Shameem et al. Journal of Analytical Science and Technology (2015) 6:36
DOI 10.1186/s40543-015-0076-z

Journal of Analytical Science and Technology
a SpringerOpen Journal

RESEARCH ARTICLE Open Access

# Antimicrobial and antioxidant activity of methanol extracts of *Arnebia benthamii* (Wall ex. G. Don) Johnston—a critically endangered medicinal plant of North western Himalaya

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## Abstract

**Background:** *Arnebia benthamii* is one of the promising folklore medicinal plants which is being traditionally used over the years for the treatment of various prevailing diseases in the area. The aim of this research work was to evaluate the antimicrobial activity and antioxidant activity of methanolic plant extract.

**Methods:** Antimicrobial activity of the plant extract (250–500 μg/ml concentration) was analyzed against *Escherichia coli* CD0006, *Pseudomonas aeruginosa* CD0023, *Shigella flexneri* CD0033, *Klebsiella pneumonia* CD0049, *Salmonella typhimurium* CD0003, *Staphylococcus aureus* CD0001, *Aspergillus versicolor* CDF0011, *Candida albicans* CDF0032, *Candida kruesie* CDF0016, *Candida parapsilosis* CDF0013, *Aspergillus flavus* CDF0024, and *Acremonium* spp. CDF0027. The free radical scavenging assay of the plant extracts was evaluated by various antioxidant methods.

**Results:** Comparative analysis reveals that the aerial part exhibited the highest antibacterial activities against almost all tested bacterial strains with the highest inhibition zone diameter (IZD) ($30 \pm 0.54$) was recorded on *P. aeruginosa* CD0023 and *E. coli* CD0006. All the fungal strains except *C. parapsilosis* CDF0013 were more or less inhibited by both aerial and root part extracts of the plant. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values recorded revealed that the *P. aeruginosa* CD0023 was inhibited by the least concentration of 75 μg/ml of the aerial part methanol extract. The antioxidant activity of the aerial and root part extracts was almost of the same strength with the root part slightly showing a higher scavenging effect in a concentration-dependent manner in superoxide anion and hydroxyl radicals.

**Conclusions:** The plant has got a broad spectrum antimicrobial and antioxidant activity and has a promising potential for treating diseases.

**Keywords:** *Arnebia benthamii*, Disk diffusion, Kahzaban, MIC, Alcoholic extract

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## Background

Diseases caused by pathogens remain a major challenge globally and particularly in Asian countries like India. Resistance to available antibiotics is increasing at a very alarming stage globally (Stuart and Bonnie 2004). Efforts are urgently needed to replace the current available antibiotics. The antibacterial activity of plants is continuously attracting global attention (Rukayadi et al. 2009). The antimicrobial activity may therefore be due to the presence of antioxidants in extracts that have the potential to prevent the activity of free radicals and reactive oxygen species thus helping in fighting diseases caused by bacteria and other pathogens (Parray et al. 2015a; Adamu et al. 2014). In a biological system, an antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. Recently, increasing attention has been focused on the use of natural antioxidants, such as ascorbic acid, tocopherols, phenolic compounds including flavonoids, phenolic acids, and volatile compounds for preventing oxidation of biomolecules which can lead to cell injury and death (Mohamed et al. 2013). The medicinal properties of some plants have been investigated throughout the world, due to their potent antioxidant activities. Reactive oxygen species (ROS) including singlet oxygen ($^1O_2$), superoxide ion ($O_2^-$), hydroxyl ion (OH), and hydrogen peroxide ($H_2O_2$) are highly reactive and toxic molecules generated in cells under normal metabolic activities. ROS can cause oxidative damage to proteins, lipids, enzymes, and DNA molecules (Li et al. 2008; Parray et al. 2011). Living cells possess powerful scavenging mechanisms to avoid excess ROS-induced cellular injury, but with aging and under the influence of external stresses, these mechanisms become inefficient, and dietary supplementation by antioxidants is required (Peschel et al. 2006). Among the treasures of medicinal plant wealth, one of the promising folklore medicinal plant, *Arnebia benthamii* or "Kahzaban", traditionally used over the years, is a perennial medicinal herb growing in the sub-alpine and alpine zones of North West Himalaya (Dar et al. 2002; Dar and Khuroo 2013). It ranks second in the list of medicinal plants prioritized for western Himalaya and also figures among the 59 medicinal plants prioritized for conservation due to high extinction threat and is being classified as threatened non-endemic plant of Kashmir (Dar and Khuroo 2013). Gule Khazaban from *A. benthamii* is a very costly medicine (Dar et al. 2002; Parray et al. 2015b; Ganie et al. 2012) and has been found to have cardiac (used in the treatment of heart problems) and febrifuge (reduces fevers) properties. The plant is considered to be useful in the treatment of diseases of the tongue and throat (Kaul 1997). The species is a major ingredient of the commercial drug available under the name Gaozaban, which has antibacterial, antifungal, anti-inflammatory, and wound-healing properties (Kaul 1997). The roots yield a red pigment, Shikonin (a dye), which has several medicinal properties and is marketed under the trade name Ratanjot (Kashiwada et al. 1995). Secondary metabolites, arnebin 1, and arnebin 3 obtained from other species of this genus are reported to possess anti-cancerous property (Harborne and Baxter 1996).

