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Antioxidant and Antibacterial Activities of Some Yemeni Medicinal Plants

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Abstract
The current study represents the investigation of 4 selected traditional plants used in Yemen, *Pulicaria jaubertii E, Rumex nervosus Vahl, Peganum harmala L, Caralluma wissmannii O. Schwartz*. The obtained extracts were tested for their antioxidant activity using scavenging activity of DPPH radical method and for their antimicrobial activity against two Gram-positive bacteria and two Gram-negative bacteria strains using agar diffusion method. In DPPH method, the 4 extracts showed antioxidant activity and the activity was concentration dependent. The *Pulicaria jaubertii leaf* extract showed more potent against *Micrococcus litius* moderately inhibited the *Bacillus subtilis* and *Salmonella choleraesuis* and the effect was comparable with the standard antibiotics. The results indicate that these plant are potential candidates to be used as antimicrobial and antioxidant. The selected extracts might be developed in order to establish new pharmacological possibilities for their applications.

Keywords: antioxidant; antibacterial; radical scavenging; Pulicaria jaubertii; Rumex nervosus Vahl; Caralluma wissmannii O. Schwartz; Peganum harmala

1. Introduction
According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases [1]. Reactive oxygen species (ROS) may caused great damage to cell membranes and DNA, including oxidation that causes membrane lipid peroxidation, decreased membrane fluidity and DNA mutations leading to cancer, degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus and others [2]. Antioxidants are compounds that inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain [3]. In recent years, the use of natural antioxidants present in plant and other biological materials has attracted considerable interest due to their presumed safely, nutritional and therapeutic value [4]. Concern has been expressed about the rising prevalence of pathogenic microorganisms, which are resistant to the newer or modern antibiotics that have been produced in the last three decades [5, 6]. Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized [7]. Coincidentally, the last decade has also witnessed increasing intensive studies on extracts isolated from plant species used for natural therapies or herbal medicine [8]. For over thousands of years now, natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents [9]. Screening of medicinal plants for antimicrobial activity is important for finding potential new compounds for therapeutic use.
Developing countries like Yemen depend on plant resources mainly for herbal medicines. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [10, 11]. Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medicines [12]. In this study, four plants used in folk medicine by Yemeni people were selected to determine their antioxidant and antimicrobial activity including, *Pulicaria jaubertii, Rumex nervosus Vahl, Peganum harmala and Caralluma wissmannii O. Schwartz*. There is a lack of scientific studies on these selected plants including antioxidant and antimicrobial studies. In this study, 4 methanolic extracts prepared from the selected plants were determined for their antioxidant activity and antibacterial activity.

2. Materials and Method

2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma-Aldrich (St. Louis, MO). Ascorbic acid was purchased from Fisher Scientific (Loughborough, UK). Methanol and DMSO were purchased from Fisher Scientific (Fisher Scientific Co Ltd., Ottawa, ON).

2.2 Plant collections

Pulicaria jaubertii, was collected from Sana'a, the *Rumex nervosus Vahl*, was collected from Taiz, whereas *Peganum harmala L* seeds and *Caralluma wissmannii* were purchase from local market in Sana`a. The four different samples were identified and authenticated by a plant taxonomist at Department of Biology, Faculty of Sciences, Sana`a University Yemen. Samples were dried, powdered, stored at 4 ºC and protected from light prior to further use. The analysis has been carried out in 2012 at the Laboratory of Food science and Technology Faculty of Agriculture Sana`a University Yemen.

2.3 Samples extraction

All samples were finely ground using an electrical grinder (Waring Blender, Tokyo, Japan) at speed 6 for 2 min and ground samples (100g) of each samples were soaked for two days in methanol at 1:10 ratio at room temperature. The mixture of samples and solvent was filtered through a filter paper (Whattman No. 2). The solvent was removed by drying at the oven at 40 °C. The extract was transferred into glass sealed amber dark bottles and then stored in at 4 °C for subsequent analyses.

2.4 Determination of DPPH radical scavenging activity

The antioxidant activity of the selected extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, were determined by the method described by Benzie and Strain (1996). L ascorbic acid was used as standard antioxidants and methanol was used as the control. An aliquot of 0.5 ml of a methanolic solution of DPPH (50 mg DPPH/100 mL MeOH) was added into the different concentration (1, 0.5, 0.25 mg/ml) of each extract and ascorbic acid as long as control samples (both extract and ascorbic acid were dissolved in methanol). All samples were incubated in the dark at room temperature for 30 min before absorbance values were read at 517 nm (Amersham 2100Pro, UV-vis spectrophotometer, UK). The decrease in absorbance was calculated as an IC_{50} and expressed as µg/ml, which is the concentration of sample required for 50% scavenging of DPPH radicals in the specified time period.

