Research Article

Antimicrobial and Antioxidant Activity of Crude Extracts of Two Medicinal Plants *Pistacia Integerrima* and *Debregeasia Salicifolia*

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Accepted on: 07-11-2012; **Finalized on:** 31-12-2012.

ABSTRACT

The methanolic extracts of different parts of the two medicinal plants *Pistacia integerrima* Stewart Ex Brandis (Anacardiaceae) and *Debregeasia salicifolia* Rendle (Urticaceae) were studied for *in vitro* antimicrobial and antioxidant activity. The antimicrobial study was carried against various species of bacteria. Gram positive bacteria consisted of *Staphylococcus aureus, Bacillus subtilis* and Gram negative of *Proteus mirabilis, Salmonella typhi, Escherichia coli* and *Citrobacter.* The antioxidant study was performed through DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity using ascorbic acid as standard. Roots and bark showed high zones of inhibition at low concentrations while the leaves showed activity at high dose concentrations of the crude extracts. *Debregeasia salicifolia* was effective against *Bacillus subtilis* with zone of inhibition 17.50 mm and at a dose of 150 µg. The highest zone of inhibition was shown by *Pistacia integerrima* leaves up to 28.00 mm against *Citrobacter* at the highest test dose of the extract, while the bark of *Pistacia integerrima* was also very effective showing a zone of inhibition of 21.80 mm against *Staphylococcus aureus* even at a very small dose of 150 µg. The crude extracts were compared against two standard drugs neomycin and doxycyclin. *Pistacia integerrima* bark and leaves were highly effective as free radical scavengers and the results were comparable to the standard ascorbic acid, while the leaves and roots of *Debregeasia salicifolia* also showed a very potent anti-oxidant activity but still less effective then *Pistacia integerrima.*

Keywords: *Pistacia integerrima, Debregeasia salicifolia*, Well Diffusion, Antimicrobial Assay, Antioxidant Assay.

INTRODUCTION

Antibacterial and antioxidants compounds are of great value for human beings. Increases in antibiotic resistant strains of clinically important pathogens have led to the emergence of new bacterial strains that are multi-resistant[1-3]. Worldwide but especially in developing countries there is a high mortality due to infectious bacterial diseases, thus there is a great need for new antibacterial compounds which are effective to these diseases[4].

Reactive oxygen species (ROS) which include free radicals such as superoxide anion radicals (O^{-2}), hydroxyl radicals ($OH^{\bullet}$) and non free radical species such as H_2O_2 and singlet oxygen ($^1O^2$) are various forms of activated oxygen generated in the body[5]. They have attracted attention because of their role in cellular injury and ageing process[6]. Under normal circumstances these free radicals and ROS can be removed by the body's natural antioxidant defense, e.g. glutathione peroxidase, catalase and superoxide dismutase[7]. However, overproduction of ROS arising from either the mitochondrial electron transport chain or excessive stimulation of NAD(P)H, or from exposure to environmental pollutants i.e. cigarette smoke, UV-rays, radiation and toxic chemicals[8], results in a weakened body defense system, hence creating the need to provide the body with a constant supply of phytochemicals through dietary supplementation. Antioxidants have the ability to prevent, delay or ameliorate many of the effects of free radicals.

Plants are considered to have a rich source of naturally occurring bioactive compounds with high medicinal activities against various diseases. Also the use of medicinal plants for local remedies is a traditional custom. As we know medicinal plants have the potency to combat all these diseases, so the current study is carried out on two medicinal plants *Pistacia integerrima* and *Debregeasia salicifolia* in order to check the probable antimicrobial and antioxidant activity of these plants.

Pistacia integerrima is a dioecious tree up to 17 m or even more tall. Leaves pari-or imparipinnate, 16-25 cm long. Leaflets opposite or subopposite, subsessile, 7-9 in number, 90-120 x 22-32, lanceolate, acuminate, glabrous, pale green on the under-surface. The species is generally distributed in E. Afghanistan, Pakistan, and N.W. & W. Himalaya to Kumaon[9].

Debregeasia salicifolia is a dioecious, evergreen tall shrub or small tree. Stem with dark brown fibrous bark scabrous, young shoots whitish tomentose. Leaves with up to 2.5 cm long, densely tomentose petiole, lamina oblong - lanceolate 2-15 cm long, 0.6-3 cm broad, silvery tomentose beneath, scabrous and rugose above, serrate, acute, stipules linear-lanceolate up to 1 cm long, brown, deciduous. Distribution is in India, Pakistan (Punjab, N.W.F.Province, and Kashmir), Afghanistan and Tropical Africa[10].