The important metabolites belonging to different class of compounds along with mass, $m/z$ ratio and abundance like artemidiol ($m/z$—235.1; abundance—12,359.8); hoslundal ($m/z$—311.1; abundance—21,354.5), shikonin ($m/z$—289.1; abundance—3817.1); ganoderiol C ($m/z$—541.4; abundance—59,593.7); and 2-hexaprenyl-6-hydroxyphenol ($m/z$—541.4; abundance—59593.7) were found to be of immense importance in curing many ailments in humans or recommended as antioxidants (Parray et al. 2015b; Jan et al. 2015). The other phytochemical constituents isolated from *Arnebia* spp. are deoxyshikonin, acetyleshikonin, and β-hydroxy-sovaleryl shikonin and napthoquinones, arnebinone, stigmasterol, arnebin-7. *Arnebia* is considered to be useful in the treatment of diseases of tongue and throat, fevers, and cardiac disorders. Its root is used as an antiseptic and antibiotic for healing of wounds and applied as a poultice. Its paste made in water is applied on fire burns for quick healer (Chauhan 1999). Dry plant yields essential oil (Arnebinus 0.37 %). Aerial parts, leaves, flowers, and roots are medicinally important. *Arnebia* is considered to be useful in the treatment of diseases of tongue and throat, fevers, and cardiac disorders. In India, it is a traditional herb of Ayurvedic and Unani system of medicine. Its flowers are reported to have soothing effect on patients with heart ailments (Kaul 1997). Secondary metabolites, arnebin 1, and arnebin 3 obtained from the other species of this genus are reported to possess anti-cancerous property (Harborne and Baxter 1996). *Arnebia euchroma* exhibits a potent anti-HIV activity (Kashiwada et al. 1995). In a very recent study, Parray et al. (2015b) evaluates the antioxidant activity of the ethyl acetate root extract of *A. benthami* and its DNA protection property, and it was observed that shikonin present in the root extract was effective in scavenging the radicals and its ability to protect the DNA from hydroxyl radicals. Some other studies pertaining to the cytotoxic effect and antioxidant activity of the whole plants of *A. benthami* credit to Ganaie et al. (2012a). In one more recent report, the shikonin and its derivatives showed a significant antioxidant activity from tissue cultures in *Arnebia hispidissima* (Singh and Sharma 2014). In this background, the present study aims to further add to the scientific knowledge by evaluating the antimicrobial activity against the different clinically isolated microbial pathogens and antioxidant activity through

different methods from the separate aerial and underground parts of *A. benthamii.*

## Methods

### Collection and identification of plant material

*A. benthamii* (wall ex. G. Don) Johnston was collected as a whole plant from Duksum and Sinthan Top, Kashmir Himalaya, J&K, India, in July-September 2012. The characters of the study area are altitude (meter above sea level) 3748, forest range Anantnag, climatic zones subalpine, direction southeast, latitude, longitude 34–20° N, 75–20° E, and snowfall (2012) 221 cm less dense. Sampling was carried out immediately after inflorescence formation, and plants were collected manually in bulk from the area. The plant was identified at Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar under accession no. 1748.

### Preparation of plant extracts

Both plant parts were separated, washed, and dried under shade, chopped, and made in powdered form in a wood grinder. Of the dried powder of the aerial and root parts, 50 g was extensively extracted in Soxhlet extractor with methanol (500 ml) (HPLC grade, Rankem). The extraction process was carried out in the Soxhlet apparatus, and the process was carried out for the time unless the cotton used in the Soxhlet apparatus became again colorless. Extracts were concentrated using rotevaporator, which were later dried, weighed, and kept for further usage in sterilized caped vials at 4 °C.

### Test microorganisms

#### *Bacterial strains*

The bacterial strains were *Escherichia coli* CD0006, *Pseudomonas aeruginosa* CD0023, *Shigella flexneri* CD0033, *Klebsiella pneumonia* CD0049, *Salmonella typhimurium* CD0003, and *Staphylococcus aureus* CD0001.

#### *Fungal strains*

The fungal strains were *Aspergillus versicolor* CDF0011, *Candida albicans* CDF0032, *Candida kruesie* CDF0016, *Candida parapsilosis* CDF0013, *Aspergillus flavus* CDF0024, and *Acremonium* spp. CDF0027.

All the strains were taken from the Department of Microbiology, CORD, University of Kashmir, with proper clinical isolation number.

#### *Phytochemical screening*

The phytochemical screening tests of the different plant extracts were performed by using standard procedures (Harborne 1973; Sofowora 1993; Ayoola et al. 2008)

#### *Quantitative estimation of phenols*

The amounts of the total phenolics in the extracts were determined according to the Folin-Ciocalteu procedure (Padmaja et al. 2011). The samples (200 μl) were introduced into test tubes, and 1 ml of Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5 %) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in μg/mg tissue as calculated from standard gallic acid graph. A standard calibration curve was prepared by plotting absorbance vs concentration, and it was found to be linear over this concentration range.

