Özata, 2014). The phytochemical profile of *P. blanda* extracts displayed the presence of terpenoids, alkaloids and steroids in both pet. ether and DCM extracts, whereas flavonoids, coumarins and alkaloids were present in MeOH extracts. Moreover, ascorbic acid was recovered from both plants. Previously, the presence of a large number of common and

Table 3. The diameters of inhibition zones of the tested plants against pathogenic microbes

Plant species	Extract	Microbial strains tested								Fungi strains tested	
		M.L	E.C	B.S	E.H	S.E	S.EP	K.P	S.A	P.A	C.A	A.N
Inhibition zone (mm)												
P. blanda	PE	13.7±2.5	NA	NA	16.8±2.9	NA	13.7±2.5	15.0±1	19.3±1.2	16.3±2.2	NA	15.0±1.4
(Leaves and stem)	DCM	19.0	NA	NA	17.7±2.5	NA	10.0±1.7	NA	18.5+2.5	NA	12.0	13.0±1.4
	MeOH	18+2.6	9.7+1.1	NA	22.5±3.5	NA	12.5±0.7	NA	10.7±0.6	27.7±1.2	NA	13.0±1.4
L. socotranum	PE	10.7±0.6	19.5±0.7	NA	27±1.7	NA	NA	13.3±1.2	NA	15. ±1.4	33.5±2.1	35.0
(Leaves and flower)	DCM	NA	NA	NA	10.3±0.6	NA	11.0	7.0	11	15.0	10.5±0.7	NA
	MeOH	22.0	NA	17.3±0.6	21.7±1.2	14.3±0.6	24.50±0.6	NA	17.7±1.2	17.0	NA	NA
L. socotranum	PE	NA	NA	NA	NA	NA	14.3±0.6	12.0	NA	NA	29.0±1.4	25.0
(Stem)	DCM	10.3±2.3	32.5±3.5	NA	NA	17.3±0.6	NA	NA	NA	13.0	NA	NA
	MeOH	20.0	NA	17.0	18.0	15.7±0.6	25.5	25.0	15.7±0.6	16.3±0.6	NA	NA
Ampicillin		22.0	NA	NA	15.0	10.0	NA	NA	21.0	NA	ND	ND
Gentamicin		25.0	18.0	26.0	NA	21.0	25.0	25.0	30.0	20.0	ND	ND
Ketoconazole		ND	ND	ND	ND	ND	ND	ND	ND	ND	25.0	10.0
Nystatin		ND	ND	ND	ND	ND	ND	ND	ND	ND	20.0	NA
Dimethyl sulfoxide		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

M.L; *Micrococcus luteus*, E.C.; *Escherichia coli*, B.S.; *Bacillus subtilis*, E.H; *Enterococcus hirae*, S.E; *Salmonella ebony*, S.EP; *Staphylococcus epidermis*, K.P; *Klebsiella pneumoniae*, S.A.; *Staphylococcus aureus*, P.A.; *Pseudomonas aeruginosa*, C.A; *Candida albicans*, A.N; *Aspergillus niger*, NA; not active, ND; not determined, Values are represented as mean ± standard deviation, Values are significantly different at $P < 0.05$.

Table 4. Minimum Inhibitory Concentration (MIC) of the investigated plants against selected microbes

MIC (µg/mL)	Extract	S.A	M.L	P.A	E.H	K.P	E.C	S.EP	A.N	C.A
P. blanda	Pet. ether	250	500	250	125	125	ND	62.5	125	ND
(Leaves and	DCM	500	250	ND	125	ND	ND	ND	ND	ND
stem)	MeOH	1000	250	1000	250	ND	ND	ND	ND	ND
L. socotranum	Pet. ether	>1000	>1000	>1000	125	ND	250	ND	125	62.5
(Leaves and	DCM	ND	ND	>1000	ND	ND	ND	ND	ND	ND
flower)	MeOH	125	15.6	125	250	ND	ND	>1000	ND	ND
L. socotranum	Pet. ether	ND	ND	ND	ND	ND	ND	>1000	62.5	250
(Stem)	DCM	ND	ND	ND	ND	ND	125	ND	ND	ND
	MeOH	125	125	500	250	1000	ND	62.5	ND	ND
Ampicillin		250	125	125	250	125	NA	125	ND	ND
Gentamicin		15.6	15.6	125	ND	15.6	7.8	15.6	ND	ND
Ketoconazole		ND	ND	ND	ND	ND	ND	ND	250	62.5

M.L; *Micrococcus luteus*, E.C.; Escherichia coli, B.S.; *Bacillus subtilis*, E.H; *Enterococcus hirae*, S.E; *Salmonella ebony*, S.EP; *Staphylococcus epidermis*, K.P; *Klebsiella pneumoniae*, S.A.; *Staphylococcus aureus*, P. A.; *Pseudomonas aeruginosa*, C.A; *Candida albicans*, A.N; *Aspergillus niger*, NA; not active, ND; not determined, Values were represent mean ± standard deviation.

rare compounds, such as secolignans (also known as peperomins), 2-acyl-cyclohexane-1,3-dione polyketide-type compounds, chromenes and new flavonoid compounds from methanol extract (Gutierrez *et al.*, 2016) were recorded from *Peperomia.* This discovery makes this genus a wealth of unique compounds for the drug discovery pipeline.