The radical scavenging effect was calculated as follows:

Radical scavenging effect (%) = Ac – As/Ac × 100

Ac = absorbance of control and as = absorbance of test sample. Where control is the absorbance of the DPPH radical+ methanol.

2.5 Antibacterial activity

Four bacterial strains were used for the study. Gram positive bacteria include of *Bacillus subtilis* (ATCC 6059), *Micrococcus luteus* (ATCC 9341) and Gram negative bacteria include *Escherichia coli* (ATCC11229), *Salmonella choleraesuis* (ATCC 10708). All the tested strains were reference strains and were obtained from Department of Biology, Division of Microbiology, Science University and Department of Food Sciences and Technology, Sana'a University. All the test strains were maintained in nutrient broth (Difco, 0003-01-6 - USA) at 37 °C and maintained on Brain Heart Infusion Agar (OXOID, CM0375) slants at 4 °C**.** These bacteria served as test pathogens for antibacterial activity assay.

Four different concentrations of each extract of selected plants (25, 50, 100 and 200 mg/ml) were dissolved in 10% dimethylsulfoxide (DMSO) in Phosphate buffered saline to be used in antimicrobial activity test. Extract solutions were prepared just before carrying out the test. Antibacterial activity of the extracts was determined by agar well diffusion method as described by (Satish *et al*., 2008) on Mueller-Hinton Agar (MHA) (Accumix-AM071, India) with some modifications. The concentration of bacterial suspension was determine by serial dilutions and pour plates technique. The bacterial suspensions containing 10^6 CFU/ml of bacteria were spread on MHA plates with a sterile swab moistened with the bacterial suspension. In each of these plates four wells were cut out using a standard cork borer (7 mm). About 50µl of each extract was added into different wells (duplicate each concentration), DMSO was used as a negative control. A positive control antibiotic disc was placed in the plate. All the plates were incubated for 24h at 37 °C. After incubation bioactivity was evaluated by measuring the zone of inhibition. All the experiment was performed in duplicates. Antibiotics standard discs (Hi-Media, India) Enrofloxacin (5mcg), Tetracycline (30 mcg), Gentamycin (10 mcg) were used as references to determine the sensitivity of each bacterial species tested and used as control positive.

3 Results

3.1 Yield of Methanolic extract

Extraction of *Pulicaria jaubertii, Rumex nervosus Vahl, Peganum harmala and Caralluma wissmannii* with 100% methanol produced 11.76 ± 0.43, 17.65± 0.76, 12± 0.46 and 9.26 ± 0.43% (w/w), respectively.

3.2 Antioxidant activity

The results of the free radical scavenging activity *Pulicaria jaubertii, Rumex nervosus Vahl, Peganum harmala, Caralluma wissmannii* and the authentic antioxidant L-ascorbic acid was summarized in Table 1. Result from this assay clearly showed that all extract exhibited high antiradical activity towards DPPH radical. The scavenging activity of the pure antioxidant standard, ascorbic acid was 45% at lower concentration of 250µg/ml. The radical scavenging activity was concentration dependent and it increased by concentration to 58 and 82% at 500 and 1000µg/ml respectively.

After 30 minutes of the reaction, both *Pulicaria jaubertii* and *Rumex nervosus Vahl*, at lower concentration (250 µg/ml) scavenged 44 and 39%, respectively of the total radicals in the reaction system. Subsequently, the scavenging activity of both *Pulicaria jaubertii* and *Rumex nervosus* were gradually increased by 84 and 71% of the total radicals respectively, at higher concentration (1000µg/ml). *Peganum harmala* and *Caralluma wissmannii* methanolic extract showed radical scavenging activity of DPPH by 62 and 51% respectively at higher concentration (1000µg/ml). Whereas the radical DPPH scavenging activity gradually decreased to less than 20% of the total radicals at lower concentration (250µg/ml). The amount of the sample needed for 50% inhibition of free radical activity is expressed by IC^{50}. Lower IC^{50} value indicates higher antioxidant activity. IC^{50} values of *Pulicaria jaubertii, Rumex nervosus, Caralluma wissmannii* leaf extracts, *Peganum harmala* seeds methanolic extract and the authentic antioxidant L-ascorbic acid are given in Table 2 and Figure 1. *Pulicaria jaubertii* exhibited significantly higher antioxidant activity

than *Rumex nervosus Vahl* and *Peganum harmala*, whereas *Caralluma wissmannii* exhibited significantly lower antioxidant activity than the other samples.