#### *Antimicrobial susceptibility tests*

The Kirby-Bauer (Bauer et al., 1966) method was followed for determination of antimicrobial (antifungal and antibacterial) activity. However, for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination of plant extracts, the method used by Sette et al. (2006) with some modifications was followed on a cell culture test plate (96 wells). The four bacterial strains, *E. coli* CD0006, *P. aeruginosa* CD0023, *S. flexneri* CD0033, *K. pneumoniae* CD0049, were selected for determination of MIC and MBC of the methanol extracts of the shoot and root parts.

#### *Antioxidant activity*

All the methods of antioxidant activity, i.e., 2,2-diphenylpicrylhydrazyl (DPPH) assay, hydroxyl scavenging activity-deoxyribose assay, lipid peroxidation method, and superoxide anion radical scavenging activity (Parray et al. 2015a),were followed for describing the scavenging potential of both the aerial and underground parts of *A. benthamii*.

#### *Calculations*

The percentage inhibition of the free radicals in all the abovementioned methods was calculated by using the following formula:

$$\% \text{ age inhibition } = \text{ Ac–As Ac } \times 100$$

where Ac is the absorbance of the controlled reaction (reaction mixture without any antioxidant substance) and As is the absorbance of the reaction mixture with the reference substance or plant extract. All the methods were repeated three times ($n = 3$).

#### *Statistical analysis*

Data were subjected to analysis of variance using SSPP software version 17.0 (SAS Institute Inc., Cary, NC, USA). The inhibitory activities of plant extracts was

considered significant according to the magnitude of the $F$ value ($P < 0.005$).

## Results and discussion

### Phytochemical screening

Different methods were followed to determine qualitatively the presence of phytochemical constituents present in the plant extracts. The amount of crude extracts varied among the parts used. Under the present study, the methanol extract of the aerial part (4.1 %) showed higher yield. The qualitative phytochemical screening of crude extracts of *A. benthamii* revealed that alkaloids, phenols, anthraquinones, and flavonoids were present in both aerial part (AP) and root part (RP) extracts. Saponins, glycosides, and tannins were absent in both the RP and AP extracts while terpenoids were present in only the RP extract (Table 1).

The results obtained in the present study revealed that the level of the total phenolic (TP) content in the plant extracts of both the AP and RP extracts were considerable. The TP content was measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: $y = 0.007 \times + 0.178$, $r^2 = 0.991$). The TP content (200 μg GAE $mg^{-1}$ dried weight (DW)) was found in the AP extracts, and in the root, MeOH extract (210 μg GAE $mg^{-1}$ DW) was present (Table 1).

### Antimicrobial activity

Antibiotics that once readily cured a wide range of infections are becoming less valuable mainly due to their misuse and also the development of antibiotic resistance (Nostro et al. 2001); therefore, there is a need to develop alternative antimicrobial drugs for the treatment of diseases from medicinal plant products Yildirim et al. (2001). Although the different parts of a plant showed varying antimicrobial activity, in the case of *A. benthamii*, both aerial as well as root parts are being frequently used for treating some of the common prevailing diseases in Himalayan area (Kaul 1997; Dar and Khuroo 2013). In our study, both plant extracts exhibited significant antimicrobial activity.

The average maximum inhibition zone diameter (IZD) (30 ± 0.54) was recorded for both *P. aeruginosa* CD0023 and *E. coli* CD0006 followed by *S. typhimurium* CD001 with an IZD of 28 ± 0.93 mm (Table 2). However, *E. coli* CD0006 was found highly susceptible with an IZD of 25 mm followed by *S. flexneri* CD0033 with an IZD of 20 mm for the root extract (Table 2). It was obvious from the results that the root extracts showed least inhibitory activity than the aerial part extracts (Table 2). Among the strains tested, *S. aureus* CD0001 was found susceptible to only methanol root extract, and it is well documented by Chien-Chang et al. (2000) who reported a strong anti-*S. aureus* activity by shikonin and alkannin derivates of *A. euchroma.* The methanol extract of the aerial part showed the highest inhibitory activity against *Acremonium* spp. CDF0027 (25 mm) followed by *A. versicolor* CDF0011 with an IZD of 22 mm and *C. albicans* CDF0032 with an IZD of 22 mm 14 mm. However, *C. parapsilosis* CDF0013 showed complete resistance towards both the aerial and root extracts of the plant (Table 3). The MIC and MBC results revealed that the lowest inhibitory concentration of 75 μg/ml and lowest bactericidal concentration of 100 μg/ml of the aerial plant extract were observed against *P. aeruginosa* CD0023. In the case of *S. flexneri* CD0033, the lowest values of inhibitory and bactericidal concentrations recorded were 200 and 400 μg/ml, respectively. Meanwhile, the root extract was found less effective in inhibiting the pathogenic organisms, and the lowest values of inhibitory and bactericidal concentration recorded were 225 and 500 μg/ml, respectively, against *S. flexnerii* CD0033 (Fig. 1). In our study, the plant extracts