MATERIALS AND METHODS

Plant materials

The leaves and barks of *Pistacia integerrima* and the leaves and roots of *Debregeasia salicifolia* were collected from local mountains of District Swat valley of Malakand division, Pakistan. These plants were collected during the season of September to October. The samples were identified by Dr. Siraj ul Haq, professor at the Botany Department, Post-Graduate Jehanzeb College, Saidu Sharif District, Swat, Khyber Pukhtoon Khwa, Pakistan. All plant parts were rinsed with tap water and air dried under shade for fourteen to twenty days. After shade drying the plant materials were cut down to as small pieces as possible.

Plant Material Extraction

The preparation of the plant extracts was performed using standard methods[11]. All the respective parts were soaked in methanol and were placed for about 15 days with occasional shaking in a glass bottle covered with cotton wool plug. After fifteen days soaking the solvent was decanted from the plant material and filtered through Whatman No. 1 filter paper. Extraction was allowed to proceed in a rotary evaporator with water bath maintained at 40 °C. The filtrate was evaporated to dryness using a rotary evaporator attached to a vacuum pump (Model: BUCHI Rotavapor R-200 Switzerland).

Microorganisms

The microorganisms were clinical pathogens obtained from PCSIR (Pakistan Council of Scientific and Industrial Research), Karachi, and were identified. Six different types of micro-organisms were tested in which G (+ve) bacteria consisted of *Staphylococcus aureus, Bacillus subtilis* and G (-ve) of *Proteus mirabilis, Salmonella typhi, Escherichia coli* and *Citrobacter.* They were maintained on Nutrient Agar medium (Merck). Twenty-four hour old pure cultures were prepared for use each time.

Culture media

Nutrient Agar (Merck) was prepared according to the manufacturer's instructions, autoclaved and dispensed in glass tubes for the preparation of pure culture from old cultures. These cultures were then maintained at normal growth temperature of 37 °C in an incubator for about 24 h, while for the preparation of plates the medium used was Antibiotic Medium #1 (Merck) which was prepared according to the manufacturer's instructions, autoclaved and dispensed 20 ml per plate in 12 x 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

Antimicrobial assay

The agar well diffusion method was used to test the antimicrobial activity of the plant extracts. A suspension of micro-organisms was prepared in sterile normal saline and adjusted to 0.5 McFarland standards (10^8 Cfu/ml)[12]. Six Petri dishes containing a particular bacterium was used. Then 20 ml of antibiotic medium # 1 at 45 °C was poured to each plate and the plates were rocked for a few seconds for even spread and proper mixing of bacteria and the medium. The contents of the plates were allowed to solidify and wells approximately equidistant to each other were bored on the surfaces of the agar medium using a sterile 5 mm cork borer. 0.1 ml of the various extract concentration were dropped into each, appropriately labeled well[13-14]. The inoculated plates were kept in the refrigerator for 1 h to allow the extracts to diffuse into the medium[13]. All the plates were then incubated at 37 °C for 24 h and the zones of inhibition were measured. The means of triplicate results were taken. The results were compared against two standard drugs doxycycline and neomycin.

DPPH assay for Antioxidant activity.

The DPPH test was used in this study to assess the free radical scavenging (antioxidant) property of the extracts[15]. DPPH (4 mg) was dissolved in MeOH (50 ml) to obtain a concentration of 80 µg/ml. This assay was carried only quantitatively using a UV-Vis spectrometer. For the quantitative assay, the stock solution of crude extracts was prepared using MeOH to achieve a concentration of 10 mg/ml[16], whereas the positive standard was prepared at a concentration of 0.5 mg/ml. Dilutions of the stock solutions of the crude extracts were prepared to obtain concentrations of 5 $\times 10^{-2}$, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/ml. Diluted solutions (1.00 ml each) were mixed with DPPH (1.00 ml) and allowed to stand for 30 min for any reaction to take place. The UV absorbances of these solutions were recorded at 517 nm[17]. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the standard drug (ascorbic acid).