Table 2: Antioxidant activities of the *selected* extracts and L-ascorbic acid using the (DPPH) free radical-scavenging assay

Antioxidant activity IC^{50}/ DPPH (µg/ml)	Samples
320±12.0 [a]	L- ascorbic acid
350±23.0 [b]	Pulicaria jaubertii
450±14.3 [c]	Rumex nervosus
550±15.7 [d]	Peganum harmala
850±12.6 [e]	Caralluma wissmannii

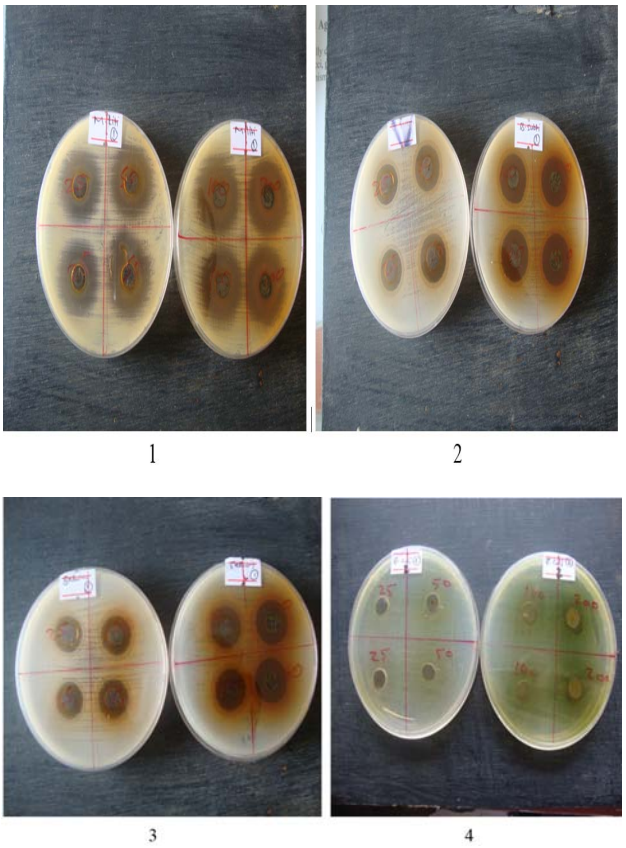

Fig 1: Inhibition zones observed with leaf methanolic extracts of Pulicaria jaubertii against

1-*Bacillus subtilis*.
2-Micrococcus litius
3-Salmonella choleraesuis
4-Escherichia coli

3.3 Antimicrobial activity

The antimicrobial activity of the methanolic extracts of *Pulicaria jaubertii, Peganum harmala*, *Rumex nervosus and Caralluma wissmannii* against tested bacteria are summarized in Table 4. In addition, the inhibition zones formed by standard antibiotics are listed in Table 3. The methanolic leaf extracts of *Pulicaria jaubertii,* inhibited the growth of most of the bacteria isolates but all the extracts did not showed any inhibition effect on *Salmonella choleraesuis*. The *Pulicaria jaubertii* leaf extract showed more potent against *Micrococcus litius* moderately inhibited the *Bacillus subtilis* and *Salmonella choleraesuis* (Figure 2). *Peganum harmala* methanolic seed extract showed more potent against *Micrococcus litius* and at concentration of 100 and 200 mg/ ml only, low effect against *Bacillus subtilis* and *Salmonella choleraesuis* (Figure 3). *Rumex nervosus* showed low inhibition effect against *Micrococcus litius*, *Bacillus subtilis* and *Salmonella choleraesuis* at higher concentration only of 200 mg/ ml (Figure 4). *Caralluma wissmannii* showed low inhibition effect against *Micrococcus litius* only (Figure 5).