**Table 1** Phytochemical analysis of different secondary metabolites present in the aerial and root part extracts of *A. benthamii* L

| Phytochemical constituents | Aerial part extract | Root extract |
|---|---|---|
| Alkaloids | + | + |
| Glycosides | – | – |
| Anthraquinones | + | + |
| Saponins | – | – |
| Tannins | – | – |
| Flavonoids | + | + |
| Terpenoids | – | + |
| Phenolic compounds | + | + |
| | (200 GAE (μg/mg) DW) | (210 GAE (μg/mg) DW) |
| Yield (%) | 4.1 | 2.9 |

(+) = present, (–) = absent

**Table 2** Antibacterial activity of methanol extract of the aerial and root parts of *Arnebia benthamii* against pathogenic bacterial strains

| Strains | Aerial part | Root part | Gentamycin |
|---|---|---|---|
| Inhibition zone diameter (mm) | | | |
| *Staphylococcus aureus* CD0001 | NA | $^{a}08 \pm 0.98$ | $^{b}23 \pm 0.23$ |
| *Shigella flexneri* CD0033 | $^{a}23 \pm 1.20$ | $^{c}20 \pm 0.12$ | $^{c}30 \pm 0.33$ |
| *Klebsiella pneumonia* CD0049 | $^{a}20 \pm 0.67$ | $^{b}13 \pm 0.65$ | $^{a}15 \pm 0.53$ |
| *Pseudomonas aeruginosa* CD0023 | $^{bc}30 \pm 0.54$ | $^{b}12 \pm 0.46$ | $^{a}16 \pm 0.44$ |
| *Escherichia coli* CD0006 | $^{bc}30 \pm 0.87$ | $^{c}25 \pm 0.15$ | $^{ab}21 \pm 0.22$ |
| *Salmonella typhimurium* CD0003 | $^{b}28 \pm 0.93$ | $^{b}12 \pm 0.34$ | $^{c}29 \pm 0.11$ |

Values are represented as mean ± SD ($n = 3$). Values along the columns with the same superscript letter are significant at $P < 0.005$; Concentration used was 250 μg/ml
*NA* no activity

**Table 3** Antifungal activity of methanol extract of the aerial and root parts of *Arnebia benthamii* against pathogenic fungal strains

| Strains | Aerial part | Root part | Nystatin |
|---|---|---|---|
| Inhibition zone diameter (mm) | | | |
| *Aspergillus flavus* CDF0024 | $^{bc}15 \pm 0.10$ | NA | $^{b}35 \pm 0.08$ |
| *Aspergillus versicolor* CDF0011 | $^{c}22 \pm 0.91$ | $^{a}12 \pm 0.23$ | $^{a}20 \pm 0.90$ |
| *Acremonium* spp. CDF0027 | $^{a}12 \pm 0.15$ | NA | $^{b}30 \pm 0.09$ |
| *Candida albicans* CD0032 | $^{a}14 \pm 1.02$ | $^{a}13 \pm 0.17$ | $^{a}20 \pm 1.00$ |
| *Candida kruesie* CDF0016 | $^{a}13 \pm 1.34$ | $^{a}13 \pm 0.07$ | $^{a}20 \pm 0.21$ |
| *Candida parapsilosis* CDF0013 | NA | NA | $^{a}20 \pm 0.32$ |

Values are represented as mean ± SD ($n = 3$). Values along the columns with the same superscript letter are significant at $P < 0.005$; *NA* no activity. Concentration used was 500 μg/ml

exhibited significant antimicrobial activity against the pathogenic organisms which are considered as a major component for diseases. The possible explanation of the plant activity may be due to the presence of alkannin, naphthoquinones, shikonin, and their derivatives in species of some genera of the Boraginaceae family such as *Arnebia*, *Alkanna*, and *Onosma* (Manjkhola and Dhar 2002). In our study, some of the strains were resistant to the plant extracts which may be due to the permeability barrier afforded by their outer membranes (Apak and Olila 2006). Pharmacologically and biologically, the *Arnebia* spp. were documented to have antibacterial (Singh and Sharma 2012), antitumor (Deng et al. 2010), antifungal (Gao 1986), and antiviral-HIV properties (Kashiwada et al. 1995). Some reports mentioned that the naphthoquinone derivative, arnebin-1 (b,b-dimethylacrylalkannin), present in *Arnebia* spp., significantly accelerated wound healing vis-à-vis the inhibiting growth of pathogenic organisms (Sidhu et al. 1999). The multidrug resistance of *K. pneumonia* as reported is a major concern nowadays, and our plant extracts exhibit a significant activity against the *K. pneumonia* which is further supported by Koca et al. (2010) who also reported the activity of *Arnebia densiflora* extracts against some of the isolated strains of *K. pneumonia*. The potent antifungal activity of the plant extracts of *A. benthamii* against *Aspergillus niger* and *C. albicans* is well documented in literature of other plants (Mathur et al. 2011; Menghani et al. 2011). It is established that the anthraquinone compounds like shikonin present in *Arnebia* spp. possess many biological activities and are likely to have an influence on biological membranes via the inhibition of protein synthesis and their activity increases with increasing lipophilicity of alkoxy group (Ding et al. 2005). The roots of *Arnebia* species contain mixture of naphthoquinones including derivatives of alkannin and shikonin. Shikonin and its derivatives were also investigated from tissue cultures of *Arnebia* species. These phytochemicals are potent pharmaceutical substances that showed significant biological activities including antioxidant and antimicrobial activity (Singh and Sharma 2014).