RESULTS AND DISCUSSION

The antibacterial activity was studied against various species of bacteria and a wide range of zones of inhibition was observed for different species at different doses. Root and bark of both medicinal plants showed good activities at a very low dose starting from 10 µg up to 150 µg. These two extracts were dissolved in water due to their high solubility in water, while the leaves of both the medicinal plants were ineffective at low dose concentration of up to 150 µg. Above 150 µg the leaves showed various zone of inhibitions which were almost comparable to the standard drugs run in parallel with the plant extracts.

Low doses of the root extract of *Debregeasia saicifolia* showed no activity against *Citrobacter* and *Proteus mirabilis* at a dose of 10 µg. The roots of this plant were also ineffective against *Citrobacter* even at a dose of 25 µg. It showed a high activity against *Bacillus subtilis* with a zone of inhibition ranging up to 17.50 mm at a dose of 150 µg, while the bark extract of *Pistacia integerrima* was highly effective against *Staphylococcus aureus* giving a zone of inhibition about 21.80 mm at a dose of 150 µg.

Bark and roots of both these medicinal plants showed a good potential as an antibacterial agent as shown in Table 1 with different zones of inhibition against other species at various doses.

Leaves of *Debregeasia saicifolia* also revealed the antibacterial potential of this medicinal plant, by showing different zones of inhibition against the tested bacteria. The highest zone of inhibition was 22.01 mm against *Citrobacter* at a dose of 1500 µg. But still at higher doses the *Debregeasia saicifolia* leaves did not showed any antibacterial activity against *Proteus mirabilis.* Similarly the leaves crude extract of this medicinal plant was also ineffective against *Escherichia coli* at a dose of 300 µg.

Although amongst all the four parts of these two medicinal plants the *Pistacia integerrima* leaves showed the highest zone of inhibition with 28.00 mm at a dose of 1500 µg, still the leaves were ineffective in preventing the growth of *Proteus mirabilis* even at a dose of 300 µg and 500 µg (Table 2).

Table 1: Antibacterial activity of *Pistacia integerrima* Bark and *Debregeasia salicifolia* Root. (Zone of inhibition in mm)

Dose (µg)	Extract	*Citrobacter*	*E. Coli*	*B. subtilis*	*S. aureus*	*P. mirabilis*	*S. typhi*
10	D.S Root	00±0.00	11.99±0.10	12.52±0.12	12.05±0.13	00±0.00	10.16 ±0.20
	P.I Bark	14.20±0.07	15.83±0.12	13.85±0.23	08.08±0.12	13.57±0.09	14.01±0.09
	Neomycin	24.59±0.01	22.09±0.08	21.16±0.05	27.22±0.03	18.75±0.15 ±0.15	23.71±0.05
	Doxycyclin	21.84±0.20	25.48 ±0.0	21.02±0.02	18.01±0.09	20.22±0.10	22.55±0.05
25	D.S Root	00±0.00	11.00±0.40	12.53±0.25	12.07±0.16	10.83±0.11	10.16±0.30
	P.I Bark	14.32±0.20	17.85±0.10	16.00±0.02	13.39±0.06	14.71±0.10	16.11±0.12
	Neomycin	24.59±0.08	22.49±0.06	22.85±0.07	29.19±0.02	21.09±0.03 ±0.03	25.42±0.08
	Doxycyclin	24.43±0.01	25.5±0.05	25.39±0.09	22.80±0.07	22.84±0.03	24.87±0.02
50	D.S Root	11.35±0.11	12.01±0.02	12.54±0.09	12.09±0.15	10.84±0.32	10.87±0.20
	P.I Bark	13.78±0.21	17.85±0.22	19.18±0.24	13.39±0.09	17.07±0.10	17.07±0.08
	Neomycin	26.37±0.10	25.65±0.09	25.71±0.11	31.76±0.08	24.04±0.02 ±0.02	25.40±0.05
	Doxycyclin	24.53±0.02	25.73±0.05	26.78±0.06	24.12±0.08	32.36±0.05	25.6±0.05
75	D.S Root	11.36±0.06	12.02±0.10	13.88±0.30	13.36±0.20	10.84±0.30	12.14±0.08
	P.I Bark	17.45±0.11	19.01±0.12	19.20±0.21	16.46±0.12	17.21±0.11	17.08±0.09
	Neomycin	26.38±0.05	28.86±0.10	26.54±0.09	32.76±0.05	25.44±0.06	26.04±0.05
	Doxycyclin	26.2±0.02	26.29±0.04	27.16±0.06	25.13±0.06	24.83±0.05	27.42±0.07
100	D.S Root	13.10±0.12	14.03±0.11	14.31±0.06	13.68±0.05	14.73±0.12	13.27±0.08
	P.I Bark	18.02±0.10	20.11±0.22	19.21±0.20	18.92±0.13	17.33±0.23	18.44±0.09
	Neomycin	26.47±0.02	28.79±0.10	27.05±0.05	33.00±0.09	25.45±0.08	26.04±0.05
	Doxycyclin	27.62±0.05	26.55±0.07	30.79±0.01	25.40±0.04	24.87±0.05	28.20±0.06
150	D.S Root	13.95±0.10	15.13±0.15	17.50±0.14	14.21±0.09	15.41±0.13	15.27±0.10
	P.I Bark	21.00±0.12	21.44±0.24	20.81±0.30	21.80±0.31	19.14±0.10	21.00±0.15
	Neomycin	29.53±0.12	29.04±0.13	28.44±0.05	33.16±0.07	25.45±0.081	28.60 ±0.04
	Doxycyclin	28.11±0.04	26.81±0.07	31.40±0.02	27.13±0.05	25.11±0.05	29.94±0.02