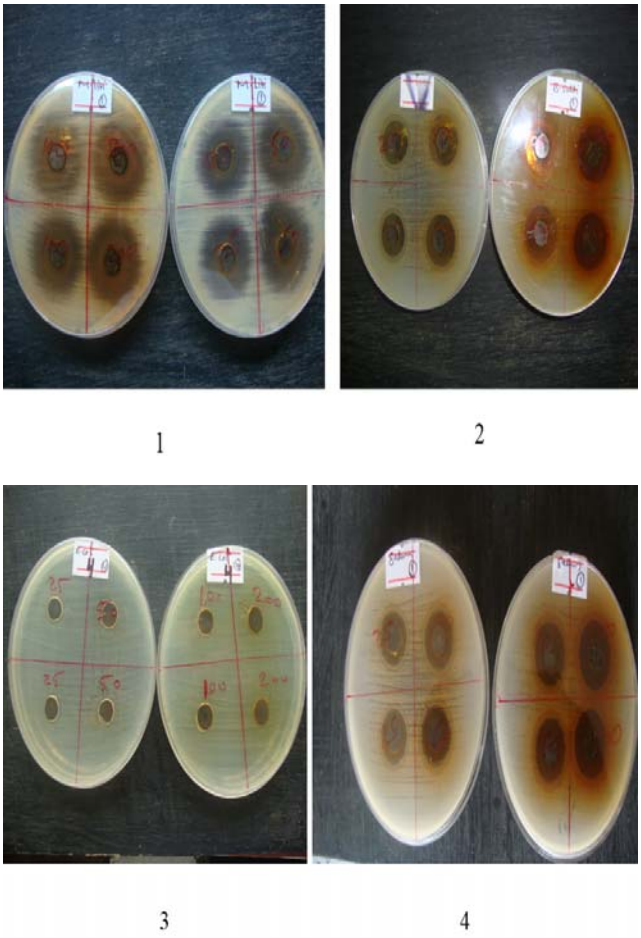

Fig 2: Inhibition zones observed with leaf methanolic extracts of Peganum harmala

1- *Micrococcus litius*
2- Bacillus subtilis.
3- Escherichia coli
4- Salmonella choleraesuis

Table 3: Antibacterial activity of standard antibiotics discs against tested bacteria the

Inhibition zones diameters (mm) of tested Antibiotic			
Bacteria	**Enrofloxacin (5mcg/disc)**	**Gentamycin (10mcg/disc)**	**Tetracycline (30mcg/disc)**
Bacillus subtilis	28.5^{a}	20^{b}	29.5^{a}
Micrococcus litius	29.5^{a}	36.5^{b}	44^{c}
Escherichia coli	23.5^{a}	12^{b}	10^{b}
Salmonella choleraesuis	24^{a}	13.5^{b}	23^{a}

Results are expressed as means. Within a row Different alphabets indicate significant difference (n=3).

Table 4: Antibacterial activity of selected plants against tested bacteria

	Inhibition zones diameters (mm) of Pulicaria jaubertii			
Bacteria	**200 mg/ml**	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**
Bacillus subtilis	20[a]	19[a]	18[a]	16.5[a]
Micrococcus litius	31.5[b]	30[b]	28[b]	26[b]
Escherichia coli	– *	–	–	–
Salmonella choleraesuis	20[a]	17.5[c]	17[a]	16[a]
	Inhibition zones diameters (mm) of Peganum harmal			
Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Bacillus subtilis	20 [a]	17[a]	15[a]	13[a]
Micrococcus litius	33[b]	30[b]	–	–
Escherichia coli	–	–	–	–
Salmonella choleraesuis	18.5[a]	16.5[a]	14[a]	13[a]
	Inhibition zones diameters (mm) of Rumex nervosus			
Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Bacillus subtilis	13[b]	– *	–	–
Micrococcus litius	16[b]	13	–	–
Escherichia coli	–	–	–	–
Salmonella choleraesuis	12.5[a]	–	–	–
	Inhibition zones diameters (mm) of Caralluma wissmann			
Bacteria	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml
Bacillus subtilis	–	–	–	–
Micrococcus litius	–	–	–	–
Escherichia coli	13.5	13	11	–
Salmonella choleraesuis	–	–	–	–

Results are expressed as means. In same column within each extract, different alphabets indicate significant difference. * (–) = no effect.