### Antioxidant activity

All the methods provide a better assessment of antioxidant properties, and the results revealed that inhibitory activity was concentration dependent. The concentration range of 50–300 μg/ml of the plant extracts as well as for the control (ascorbic acid and BHT) were used. The 10 % aq DMSO was used as negative control in all experiments. Free radical scavenging potential of the plant extracts at different concentrations was tested by the DPPH method. The percent inhibition of the aerial and root plant extracts (50–300 μg/ml) are about 50, 55, 69, 73, 80, and 86 % and 58, 64, 70, 75, 82, and 88 %, respectively, and it was obvious from the results that values of the standard antioxidant were equal with our plant extracts (Table 4). In support to our results, a

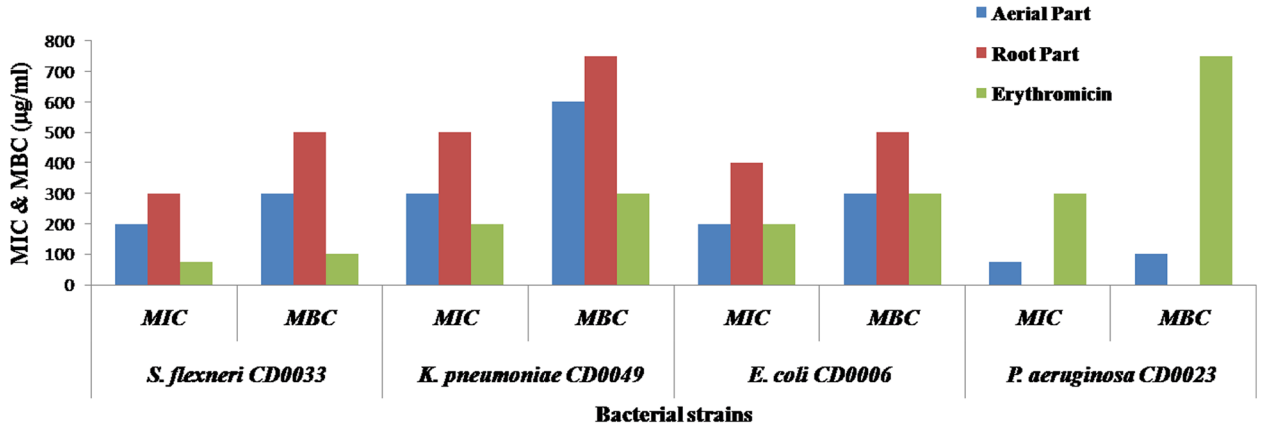


**Fig. 1** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (μg/ml) of the methanol extract of the aerial parts and root parts of *Arnebia benthamii* against standard pathogenic bacterial strains