*DSR (*Debregeasia salicifolia* Roots), PIB (*Pistacia integerrima* Bark)

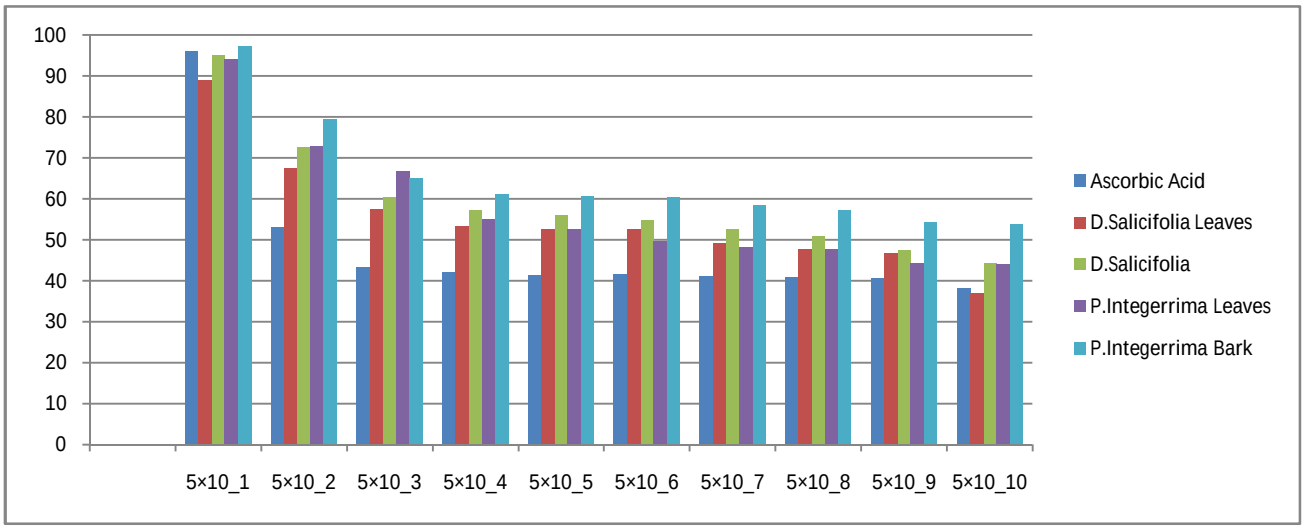

Figure 1: Antioxidant profile for various dilutions of crude extracts of the two plants.

Table 2: Antibacterial activity of *Pistacia integerrima* and *Debregeasia salicifolia* leaves. (Zone of inhibition in mm).