Natural products are used not only in direct medical applications as drug entities but also as chemical models for the design, synthesis and semi-synthesis of patented anti-cancer drugs, as in the cases of paclitaxel (Taxol®), vincristine (Oncovin®) and camptothecin (Amin *et al.*, 2009). So, as the first step to discovering bioactive compounds, the cytotoxic activities of plant extracts were investigated. Therefore, the cytotoxic activities of different plants against MCF-7 and HepG2 were evaluated. Based on the National Cancer Institute (NCI) plant screening program, a plant crude extract with an IC_{50} value less than 20 µg/mL after 48–72 hours of incubation is considered to have cytotoxic activity (Kuete *et al.*, 2011). The results of the cytotoxic activity of *L. socotranum* against MCF-7 and HepG2 cells are in agreement with previously reported results against different cell lines such as FL-cells where the chloroform extract of *L. socotranum* leaves showed high to moderate cytotoxic activity against FL-cells compared with the reference compound, etoposide (Kandil *et al.*, 2000). Moreover, previous studies described that other species of *Peperomia*, such as *Peperomia pellucida* (Al-Fatimi *et al.*, 2007; Xu *et al.*, 2006) and their isolated compounds displayed antimicrobial effects and cytotoxic activities (Gutierrez *et al.*, 2016).

Due to the antibiotic resistance developed by pathogenic organisms, there has been excessive interest in searching for novel antimicrobial drugs derived from nature. This is because the herbal crude drug extracts and bioactive compounds derived from plant species employed in folk remedies can be prolific resources for potential new drugs (Al-Fatimi *et al.*, 2007). The present study is used as the first step to finding active antimicrobial extracts for further assay. It is fascinating to note that these plant extracts demonstrated strong antibacterial

activity against tested pathogens (*E. coli*, *P. aeruginosa*, *M. luteus* and *S. epidermis*) with inhibition zones greater than those of positive controls (gentamicin and ampicillin). In addition, the potency of the plant extracts against *Salmonella*, *E. coli* and *Pseudomonas* were important because of the ability of these pathogens to develop resistance and toxin production that cause different types of enteritis, septicaemia and urinary tract infections (Medini *et al.*, 2014).

The screening assay results support the utilization of the examined plants in Yemen for ethnomedicinal purposes. The potent antifungal activity of *L. socotranum* against *A. niger* and *C. albicans* supports the traditional use of *Limonium* species as an antifungal agent among the locals. The antimicrobial activity of *P. blanda* crude extracts was higher than the reported results from *P. vulcanica* and *P. fernandopoioana* against *S. aureus* (ATTC 33862) (Mbah *et al.*, 2012) and close to the recorded data from *P. pellucida* against *P. aeruginosa* (Khan & Omoloso, 2002). The presence of common phytochemical components of *Peperomia*, such as secolignan, cyclohexane-1,3-dione and prenylated quinone, between *Peperomia* species may account for their similar activities, whereas the higher activity reported in our study may be because we used a different strain of *S. aureus* (ATTC 25923), and the percentage or type of bioactive compounds may vary among strains. On the other hand, *Limonium* species such as *L. delicatulum*, *L. avei* (Medini *et al.*, 2014; Nostro *et al.*, 2012) and *L. californicum* (Sakagami *et al.*, 2001) have also been reported to have antibacterial activity. These reported activities support the activity displayed in the methanol extract of *L. socotranum*. Therefore, the antibacterial activity of the two plants could be due to the protective role of flavonoid and phenolic compounds in methanol extracts against microbial attack. The presence of tannin in petroleum ether extracts may have a direct damaging effect on the bacterial cell wall because of its astringent activity.

CONCLUSION

The results of the present study indicate that the investigated plant extracts could be potentially valuable for the development of therapeutic agents against microbial infections as well as for cancer therapy. These extracts are good candidates for further investigation in the potential discovery of new natural bioactive compounds. Further research on the plant's extracts including isolation, structure elucidation and identification of anticancer, antibacterial and antifungal active components are required for potential drug development.

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