similar type of work has also been carried out using the whole plants of *A. benthamii* by Ganaie et al. (2012) and significant DPPH activity has also been documented against *A. densiflora* root extracts (Orhan et al. 2008). The root extracts were found to be more effective in scavenging the superoxide radicals, and the percentage inhibition of the aerial and root extracts are 54, 58, 62, 66, 68, and 70 % and 58, 64, 70, 75, 82, and 88 %, respectively (Table 4). It has been established that the presence of compounds like anthraquinones, shikonins, and alkanins in the family Boraginaceae is a possible reason for effective scavenging or chelating of superoxide radicals (Kessler et al. 2003; Parray et al. 2015b). Hydroxyl radical is an extremely reactive species formed in biological systems. The OH is known to cause DNA damage by degradation of deoxyribose moiety (Kumar 2011); however, the scavenging or chelation of radicals by any substance attributes the antioxidant capacity of that particular substances (Ganie et al. 2012a, Parray et al. 2010; Parray et al. 2011), and in our study, both the alcoholic plant extracts showed a good scavenging activity of OH radicals. Similarly, Saenjum et al. (2010) reported the protective effect of *Caesalpinia sappan* extracts on DNA damage induced by hydroxyl radical at the same concentration tested. The alcoholic aerial part and root extract (50–300 μg/ml) showed a protecting effect of DNA from OH radicals of the highest of 57, 66, 72, 77, and 85 % and 56, 64, 70, 77, and 86 %, respectively, while BHT exhibited about 95 % inhibitory effect of radicals at 300 μg/ml (Table 3). However, similar studies conducted by Ganaie et al. (2012) on *A. benthamii* reported that the methanolic extract exhibited 72 % protection effect at 800 μg/ml concentration. The inhibition of $FeSO_4$-induced lipid peroxidation was high in the presence of the positive control (ascorbic acid 95.78 ± 1.0 %) compared to the plant extracts of *A. benthamii*. The aerial part and root extract showed considerable inhibitory activity of 84 and 72 %, respectively, at a higher concentration of 300 μg/ml (Table 4). The alkannin and shikonin obtained from *Arnebia* species have been earlier reported to inhibit lipid peroxidation in vitro (Kourounakis et al. 2002); in support to our studies, Ganai et al. (2012) reported the similar type of observations from the extracts of *A. benthamii*. Our study showed that the plant extracts form a strong hydroxyl radical, scavenging DPPH, and superoxide anion and inhibition of yolk lipid peroxidation in a dose-dependent manner as reported earlier (Ganie et al. 2012; Parray et al. 2011) and its possible mechanism is may be the presence of the alkaloids and phenolic substances that helps to capture lipid peroxidation chain reactions triggered by reactive oxygen species, reduce the lipid peroxidation chain length, and block or slow down the lipid peroxidation (Huang et al. 2006). The *A. benthamii* extracts seems to have good potential as a source for natural antioxidants. In addition, the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation Rekka and Kourounakis (1991). The antimicrobial and antioxidant activity may be either due to the individual or additive effect of the phytoconstituents. The result of the present study offers pharmacological evidence on the folkloric use of *A. benthamii*. Our study is also supported by some recent literature regarding the antioxidant activity of ethyl acetate fraction of *A. benthamii* (Parray et al. 2015b).

## Conclusions

The results of this investigation, which determined the radical scavenging and antioxidant activity of the aerial and root extracts of *A. benthami*, demonstrate that these

**Table 4** Antioxidant activities of methanol extract of the aerial and root parts of *Arnebia benthamii*

| Conc. μg/ml | DPPH scavenging activity | | | Superoxide dismutase (SOD) activity | | | Hydroxyl scavenging assay | | | Lipid peroxidation assay | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % scavenging of radicals | | | | | | | | | | | |
| | AP | RP | Aa | AP | RP | Aa | AP | RP | BHT | AP | RP | BHT |
| 50 | $^{a}50 \pm 2.5$ | $^{a}58 \pm 1.32$ | $^{b}55 \pm 1.9$ | $^{a}54 \pm 1.4$ | $^{a}58 \pm 1.3$ | $^{a}65 \pm 1.43$ | $^{a}51 \pm 2.1$ | $^{a}50 \pm 1.4$ | $^{a}65 \pm 1.3$ | $^{b}45 \pm 1.4$ | $^{a}53 \pm 1.4$ | $^{a}70 \pm 1.3$ |
| 100 | $^{a}55 \pm 2.4$ | $^{ab}64 \pm 2.31$ | $^{ab}61 \pm 1.4$ | $^{a}58 \pm 1.0$ | $^{bc}64 \pm 2.3$ | $^{ba}70 \pm 1.09$ | $^{ab}57 \pm 1.4$ | $^{ab}56 \pm 1.0$ | $^{ab}70 \pm 1.23$ | $^{a}53 \pm 1.0$ | $^{ab}57 \pm 1.0$ | $^{b}77 \pm 1.23$ |
| 150 | $^{b}69 \pm 2.0$ | $^{b}70 \pm 2.03$ | $^{b}67 \pm 1.0$ | $^{ab}62 \pm 1.0$ | $^{c}70 \pm 2.0$ | $^{c}79 \pm 1.9$ | $^{c}66 \pm 0.12$ | $^{c}64 \pm 1.12$ | $^{c}76 \pm 1.54$ | $^{b}59 \pm 1.0$ | $^{b}59 \pm 1.0$ | $^{c}85 \pm 1.54$ |
| 200 | $^{b}73 \pm 1.8$ | $^{b}75 \pm 1.65$ | $^{c}74 \pm 1.1$ | $^{b}66 \pm 1.65$ | $^{c}75 \pm 1.65$ | $^{cd}84 \pm 1.76$ | $^{cd}72 \pm 1.65$ | $^{cd}70 \pm 1.65$ | $^{d}83 \pm 1.7$ | $^{bc}63 \pm 1.65$ | $^{bc}63 \pm 1.65$ | $^{d}91 \pm 1.7$ |
| 250 | $^{bc}80 \pm 3.0$ | $^{c}82 \pm 2.54$ | $^{cd}82 \pm 1.7$ | $^{bc}68 \pm 1.89$ | $^{d}82 \pm 2.54$ | $^{d}90 \pm 1.54$ | $^{d}77 \pm 1.89$ | $^{d}77 \pm 1.89$ | $^{de}89 \pm 1.2$ | $^{cd}69 \pm 1.89$ | $^{c}65 \pm 1.89$ | $^{d}95 \pm 1.6$ |
| 300 | $^{c}86 \pm 2.9$ | $^{c}88 \pm 1.09$ | $^{e}90 \pm 1.9$ | $^{c}70 \pm 1.34$ | $^{d}88 \pm 1.09$ | $^{d}94 \pm 1.33$ | $^{e}85 \pm 1.0$ | $^{e}86 \pm 1.34$ | $^{e}95 \pm 1.0$ | $^{d}72 \pm 1.34$ | $^{d}84 \pm 1.34$ | $^{e}98 \pm 1.0$ |