Dose (µg)	Extract	*Citrobacter*	*E.Coli*	*B.subtilis*	*S.aureus*	*P.mirabilis*	*S.typhi*
300	P.I Leaves	23.19±0.21	20.34±0.11	21.47±0.09	18.75±0.13	00±0.00	14.63 ±0.11
	D.S Leaves	14.38±0.07	00±0.00	11.79±0.11	12.01± 0.12	00±0.00	10.61 ±0.07
	Neomycin	24.59±0.01	22.09±0.08	21.16±0.05	27.22±0.03	18.75±0.15 ±0.15	23.71±0.05
	Doxycyclin	21.84±0.20	25.48 ±0.0	21.02±0.02	18.01±0.09	20.22±0.10	22.55±0.05
500	P.I Leaves	23.20±0.13	20.36±0.09	21.48±0.10	18.49±0.13	00±0.00	14.64± 0.10
	D.S Leaves	14.40±0.09	13.74±0.10	13.51±0.10	13.86± 0.02	00±0.00	14.71±0.02
	Neomycin	24.59±0.08	22.49±0.06	22.85±0.07	29.19±0.02	21.09±0.03 ±0.03	25.42±0.08
	Doxycyclin	24.41±0.01	25.52±0.05	25.39±0.09	22.80±0.07	22.84±0.03	24.87±0.02
600	P.I Leaves	23.21±0.11	22.36±0.15	21.49±0.14	23.00±0.09	15.80±0.11	15.32±0.07
	D.S Leaves	15.65±0.12	13.76±0.10	13.53±0.04	14.93±0.09	00± 0.00	14.73±0.17
	Neomycin	26.37±0.10	25.65±0.09	25.71±0.11	31.76±0.08	24.04±0.02 ±0.02	25.40±0.05
	Doxycyclin	24.53±0.02	25.73±0.05	26.78±0.06	24.12±0.08	32.36±0.05	25.61±0.05
800	P.I Leaves	23.89±0.12	24.72±0.11	22.60±0.12	23.20±0.10	16.80±0.14	15.39±0.09
	D.S Leaves	17.57±0.13	15.13±0.14	15.20±0.10	16.20±0.11	00±0.00	14.73±0.11
	Neomycin	26.38±0.05	28.86±0.10	26.54±0.09	32.76±0.05	25.44±0.06	26.04±0.05
	Doxycyclin	26.21±0.02	26.29±0.04	27.16±0.06	25.13±0.06	24.83±0.05	27.42±0.07
1000	P.I Leaves	25.55±0.17	25.88±0.16	23.31±0.11	24.92±0.10	18.20±0.14	18.64 ±0.12
	D.S Leaves	20.81±0.12	15.14±0.09	15.25±0.17	17.20±0.11	00±0.00	16.49±0.13
	Neomycin	26.47±0.02	28.79±0.10	27.05±0.05	33.00±0.09	25.45±0.08	26.04±0.05
	Doxycyclin	27.62±0.05	26.55±0.07	30.79±0.01	25.40±0.04	24.87±0.05	28.20±0.06
1500	P.I Leaves	28.00±0.13	27.97±0.09	24.80±0.12	27.00±0.19	24.66±0.18	19.79±0.09
	D.S Leaves	22.01±0.11	16.10±0.14	20.21±0.13	19.01±0.12	00±0.00	18.20±0.13
	Neomycin	29.53±0.12	29.04±0.13	28.44±0.05	33.16±0.07	25.45±0.08	28.60 ±0.04
	Doxycyclin	28.11±0.04	26.81±0.07	31.40±0.02	27.13±0.05	25.11±0.05	29.94±0.02

*DSR (*Debregeasia salicifolia* Roots), PIB (*Pistacia integerrima* Bark)

Table 3: Antioxidant Activity of *Pistacia integerrima* and *Debregeasia salicifolia.*

Concentration	Ascorbic Acid	*D.Salicifolia* Leaves	*D.Salicifolia* Root	*P.Integerrima* Leaves	*P.Integerrima* Bark
5×10^{-1}	96.08592	88.86765	95.12661	94.07549	97.18108
5×10^{-2}	53.07876	67.51075	72.57525	72.9097	79.50311
5×10^{-3}	43.42721	57.42953	60.24845	66.65074	65.12183
5×10^{-4}	42.1957	53.22504	57.09508	54.94505	61.06068
5×10^{-5}	41.28878	52.60392	56.13951	52.4128	60.63067
5×10^{-6}	41.65919	52.4128	54.70616	49.59388	60.24845
5×10^{-7}	41.09785	49.06832	52.69947	48.20831	58.38509
5×10^{-8}	40.94749	47.5872	50.83612	47.68275	57.09508
5×10^{-9}	40.52745	46.67941	47.53942	44.29049	54.37172
5×10^{-10}	38.18616	36.83708	44.33827	44.0516	53.89393

By overviewing the tables given below it is clear that among both the plant extracts applied during this antibacterial study *Pistacia integerrima* bark was very effective against all the species of bacteria tested even at low doses (Table 1). During this study the *Pistacia integerrima* leaves showed the most promising results which were comparable with the results of the standard drugs neomycin and doxycyclin. *Debregeasia saicifolia* leaves extract, although less potent than the *Pistacia integerrima* leaves extracts, were still also very effective (Table 2) against all other species of bacteria.