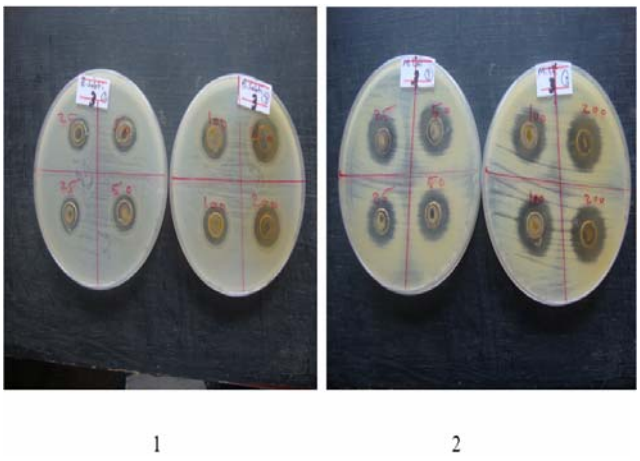

Fig 3: Inhibition zones observed with leaf methanolic extracts of Rumex nervosus Vahl

1. Bacillus subtilis
2. Micrococcus litiu
3. Escherichia coli
4. Salmonella choleraesuis

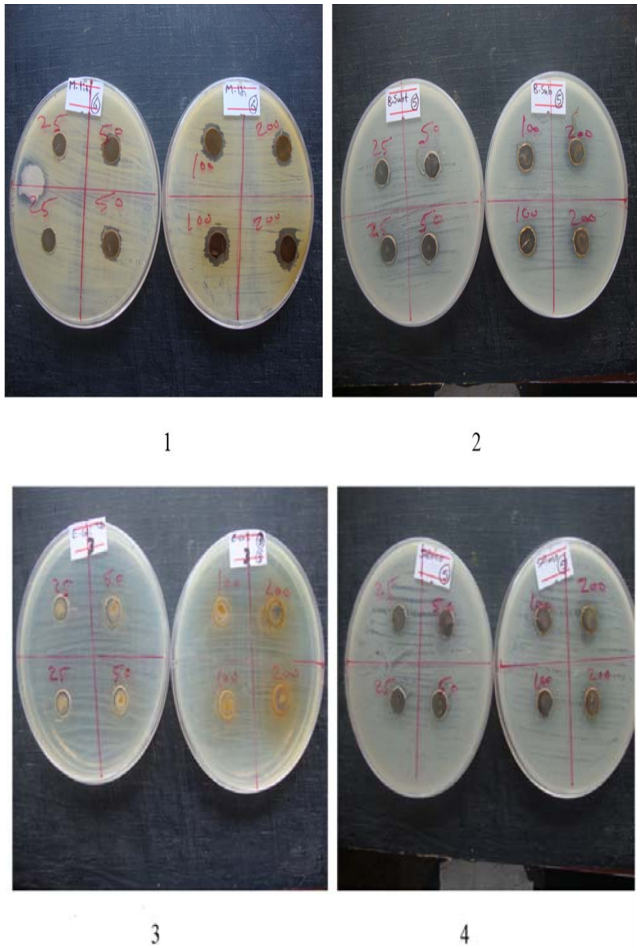

Fig 4: Inhibition zones observed with leaf methanolic extracts of Caralluma wissmannii O. Schwartz.