Values are represented as mean ± SD ($n = 3$). Values along the columns with same superscript letter are significant at $P < 0.005$. Ten percent of aq DMSO was used as negative control in all treatments

*AP* aerial part, *RP* root part, *BHT* butylated hydroxy toluene, *Aa* ascorbic acid

might be proposed as a dietary supplements as antioxidant for the prevention and/or treatment of conditions that occur due to oxidative damage and can protect DNA damage by hydroxyl radical. The plant has got a broad spectrum antimicrobial and antioxidant activity and could be a potential alternative for treating various diseases.

#### Competing interests
The authors hereby declare no conflict of interest

#### Authors' contributions
NS and JAP carried out the experimental work, ANK, RH And JAP designed the experiment, NS, JAP and SAB drafted the manuscript. All authors read and approved the final manuscript.

#### Acknowledgements
The authors are highly thankful to the Department of Botany for identifying the plant and the Department of Microbiology, SKIMS, Soura J&K, for providing the clinical strains.

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Received: 24 January 2015 Accepted: 1 December 2015
Published online: 10 December 2015

#### References
Adamu M, Vinny N, Elof JN. The antibacterial activity, antioxidant activity and selectivity index of leaf extracts of thirteen South African tree species used in ethnoveterinary medicine to treat helminth infections. BMC Vet Res. 2014;10:52.

Apak L, Olila D. The *in-vitro* antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea*—Ugandan medicinal plants. African Health Sci. 2006;6(1):31–5.

Bauer AW, Kirby E, Sherris EM, Turk M. Antibiotic by standardized single disk method. Am J Clin Path. 1966;45:493–6.

Chien-Chang SS, Wan-Jr L, Shyh-Yuan L, Chia-Hung L, Gum H. Antimicrobial activities of naphthazarins from. Arnebia euchroma. 2000;65(12):1857–62.

Dar, G. H., Bhagat, R. C. and Khan, M. A. 2002.Biodiversity of Kashmir Himalaya. Valley Book House, Srinagar, India.

Dar GH, Khuroo AA. Floristic diversity in the Kashmir Himalaya: progress, problems and prospects. Sains Malaysiana. 2013;42(10):1377–86.

Deng R, Tang J, Xie BF, Feng GK, Huang YH, Liu ZC, et al. Int J Cancer. 2010; 127(1):220–9.

Ding Y, Tong M, Liu S, Moscow JA, Tai HH. NAD-linked 15-hydroxyl prostaglandin dehydrogenase (15-PGDH) behaves as a tumor suppressor in lung cancer. Carcinogenesis. 2005;26:65–72.

Ganie SA, Jan A, Muzaffar S, Zargar BA, Hamid R, Zargar MA. Radical scavenging and antibacterial activity of *Arnebia benthamii* methanol extract. Asia Pac J Trop Med. 2012;5(10):766–72.

Ganie SA, Dar, TA, Hamid R, Zargar O, Abeer S., et al. (2014). In vitro antioxidant and cytotoxic activities of Arnebia benthamii(Wall ex. G. Don): a critically endangered medicinal plant of Kashmir valley. Oxid. Med. Cell.
Longev, DOI:10.1155/2014/792574

Gao J. A general review of resources, chemistry, pharmacology and clinical application of Gromwell. Zhongcaoyao. 1986;17:28–31.

Harborne JB (1973) Phytochemical methods, London Chapman Hall Ltd, New York, Pp 49–188

Harborne JB, Baxter H. Dictionary of plant toxins. New York: Wiley; 1996.

Huang WS, Lee AR, Yang CH. Antioxidative and anti-inflammatory activities of polyhydroxy flavonoids of *Scutellaria baicalensis* Georgi. Biosci Biotechnol Biochem. 2006;70:2371–80.

Jan S, Kamili AN, Parray JA, Bedi YS (2015) Differential response of terpenes and anthraquinones derivatives in Rumex dentatus and Lavandula officinalis to harsh winters across North Western Himalaya. Natural Product Research DOI: 10.1080/14786419.2015.1030404

Kashiwada Y, Nishizawa M, Yamagishi T, Tanaka T, Nonaka G, Cosentino LM, et al. Anti-aids agent, 18. Sodium and potassium salts of caffeic acid tetramers from *Arnebia euchroma* as anti-HIV agents. J Nat Prod. 1995;58(33):392–400.

Kaul MK. Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya. New Delhi: Indus publishing co; 1997. p. 173.

Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol. 2003;55:131–42.