Antioxidant studies performed showed that both of these medicinal plants have a high potential as free radical scavengers. *Pistacia integerrima* bark showed antioxidant activity almost as potent as the standard drug ascorbic acid. *Pistacia integerrima* leaves also showed a high potential for free radical scavenging and antioxidant activity. *Debregeasia salicifolia* roots were more effective than *Debregeasia salicifolia* leaves. As a whole the

Pistacia integerrima plant was more potent than *Debregeasia salicifolia* as antioxidant. Table 3 shows the various dilutions of the extracts with high antioxidant activity, in some cases even better than the standard drug ascorbic acid. Figure 1 outlines the percent inhibition of all the crude extracts compared to the standard drug ascorbic acid, in relation to the concentration used.

After observing these results and their comparison with the standard drugs, it is quite clear that these plants show high antibacterial and antioxidant activity. These plants have the potential for new bioactive compounds to be explored, and should be studied phytochemically for isolation of various new compounds and establishing their activities. Antioxidant compounds and new drugs are one of the areas of prime concern nowadays because of their role in various pathologies such as heart diseases, atherosclerosis, cell membrane integrity, ageing processes and cancer.

REFERENCES

1. World Health Organization (WHO), Traditional Medicine, 2001, Fact sheet number 134. Revised May, 2003.
2. Aibinu IE, Ohaegbulam VC, Adenipekun EA, Ogunsola FT, Odugbemi TO, and Mee BJ, Extended-Spectrum Beta-Lactamase Enzymes in Clinical Isolates of *Enterobacter* species from Lagos, Nigeria. *Journal of Clinical Microbiology*, 41(5), 2003, 2197-2200.
3. Aibinu IE, Adenipekun EA, and Odugbemi T, Emergence of Quinolone Resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos State Hospitals in Nigeria, *Nigerian Journal of Health and Biomedical Science,* 3(2), 2004, 73-78.
4. Nathan C, Antibiotics at the crossroads, *Nature*, 431, 2004, 899-902.
5. Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V, Comparison of antioxidant and antimicrobial activities of tilia (*Tilia Argentea* Desf Ex DC), sage (*Salvia Triloba* L.) and black tea(*Camellia sinensis*) extracts, *J. Agric. Food Chem,* 48, 2000, 5030-5034.
6. Lai LS, Chou ST, Chao WW, Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens* Hems) leaf gum, *J.Agric. Food Chem,* 49, 2000, 963-968.
7. Aruoma OI, Nutrition and Health aspect of free radical and antioxidants, *Food Chem. Toxicol*, 32, 1994, 671-683.
8. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J, Free radicals and antioxidants in normal physiological functions and human disease, *Int. J. Biochem. Cell. Biol,* 7(1), 2006, 45-78.
9. www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=250077141
10. www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=242317014
11. Trease and Evans WC, Pharmacognosy, 11th Edition, Braillar Tiriden Company. Macmillan publishers, 1996, pp. 56-109.
12. National committee for clinical laboratory standards, Methods for dilution, antimicrobial susceptibility tests for bacteria that grow aerobically, 5th Ed, 1996, Pp 30.
13. Atata R, Sani A, and Ajewole SM, Effect of stem back extracts of *Enantia chloranta* on some clinical isolates. *Biokemistri*, 15 (2), 2003, 84-92.
14. Bonjar S GH. Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus, Serratia marcescens*, *Klebsiella pneumonia* and *Bordella bronchoseptica*, *Asian Journal of Sciences*, 3(1), 2004, 82-86.
15. Takao T, Watanabe N, Yagi I, and Sakata K, A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish, *Biosci. Biotechnol Biochem*, 58, 1994, 1780–1783.
16. Sarker SD, Eynon E, Fok K, et al, Screening the extracts of the seeds of *Achillea millefolium, Angelica sylvestris and Phleum pratense* for antibacterial, antioxidant activities and general toxicity, *Orient. Phar. Exp. Med*, 33, 2003, 157–162.
17. Sarker SD, Latif Z, Satyajit D, and Alexander I, Gray, Natural Products Isolation, Second ed, Published by, Humana press, New Jersey, 2005, p-20.

Source of Support: Nil, **Conflict of Interest:** None.