1. Micrococcus litiu
2. Bacillus subtilis
3. Escherichia coli
4. Salmonella choleraesui

4. Discussion

In recent years, the searches for natural sources possessing antioxidant and antimicrobial properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Our attention has been focused, in particular, on the parts of 4 commonly medicinal plants used in Yemen. In the present experiment, methanolic extracts of 4 plants were evaluated for their free radical scavenging activity using the DPPH radical assay. The plant extracts tested showed low absorbance values compared to control samples, which indicated a high level of antioxidant activity. A concentration dependent reducing activity was observed for the methanolic extract of selected plant and for L-ascorbic acid as shown in Table.1. All extracts showed increased reducing power with the increased in the extract's concentration. The methanolic leaf extract of *Pulicaria jaubertii* however, contain overall higher reducing activity compared to the other extracts. Radical scavenging activity of the extracts is compared using their respective IC^{50} values. IC^{50} is used to express the amount or concentration of extracts needed to scavenge 50% of the free radicals. The value of IC^{50} is inversely proportional to the scavenging activity of the leaf extract. The scavenging activity between the 4 extracts was determined by comparing their scavenging activity. The L-ascorbic acid used in this study is a well-known antioxidant and thus can be used as a good indicator to compare scavenging activity between the extracts. The L-ascorbic acid used has highest scavenging activity (IC^{50}= 320±2.0µg/ml), followed by *Pulicaria jaubertii* extract (IC^{50}= 350±23.0µg/ml) then *Rumex nervosus Vahl* extract (IC^{50}= 450±4.3µg/ml) and *Peganum harmala* seed extract (IC^{50}= 550±5.7µg/ml) whereas, the *Caralluma wissmannii* showed the lowest scavenging activity (IC^{50}= 850±12.6µg/ml). The results of our screening assay for the antioxidant confirmed the use of the investigated plants in Yemeni traditional medicine. The existing knowledge about these investigated plants is in many cases very limited. The genus *Pulicaria* which belongs to the Asteraceae family (Compositae, trible Inuleae, subtrible Inulinae), comprises more than 77 species widespread all around the world [13, 14]. Chemically, this genus is not homogenous. As pointed out previously some species contain monoterpenes, diterpenes, sesquiterpene lactones [15] and caryophyllane derivatives [16]. Also the literature reports that *Pulicaria* species afforded different flavonoid profiles [17]. The *Pulicaria* species proved various activities such as antiinflammatory, antilukemic [18], potential cancer chemopreventive and cytotoxic agents [19]. The *Pulicaria jaubertii* indigenous to Yemen, locally known as Anssif, is traditionally used as diuretic, pyritic conditions in urogenetial organs, and to cure fever. The flowers of *Pulicaria jaubertii* was also used as spice and to make various delicious foods. Some investigation reported that this species reveal antimalarial properties [20]. The higher antioxidant and antimicrobial activity shown in the *Pulicaria jaubertii* leafs methanolic extract might be developed in order to establish new pharmacological possibilities that we are going to carry on for its application such as hypocholesterolemic and hypoglycemic effect in animal study.

Rumex nervosus Vahl has been used traditionally for treatment of inflammatory and painful condition in Yemen. It was investigated that the methanolic extract of *Rumex nervosus Vahl* produced an important peripheral analgesic effect, with a power of protection against the abdominal cramp, via oral pathway [21]. In our study, *Rumex nervosus Vahl* showed antioxidant activity and also antimicrobial activity against the *Micrococcus litius*, *Bacillus subtilis* and *Salmonella choleraesuis*. In supporting to our study, the crude methanolic extracts of the leaves of *Rumex nervosus Vahl* was shown to have anti-microbial effect against *Streptococcus pyogenes* and *Staphylococcus aureus* [22], suggesting the potential of this plant to treat bacterial infections of the skin.

Peganum harmala, is a wild-growing flowering plant belonging to family Zygophyllaceae and is found abundantly in Middle East and North Africa [23]. It was claimed to be an important medicinal plant. It was reported to have a hepatoprotective effect [24]. *Peganum harmala* seeds are known to possess antimicrobial action. The methanolic fraction was found to be most effective against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [25]. The results from this study showed that *Peganum harmala* methanolic seed extract posse's antioxidant and antimicrobial effect against *Micrococcus litiusand*, *Bacillus subtilis* and *Salmonella choleraesuis.*

Based on our objectives in screening for the biological activities of traditional Yemeni plant, the endemic plant *Caralluma wissmannii*, was evaluated. It has been used in Yemeni traditional medicine, for reducing blood glucose level. Nevertheless, no scientific research was done to investigate the antioxidant and antimicrobial of this Yemeni plant. We report here for the first time some biological properties of this Yemeni endemic plant. *Caralluma wissmannii* was active in inhibition of *Escherichia coli* growth only whereas, no inhibition effect was observed against other used bacteria.

Table 1: The DPPH free radical scavenging activity of the methanolic extract of selected plants and L-ascorbic acid.

Samples	Radical scavenging effect (%)		
	Concentration (mg/ml)		
	1.00	**0.5**	**0.25**
L- ascorbic acid	82±4	58±3	45±3
Pulicaria jaubertii	84±5	56±4	44±3
Rumex nervosus	71±5	49±5	39±2
Peganum harmala	62±4	48±6	20±1
Caralluma wissmannii	51±3	27±2	14±2

5 .Conclusion

In conclusion, the present results therefore offer a scientific basis for the use of the selected plants used as traditional medicine in Yemen. Our findings suggest that *Pulicaria jaubertii, Rumex nervosus, Peganum harmala, Caralluma wissmannii* are a potential antioxidants sources. Both *Pulicaria jaubertii, Rumex nervosus* are effective against *Bacillus subtilis*, *Micrococcus litius* and *Salmonella choleraesuis*. Further studies on these plants are necessary and should seek to determine pharmacokinetic properties of the selected plants.

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