Koca U, Ozcelik B, Ozgen S. Comparative in vitro activity of medicinal plants Arnebia densiflora and Ecballium elaterium against isolated strains of Klebsiella pneumoniae Turk J Pharm Sci. 2010;7(3):197–204.

Kourounakis AP, Assimopoulou AN, Papageorgiou VP. Alkannin and shikonin: effect on free radical processes and on inflammation—a preliminary pharmacochemical investigation. Arch Pharm. 2002;335:262–6.

Kumar A. Biomedical studies on lipid peroxidation and erythrocyte fragility during the process of aging. Asian Pac J Trop Biomed. 2011;1(1):6–7.

Li HB, Wong CC, Cheng KW, Chen F. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT—Food. Sci Technol. 2008;41:385–90.

Mathur A, Singh R, Yousuf S, Bhardwaj A, Verma SK, Babu P, et al. Antifungal activity of some plant extracts against Clinical Pathogens. Adv Appl Sci Res. 2011;2(2):260–4.

Menghani E, Pareek A, Neghi RS, Ojha CK. Search for Antimicrobial potentials from certain Indian medicinal plants. Res J Med plants. 2011;5(3):295–301.

Mohamed A A, Ali S I, El-Baz F K (2013) Antioxidant and antibacterial activities of crude extracts and essential oils of Syzygium cumini leaves. Plos one DOI: 10.1371/journal.pone.0060269

Nostro A, Cannatelli MA, Giuseppe C, Alonzo V. The effect of *Nepeta cataria* extract on adherence an enzyme production of *Staphylococcus aureus*. Int J Antimicrob Agents. 2001;18:583–5.

Orhan I, Tosun F, Şener B. Coumarin, anthroquinone, and stilbene derivatives with anticholinesterase activity. Z Naturforsch 2008;63:366–370.

Padmaja M, Sravanthi M, Hemalatha KPJ. Evaluation of antioxidant activity of two Indian medicinal plants. J Phytology. 2011;3(3):86–91.

Parray A, Kamili AN, Hamid R, Ganai BA, Mustafa KG, Qadri RA. Phytochemical screening, antifungal and antioxidant activity to *Euryale ferox*- SALISB. a threatened aquatic plant of Kashmir Himalaya. J Pharm Res. 2011;4(7):2170–4.

Parray JA, Kamili AN, Hamid R, Reshi ZA, Qadri RA (2015a). Antibacterial and antioxidant activity of methanol extracts of Crocus sativus L. c.v. Kashmirianus. Front Life Sci 8 (1), 40–46, http://dx.doi.org/10.1080/21553769.2014.951774

Parray JA, Hamid R, Kamili AN, Shameem N, Jan S, Ganai BA. Biological efficacy and radical scavenging potential of shikonin in *Arnebia benthamii* (Wall ex. G Don). Johnston: Industrial Crops and Products; 2015b. p. 434–9. (Impact Factor: 3.21). 04/2015 74.

Peschel W, Sanchez-Rabaneda F, Dieckmann W, Plescher A, Gartzia I, Jiménez D, et al. An industrial approach in the search of natural antioxidants from vegetable and fruits wastes. Food Chem. 2006;97:137–50. doi:10.1016/j.foodchem.2005.03.033.

Rekka E, Kourounakis PN. Effect of hydroxyl ethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity. Some structural aspects J Pharm Pharmacol. 1991;43:486–91.

Rukayadi Y, Lee K, Han S, Yong D, Hwang J-K. In vitro activities of panduratin a against clinical staphylococcus strains. Antimicrob Agents Chemother. 2009; 53:4529–33.

Saenjum C, Chaiyasut C, Kadchumsang S, Chansakaow S, Suttajit M. Antioxidant activity and protective effects on DNA damage of *Caesalpinia sappan* L. extract. J Med Plant Res. 2010;4(15):1594–600.

Sette LD, Passarini MRZ, Delarmelina C, Salati F, Duarte MCT. Molecular characterization and antimicrobial activity of endophytic fungi from coffee plants. World J Microbiol Biotechnol. 2006;22:1185–95.

Sidhu GS, Singh AK, Banaudha KK, Gaddipati JP, Patnaik GK, Maheshwari RK. Arnebin-1 accelerates normal and hydrocortisone-induced impaired wound healing. J Invest Dermatol. 1999;113:773–81.

Singh B, Sharma A. Anti-inflammatory and antimicrobial activity of Shikonin derivatives from Arnebia hispidissima (Lehm.) DC. Phytopharmacol. 2012;3(1): 68–81.

Singh B, Sharma RA. Antioxidant and antimicrobial activities of Arnebia hispidissima. Am J Adv Drug Deliv. 2014;2(2):224–37.

Sofowora A. Medicinal plants and traditional. Ibadan Nigeria: medicine in Africa Spectrum books Ltd; 1993. p. 289.

Stuart BL, Bonnie M. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10:S122–9.
Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumes crispus* L. extracts. J Agric Food Chem. 2001;49:4083–9.